Herbicides in fenugreek (Trigonella foenum-graecum, L) with particular reference to diosgenin and protein yields.

Mohamed, El-Sadig Suliman

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HERBICIDES IN FENUGREEK (*TRIGONELLA FOENUM-GRAECUM*, L) WITH PARTICULAR REFERENCE TO DIOSGENIN AND PROTEIN YIELDS

THESIS

Submitted by EL-Sadig Suliman Mohamed

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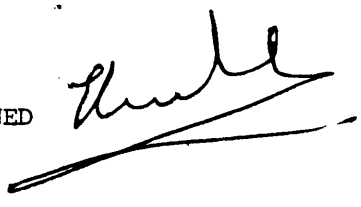

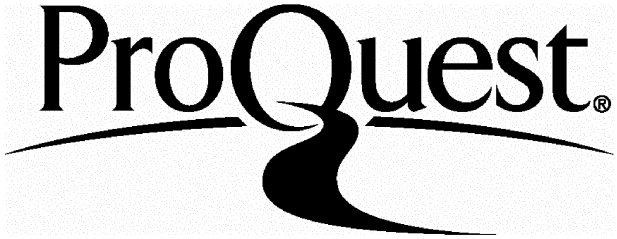

Dedication

To my brother, Basheer, for his encouragement,

my wife, Hayat, for her support and sacrifice,

and my son Mohamed, whose presence gave me comfort.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Drs. Roland Hardman and Raymond J.Hance for their wonderful supervision and encouragement in the course of this work.

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I thank Mrs Judy Harbutt for her excellent typing.

Finally, I wish to express my sincere gratitude to the Sudan Government for the study leave and to the International Centre for Agricultural Research in the Dry Areas (ICARDA) for providing me with the scholarship to pursue this work.

ABSTRACT

The tolerance of four varieties of fenugreek *(Trigonella foenum-graecum* L.) to seven pre-emergence and six post-emergence herbicides was tested in the glasshouse and some were studied further in the field.

Because fenugreek is slow to establish, pre-emergence herbicides will usually be necessary, supplemented perhaps by a post-emergence treatment. Of the pre-emergence compounds tested, fenugreek tolerated trifluralin; methazole; chlorthal-dimethyl plus methazole; metamitron; and nitrofen at realistic rates of application.

Trifluralin was particularly promising. The tolerance of fenugreek to this and other dinitroaniline herbicides was studied both in the glasshouse and in the field. A simple technique was used to investigate the site of uptake of these herbicides. Entry through the root produced a greater response than shoot entry. Laboratory and glasshouse studies of the effect of soil properties on the phytotoxicity of these compounds showed there was a negative correlation between activity and soil organic matter content. The selectivity values of the dinitroanilines between fenugreek and weeds were compared. Trifluralin and isopropalin showed good selectivity except with cruciferous weeds which were very resistant.

EPTC is very effective against a wide spectrum of weed

species including many which are unaffected by the dinitroanilines, but it is marginally tolerated by fenugreek. In an attempt to improve its selectivity,the effect of herbicide safeners was examined. R25788 and MON4606 as seed treatments gave good results in pot experiments. They protected fenugreek from up to 5 kg/ha EPTC. However, high rates of safeners adversely affected fenugreek growth. Eradicane (EPTC + R25788) as a seed dressing was effective against certain weeds,but it injured the crop.

In pot and field experiments, nodulation of fenugreek was affected only when plant growth was reduced by the herbicides. Abnormally low protein content was associated with high diosgenin yield. In pot experiments EPTC with R25788 or with MON4606 reduced diosgenin yield but not protein content.

Diquat was used as a desiccant to enhance maturity and reduce post-harvest fungal attack. Seed from desiccated plants yielded more diosgenin than from non-desiccated plants.

Pre-planting soil incorporated trifluralin or isopropalin is recommended for weed control in fenugreek. Either MCPB or bentazone plus MCPB is recommended as a supplementary post-emergence treatment for resistant weeds.

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INTRODUCTION

CHAPTER 1

a) Agriculture in The Sudan

With an area of 2,505,805 km^2, the Sudan is the largest state in Africa, located between 22°E and 39°E and 4°N to 22°N. It includes within its territory a series of zones transitional from sub-equatorial forests in the south to arid desert in the north. It occupies part of a vast basin, its surface being largely a plain, sloping generally downwards from south to north and towards the line of the River Nile.

The climate ranges from a tropical continental type in the northern desert to equatorial on the southern border. Rainfall increases generally southwards and eastwards. Average temperatures are everywhere high, for example Northern zone = 29°C, Central zone = 31°C and Southern zone = 28°C.

The Central Clay Plain covers most of the east, central and south-east parts of the country and occupies about one third of the total area. From Khartoum westwards, there is a broad zone of wind blown sand known as "Qoz". This area of light sandy soil contains pockets of heavier textured soils. North of the "Qoz" and the Central Clay Plain lies an area of stony and sandy thin desert soils.

The Sudan is very largely dependent on agriculture both for domestic food supplies and cash earning exports. In irrigated areas, cotton *(Gossypium barbadense)*, sugar cane *(Saccarum officinalis)*, wheat *(Triticum* sp.) and vegetables are the major crops. In rain-fed

areas, cotton *(G. hirsutum)*, sorghum *(Sorghum vulgaris)* and sesame *(Sesamum indicum)* are grown annually. Irrigation is essential in many areas. The methods are (1) Gravity flow irrigation, by canals led from dams on rivers, for example Gezira and Khasm El-Girba schemes; (2) Pump irrigation, for example Agricultural Reform Schemes along the White and Blue Niles and Pump schemes in northern parts of the country; (3) Flush irrigation, using the natural flood of the rivers, for example Gash and Baraka deltas. In contrast cultivation away from the Nile is possible on land receiving 350 mm or more of rain annually, for example "Qoz" land and the Gadarif, Fung and Nuba mountain areas where there are now Mechanized Crop Production Schemes.

b) Legumes in The Sudan

In irrigated areas, the groundnut *(Arachis hypogaea)* which was initially included in the rotation as a break and oil crop has now become second to cotton as a cash crop. In the rain-fed "Qoz" land the groundnut is mainly a cash crop. Other legumes in irrigated areas include pigeon pea *(Cajanus cajan)* and "Lubia" *(Dolichos lablab)* which are grown around cotton and tomato as guard, windbreak, fodder and/or vegetable crops. The necessity of introducing a legume, as a break crop, in The Mechanized Crop Production Schemes has now been realized. Soybean *(Glycine max)* has been selected as the best candidate and research is going on to allow its introduction into the rotation.

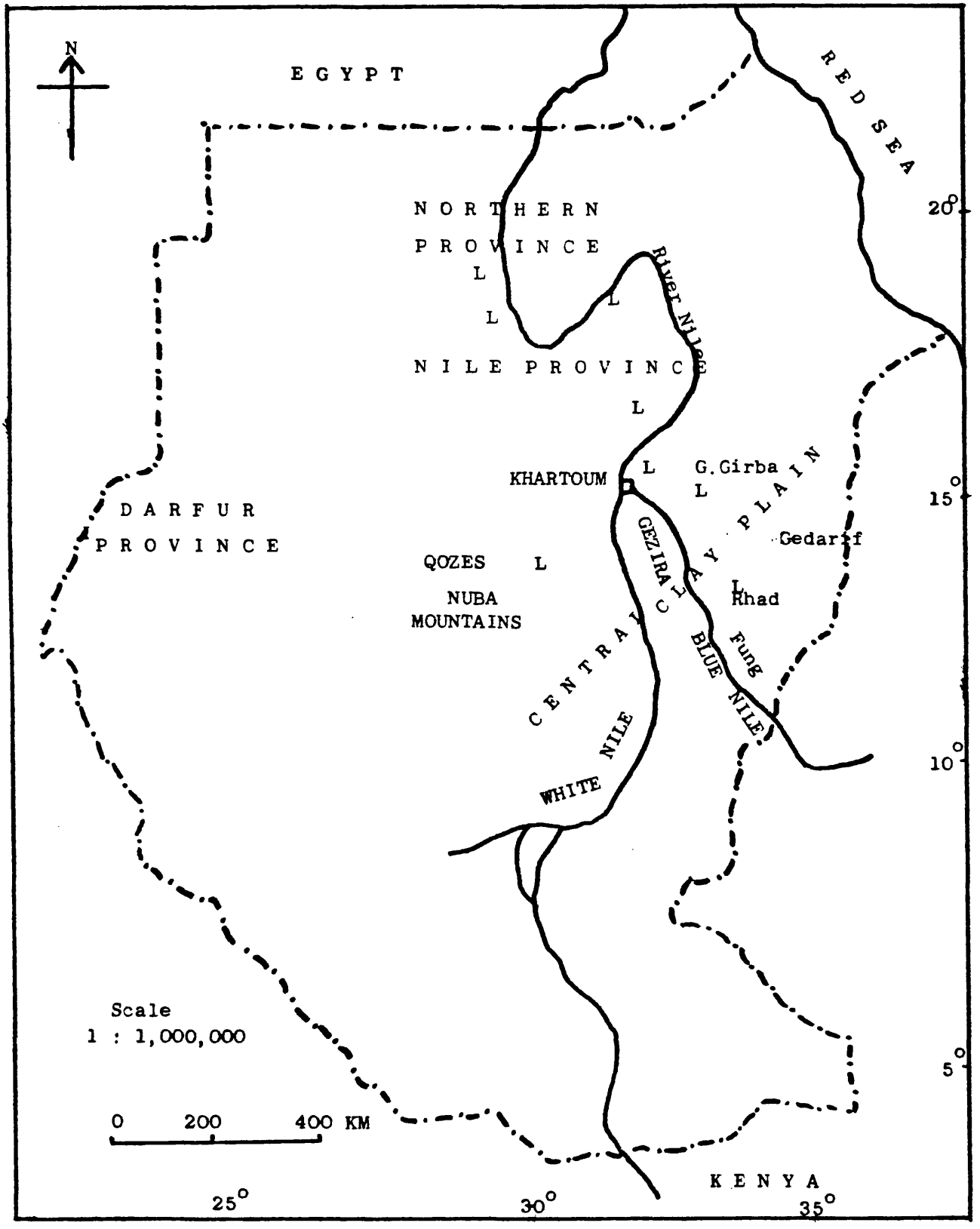
Around Khartoum, lucerne *(Medicago sativa)* is grown as a forage crop. Pigeon pea is also grown around tomato and other vegetables

as a wind break crop.

Most of The Sudan's legumes are grown in the northern part of the country along the Nile Valley as winter crops. In this part of the country, crop farming is entirely dependent on the supply of river water. Cultivation within the Nile valley, making use of annual flood and deposition of silt in basins and on the low terraces of the valley, has a very long history. Irrigation was almost entirely dependent on simple and primitive water-lifting devices such as the "Shaduf", a hand operated lever, and the "Sagia", an ox-turned wheel. These traditional methods are still in use, though supplemented by newer techniques. In this area the Nile flows through a desert region and the river valley is cut into a featureless sandstone plain to form a narrow channel of inhabited and cultivated land. The climate is characterized by a prevailing northerly wind and very low and unreliable rainfall which is less than 25 mm and limited to August. Relative humidity is very low. From May to September, day temperatures are very high, the highest monthly mean is about 40^{o}C. Winters are mild and dry, though days occur with a temperature of less than 15^{o}C. Most of The Sudan's legumes are grown in this region including broad bean *(Vicia faba)*, chickpea *(Cicer arietinum)*,cowpea *(Vigna unguiculata)* peas *(Pisum sativum)*, bean *(Phaseolus vulgaris)*, lupins *(Lupinus* sp.), lentils *(Lens culinaris)*, lucerne and fenugreek *(Trigonella foenum-graecum)*.

The policy of The Sudan government is to modernize the traditional agriculture in this region, so new techniques of irrigation have been introduced and research stations have been established along the Nile.

Map of the Sudan showing main legume producing areas.



L = legumes

Only manual methods of weed control are used. Hand weeding is difficult, time consuming, expensive and causes mechanical damage to the crop. The weed control situation is getting worse because of the labour shortage resulting from an increase in the area of cultivated land and the migration of people to big cities, or, recently, to the Arab oil-producing countries. Mechanical weed control is impossible since crops are not drilled to allow the use of inter-row cultivators. Very little work on chemical weed control in legumes has been done. There are only a few weed control specialists in The Sudan and their work is directed mainly towards cotton and sorghum. However, weed control in groundnut in irrigated areas has received a considerable amount of work.

c) Fenugreek in The Sudan

Fenugreek is grown as a winter crop in the extreme northern part of the country by private farmers in a very small area. It is a winter crop, sown in October and harvested in March. Seeds are distributed to all parts of the country. Hay, (stems and leaves), is fed to animals. Seeds are used for their protein and flavour in soft drinks, tea and coffee. They are also used with milk and wheat flour to prepare a delicious dish which is specially used for fattening girls before their wedding and by nursing mothers to increase milk production. In folk medicine, fenugreek seed has many uses including use as an antirheumatic, for cleaning the blood, for strength, to increase lactation, to alleviate dysentry and stomach troubles.

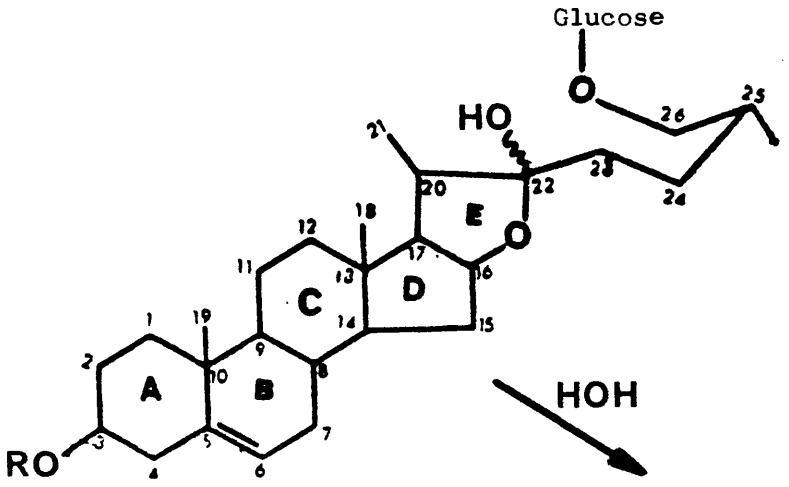
The National Council for Research in The Sudan has established a special unit, The Medicinal and Aromatic Herb Research Unit.

Its purpose is to study, isolate and utilize the valuable compounds occurring in medicinal and aromatic plants as well as to point out the importance of these natural resources to the economy, health and social life. Fenugreek is amongst the Unit's top ten plants.

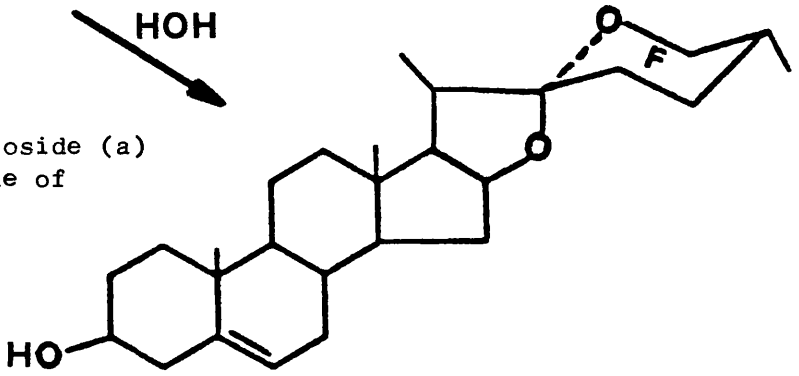
d) Fenugreek as a Multipurpose Crop

Fenugreek seed contains 30 - 35% protein and 7 - 9% vegetable oil. The seed is used as a spice in human food. Oleo-resin is used by the human food industry and in animal concentrates. The tender pods and leaves are cooked and used as a vegetable in India and Ethiopia. The fenugreek shoot is used as a forage and for silage. The Romans grew fenugreek for their horses and cattle. It can also be used as an appetizer for animals when interplanted with grasses or cereals. Because of its high nitrogen fixing capacity, fenugreek could be used as a break crop in cereal growing areas and it may also be used as a green manure. In India, the dried plant of fenugreek is mixed with grain (rice, wheat or sorghum) to protect it from insect attack during the rainy season (Nayar, 1955). Recently, the Chinese have started to interplant cotton with fenugreek to control cotton aphids. It is considered that fenugreek releases volatile substances that repel the aphid (Yu-Sing and He-Ting, 1982).

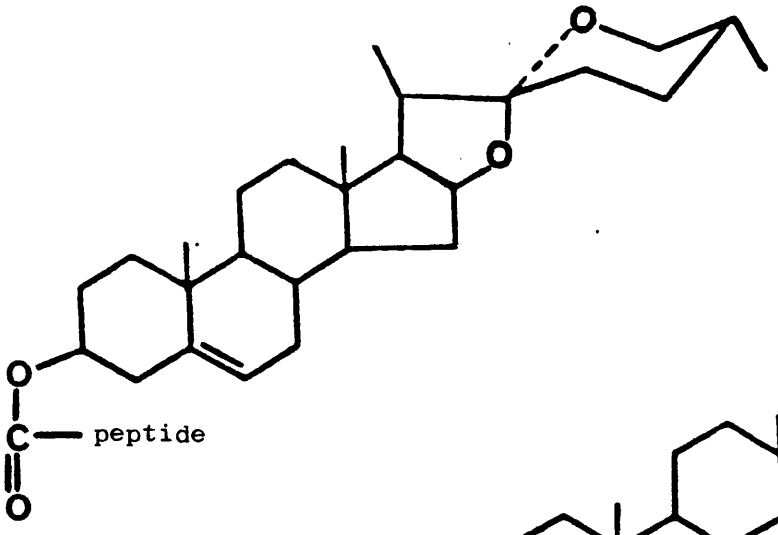
Hardman *et al.* (1980) and others have independently shown that the embryo of the seed contains furostanol type precursor glycosides (a) which affords the spirostanol diosgenin (b) on enzymic and/or acid



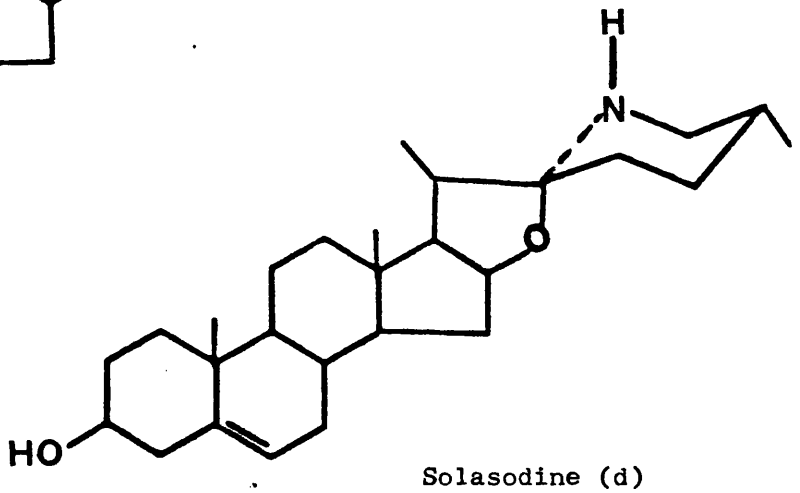
Furostanol precursor glycoside (a)
(3β-glycoside,26-glucoside of
16β,22-epoxycholesterol)



Diosgenin (b)
(25R)-spirost-5-en-3β-ol
(16,22,22,26-diepoxycholesterol)



Fenugreekine (c)



Solasodine (d)

hydrolysis. Hardman has been unable to find any evidence of free diosgenin in the seed. A peptide ester of diosgenin called fenugreekine (c) has been reported from the seed (Ghosal *et al.*, 1974), but Hardman considers fenugreekine may be an artefact resulting from the isolation procedure used and that fenugreekine probably exists in the seed as its furostanol-26-glucoside precursor molecule (R is the peptide ester in (a)).

The large traditional use in many countries of the seed in the diet (including beverages) mentioned above, suggests the seed is harmless to humans. The seed is regarded as too valuable to be fed to animals (except as a minor, but important, ingredient ... to increase palatability of animal feed). Keeler *et al.* (1976) showed that whereas solasodine (d), the 26-nitrogen analogue of diosgenin, caused teratogenic effects in hamsters, such as spina bifida and cranial bleb, diosgenin did not.

e) The Importance of Diosgenin in the Pharmaceutical Industry

Hardman has described plant steroids and their relationship to the pharmaceutical industry (1969; 1974) and reviewed the history of the steroid industry (1982).

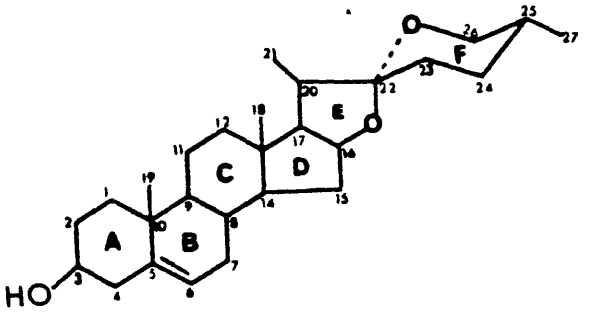
In 1944 Marker established the company Syntex in Mexico to produce progesterone from diosgenin (1) extracted from yam *(Dioscorea* spp.). At that time, the demand for the product was not large. However, in 1949 the discovery of the ability of cortisone to suppress the symptoms of rheumatoid arthritis stimulated a much greater demand. In 1952, it was found that certain microorganisms were capable of introducing an 11-oxygen function into the steroid structure, so

allowing corticosteroids as well as the sex hormones to be produced from the same precursor, diosgenin. In 1967, the contraceptive pill was approved by the World Family Planning Association. Contraceptive pills are usually composed of a combination of a progestogen (7) and an oestrogen (8) obtained through plant steroids. As a result of the introduction of such materials, a very large market for steroidal drugs was established.

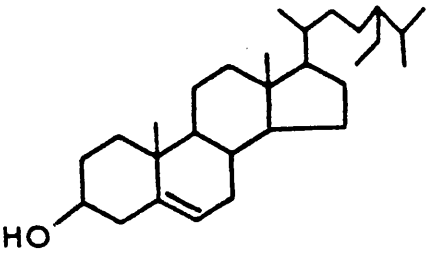
Over 80% of the World's steroids is obtained from plant steroids, the cheapest one is diosgenin (1). Removal of side chain (C-22 to C-27) gives pharmaceutical products which chemically are modifications of human steroids (made by the body from cholesterol). The modifications are chosen to give oral activity (by mouth), or topical activity (through the skin) instead of by injection. Modifications are effected by chemical and microbial methods. For example, fungi are used to introduce the 11 -oxygen atom at position 11 of the corticosteroids (3) or anaesthetic (5) and bacteria are used to remove the hydrogens from positions 1 and 2 to give corticosteroids (3) or anabolic steroids (6). In 1976 (latest figures available) 1224 tonnes of diosgenin equivalent (tde) were used to produce corticosteroids (of which 199 tde were for spironolactone (4)), 235 tde for contraceptives and 162 tde for oestrogens and androgens giving a total consumption of 1621 tde and a World wide market of over $300 million per year (Coppen, 1979).

Although the contraceptive pill is approved by the World Health Organization, the World Family Planning Association and such countries as India and China, the World demand for raw steroids for cortico-

Raw Steroids (Made by the plant from Cholesterol)

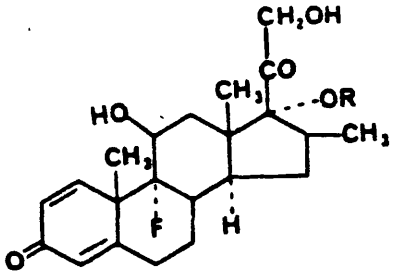


Diosgenin (1)

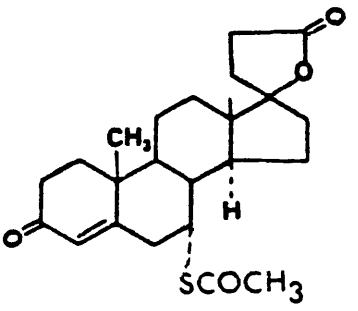


Sitosterol (2)
(24β-ethylcholesterol)

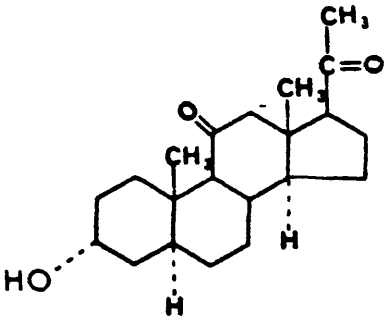
Pharmaceutical Products



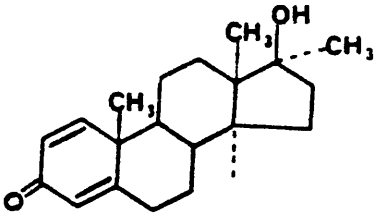
Glucocorticoids (3)
(Anti-inflammatory)
(Anti-asthmatic)
e.g. R = $CO(CH_2)_3CH_3$



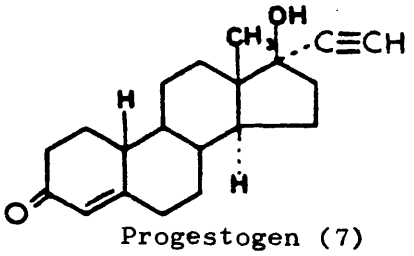
Spironolactone (4)
(Regulator of mineral metabolism)



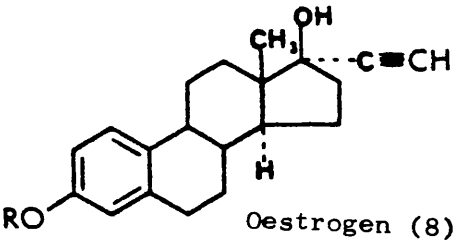
Anaesthetic steroid (5)



Anabolic steroid (6)
(Methandienone)



Progestogen (7)



Oestrogen (8)
R = H or CH_3

steroids (3 and 4) exceeds that for contraceptive pills (6 and 7). The monthly dosage of hormone in contraceptive pills has been reduced now to about one-tenth of that in the original formulation first tested in Puerto Rico in 1956.

In India the wild tubers of *Dioscorea deltoidea* and *D. prazeri* are collected from the lower slopes of the Himalayas. Because of over collection (the plants are shallow rooted) the supplies are becoming exhausted. Reports were made in 1980 of trial cultivation of native and Mexican Dioscorea in India. The export of Dioscorea tubers and diosgenin is prohibited because of India's own urgent and enormous need. China, with her access to the same Himalayan species of Dioscorea, produces diosgenin and until 1968 supplied diosgenin to the United Kingdom companies. China is trying to get its population down to 1000 million by the year 2000. Following on the contraceptive tablet it introduced an edible pill stamp in 1973 and in 1982 limited a family to one child.

There was a World shortage of diosgenin in 1974. The Mexican government in 1976, nationalized the relevant Dioscorea tubers of plants growing wild and raised the price of the raw material excessively. This caused industry, outside of Mexico, to turn to plant steroids other than diosgenin such as sitosterol (2) and its Δ22-derivative stigmasterol from such sources as seed oil of, for example, soybean. This is now a competitive raw material to diosgenin ,particularly in the United States of America.

Other sources of steroids are (1) animal source, such as the bile acid from cattle gall bladder,and cholesterol from cattle and sheep

brain and spinal cord. However, it is uneconomic to use animals solely as a source of raw steroids.(2) Synthetic steroids; the major problem in steroid synthesis is formation of the correct isomer with the natural active configuration. Total synthesis is used only in a few cases and is uneconomic for corticosteroids. Schering Co.Ltd. which produces a progestogen (Norgestrel) by total synthesis also produces it from diosgenin when this is available at a favourable price (Hardman, 1983, Per. Comm.).

f) Fenugreek as a British Crop

Fenugreek is of interest as a source of diosgenin because it can be cultivated in temperate countries and is planted and harvested in a single season. Although the diosgenin content of fenugreek seed is low (1 - 1.5% on moisture free basis) compared with that of the yam *(Dioscorea* sp.) (3 - 6%) which has been used as a major source of diosgenin, the yam needs 3 - 5 years growth before harvest and is a tropical species. Other advantages of fenugreek compared with the yam are that it can be used as a spice, as food for humans, forage for animals, for other medicinal purposes and is a potential break crop in arable rotations.

Intensive research has been carried out at the University of Bath by Dr. Roland Hardman to improve yield of the raw steroid, diosgenin, for the pharmaceutical industry and to provide an annual legume of improved agricultural merits. Five new varieties have been developed and passed to the National Seed Development Organization (NSDO) Ltd.,

Cambridge for multiplication.

There are certain problems with fenugreek as a crop in temperate regions. The two main ones are (1) slow establishment of the crop, so it suffers from weed competition, (2) late maturity which can lead to fungal attack and rapid deterioration of the seeds.

g) The Potential Weed Problems in Fenugreek

Fenugreek is a drought resistant crop and makes root growth in preference to aerial growth in the early stage of its development. Hence its ability to cover the ground at an early stage is very poor and its competition against weeds is therefore not very effective. Annual weeds, such as *Stellaria media* (chickweed), *Chenopodium album* (Fat-hen), *Convolvulus arvensis* (bindweed), *Fumaria officinalis* (fumitory), *Matricaria* spp. (mayweed), *Polygonum aviculare* (knotgrass), *Lolium* spp. (Ryegrass) and *Capsella bursa-pastoris* (Shepherd's purse) are likely to have greatest competitive effect on the crop. *F. officinalis* and *C. bursa-pastoris* are expected to make harvesting difficult. Other weeds, for example *P. aviculare,* would be expected to delay the ripening of the pods and stimulate and/or increase fungal attack by creating more humid conditions around the crop. The use of pre-emergence residual herbicides is being used successfully in other legumes, for example peas and beans. These herbicides are potentially of great importance in fenugreek to eliminate the early competition of weeds. However, this entirely depends on the tolerance of fenugreek to these herbicides. The use of post-emergence herbicides seems

unfavourable since the elimination of weeds is needed at a very early stage of the crop development. This assumption is supported by the results of these herbicides on other legumes. However, they might be used as supplements for pre-emergence herbicides to control weeds that emerge later in the season and/or those which are resistant to particular pre-emergence herbicides.

h) Aims of the Investigation

The objectives of this work with fenugreek were (1) to find suitable herbicides, (2) to investigate the effect of these on the yield of protein and of diosgenin from the seed and on root nodulation and (3) to assess the value of desiccants to enhance maturity in an attempt to minimize attack by fungi on the seed.

CHAPTER TWO

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

A. Herbicides in fenugreek

a) Introduction

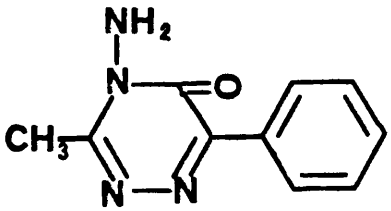
Very little information is available on weeds and weed control in fenugreek. The tolerance of fenugreek to herbicides was first reported by Gad and El-Mahadi (1972a) who found that fenugreek could tolerate nitralin up to 0.75 kg/F, where F = 1.04 acre, and classified it as a less tolerant crop to this herbicide. The Egyptian local herbicide M15, 65% calcium and iron trichloroacetate plus an abrasive, was found to be safe in fenugreek (Gad and El-Mahadi, 1972b). Under field conditions, Petropoulos (1973) found that prometryne at 1.25 lb/acre was very injurious to fenugreek (Kenyan, Moroccan and Ethiopian varieties), dinoseb at 2 lb/acre was quite safe as a pre-emergence treatment but not when used post-emergence and MCPB at 2 lb/acre was well tolerated post-emergence. The growth of fenugreek seedlings was inhibited when the seeds were soaked for a period of 16 hours in suspensions containing 5 g ℓ^{-1} of monuron, diuron, bromacil or terbacil (Tewari and Balasimha, 1976) or 0.005 - 5 g ℓ^{-1} of atrazine or simazine (Tewari *et al.*, 1976). In pot experiments, Naryana and Jain (1978) studied the effect of nitrofen and alachlor on the growth and nodulation of fenugreek. The herbicides, each at 6 concentrations (0.01 - 0.8 g kg^{-1}), were applied to the soil 15 days after emergence of the crop. Alachlor reduced the growth and nodulation of fenugreek, but with the higher rates of nitrofen were promoted. Alloxydim-sodium and pyridate were tolerated by fenugreek (Richardson and Parker, 1978). In a series of outdoor pot experiments, Richardson

(1979) tested 40 pre-emergence herbicides and 45 post-emergence ones with fenugreek, variety Paul. Each herbicide was tested at two rates, the recommended rate and twice that. In his experiments, he classified the herbicides as tolerated, moderately tolerated and non-tolerated by fenugreek. Post-emergence herbicides to which fenugreek showed good tolerance were bentazone and MCPB alone or in mixture, chlorthal-dimethyl, propyzamide, barban, dichlofop-methyl and alloxydim-sodium. Those to which the crop showed good pre-emergence tolerance included nitrofen, methazole, chlortoluron, aziprotryne, chlorthal-dimethyl, propyzamide, butam, propachlor, alloxydim-sodium and trifop-methyl. Trifluralin and tri-allate were also well tolerated as pre-planting incorporated treatments. Hardman (Pers. Comm., 1980) found that metamitron and chlorthal-dimethyl plus methazole, at normal field rates, applied pre- and/or post-emergence were well tolerated by fenugreek.

b) Specific Herbicides

Metamitron:

Metamitron is used for the control of annual weeds as a pre-emergence or as a post-emergence treatment and is particularly useful in beet crops. Structure:



(4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazine-5-one)

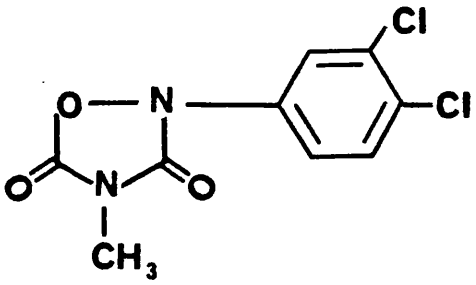
The mode of action of metamitron is by the inhibition of photosynthetic electron flow in the chloroplast (Schmidt *et al.*, 1975; Schmidt, and Fedtke, 1977). The inhibition of photosynthesis in plants exposed to metamitron in the rooting medium and its subsequent recovery after the transfer of the roots to herbicide-free nutrient solution was measured in 8 plants (Van Oorschot and Vanleeuwen, 1979). Recovery was fast with sugar beet, slow and incomplete with rye grass, slow in bean and undetectable in maize. After leaf application sugar beet plants gradually resumed the normal rate of photosynthesis, but bean plants did not. The selectivity of metamitron is attributed to an enzymic, light-independent, deamination which produces an inactive metabolite (Schmidt, 1977). This process is rapid in plants tolerant to metamitron, for example sugar beet, but slower in susceptible plants, for example bean.

Metamitron is well tolerated by fodder and red beet (Morris *et al.*, 1976) and to a lesser extent by fenugreek, bean and peas (Morris *et al.*, 1976; Richardson *et al.*, 1976; Richardson, 1979) and has potential activity against many weed species (Richardson *et al.*, 1976; Morris *et al.*, 1976, 1978). At rates of 2.8 - 7 kg/ha applied pre-emergence or post-emergence, metamitron has been shown to be quite effective against a number of weeds (Morris *et al.*, 1978) including *Chenopodium album, Poa annua, Polygonum aviculare* and *Stellaria media.* The activity of the herbicide was improved when it was applied at 3.5 kg/ha both pre- and post-emergence and the susceptibility of weeds,such as *Polygonum convolvulus,* was increased. This improvement of the effectiveness of metamitron as a result of the sequential application has also been reported by Hack and Schmidt (1976).

Methazole:

Methazole is a selective, pre-plant, pre-emergence or post-emergence, herbicide used in cotton and many other crops. It is also being used combined with chlorthal-dimethyl for weed control in many crops including peas and beans (King and Knott, 1979).

Structure:



2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-2,5-dione

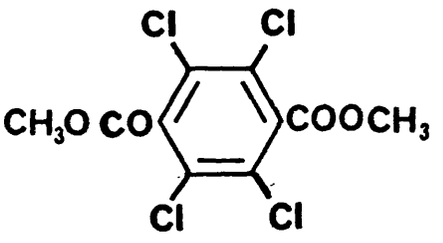
Very little is known about the mode of action of this herbicide. According to Ashton and Crafts (1981), methazole does not significantly affect photosynthesis, but DCPMU (1-(3,4-dichlorophenyl)-3-methylurea), which is the first metabolite of methazole, is a strong inhibitor of the Hill reaction. The review of Ashton and Crafts (1981) suggests that the inherent herbicidal activity may be similar to carbamates

in relation to the inhibition of RNA synthesis and the inhibition of phosphorylation. Methazole metabolizes to DCPMU much faster in susceptible plants than in tolerant ones and DCPMU metabolizes to the less toxic DCPU (1-(3,4-dichlorophenyl)urea). This further metabolism of methazole to DCPU is slower in susceptible plants than in tolerant ones.

Chlorthal-dimethyl:

Chlorthal-dimethyl is used to control annual grasses and certain annual broadleaved weeds in turf, ornamentals, lucerne, carrot and soybean.

Structure:



(Dimethyl tetrachloroterephthalate)

Frear (1976) reviewed the mode of action and selectivity of this herbicide. It inhibits seedling growth soon after emergence and affects nuclear activity and cell division. Effects on mitochondria and chloroplasts have also been reported. Emerging seedlings of resistant plants absorb and translocate chlorthal-dimethyl less effectively than sensitive plants. Different rates of metabolism to non-phytotoxic hydrolysis products may also play a role in selectivity. The placement of the herbicide in the soil may significantly affect selectivity, for example the growth of grass roots will not be affected if it is below the treated zone.

Several reports have suggested that chlorthal-dimethyl injury is primarily restricted to the treated tissues as its uptake and limited translocation result in localized concentration of the herbicide at or near the points of application (Ashton and Crafts, 1981). Chlorthal-dimethyl inhibits root growth more than shoot growth and causes swelling of hypocotyls.

Phenoxyalkanoic acids:

The phenoxyalkanoic acid compounds are formulated as the parent acids or more usually as salts and esters. They are usually used to control broadleaved weeds in cereals and grasses. They are considered as growth regulators with hormone-like activity. The structure and properties of these herbicides are summarized in Table (2.1).

Several reviews are available on the mode of action of this group of herbicide (Bovey, 1980; Ashton and Crafts, 1981; Fedtke, 1982).

Table 2.1 Structures, chemical names and water solubility of some phenoxyalkanoic acids

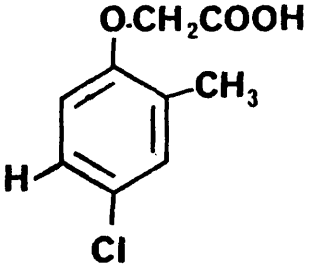
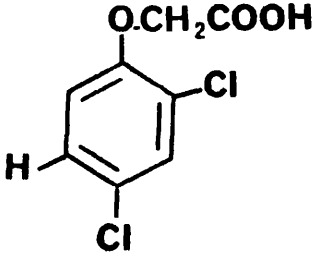
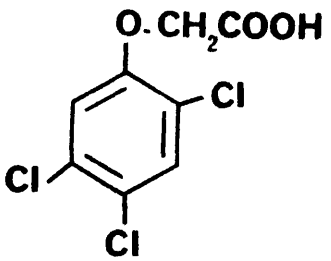
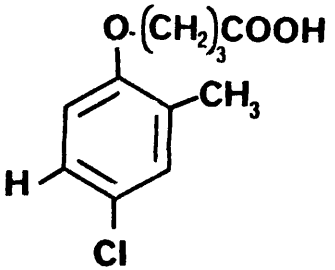
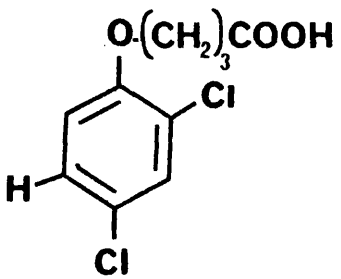
Common name	Structure	Chemical name	Water solubility (mgL^{-1}) at room temperature
MCPA		(4-Chloro-o-tolyloxy)acetic acid	825
2,4-D		(2-4-Dichlorophenoxy)acetic acid	725

Table 2.1 (continued)

Common name	Structure	Chemical name	Water solubility (mg L^{-1}) at room temperature
2,4,5-T		(2,4,5-Trichlorophenoxy)acetic acid	280
MCPB		4-(4-Chloro-o-tolyloxy)butyric acid	44
2,4-DB		4-(2,4-Dichlorophenoxy)butyric acid	46

Many investigators have found no effect of these herbicides on photosynthesis, but some consider these herbicides to be very weak inhibitors of the Hill reaction. However, some results suggest that photosynthesis may be inhibited indirectly as a result of a decreased rate of sugar diffusion from the site of photosynthesis (as a result of destruction of phloem by herbicide-induced lesions). These herbicides induce several abnormalities in the growth and the structure of the plant. These include dedifferentiation and initiation of cell division in certain mature cells and inhibition of cell division in primary meristems. They also affect nucleic acid, protein and lipid synthesis, respiration and plant hormones. The growth responses suggest that nucleic acid metabolism and metabolic aspects of cell wall plasticity are most likely to be involved in the mechanism of action of this group of herbicides.

Morphological and anatomical changes in plants susceptible to these herbicides can be observed within a few hours or days after treatments. A common response is epinastic bending of leaves and stems as a result of uneven cellular growth, meristematic cells cease dividing, elongation cells stop longitudinal growth, but continue radial expansion and mature plant parts (parenchyma cells) swell and soon begin to divide producing callus tissues and expanding root primordia.

Certain plants are resistant to foliar application of the phenoxy herbicides because of certain biochemical and physiological mechanisms inherent to those species. These include differences in absorption, translocation and/or detoxification. For example the presence of

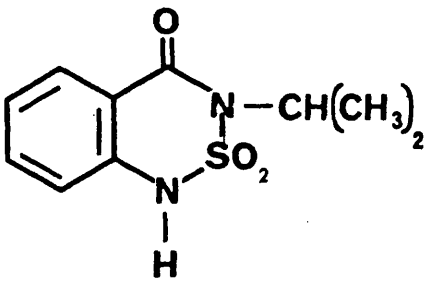
intercalary meristems may interfere with the translocation of these herbicides in monocotyledons. The absence of auxin-sensitive cambium and pericycle from vascular bundles of monocotyledons is likely to be the main factor in the selectivity of these herbicides. The phloem of the dicotyledons is plugged by abnormal herbicide-induced tissues, but in monocotyledons the phloem is scattered in bundles and protected by schlerenchyma tissues. The morphological characteristic of the plant also play a great role in the selectivity of these herbicides.

Relatively high translocation of these herbicides is likely to be responsible for the sensitivity of some monocotyledons, for example 2,4-D-sensitive maize and *Cyprus* sp. However, the high rate of detoxification in the plant is responsible for the tolerance of some dicotyledons.

Bentazone:

Bentazone is used for post-emergence control of certain broadleaved weeds in cereals and some broadleaved crops, for example soybean.

Structure:



3-isopropyl-(1H)-benzo-2,1,3-thiadiazin-4-one 2,2 dioxide

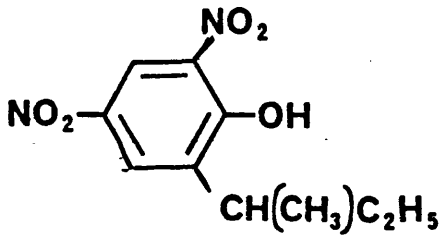
Very little is known about the mode of action of bentazone. The symptoms it produces are very similar to those caused by photosynthetic inhibitors and it is believed to inhibit the Hill reaction and photosynthetic CO_2-fixation (Mine and Matsunaka, 1975; Hays and Wax, 1975; Boger *et al.*, 1977; Retzlaff and Hamm, 1976). Mine and Matsunaka (1975) found that bentazone did not inhibit germination and early growth of radish *(Raphanus sativa)* when incorporated into the soil at 6 mg kg^{-1}, but severe desiccation and death of the plants occurred 9 days after emergence. The results indicated that the injury occurred after the carbohydrates stored in the seeds were exhausted. The same authors found the same delayed effect of bentazone on *Cyprus serotinus* when applied either as a flood - water or soil treatment at 2 kg/ha, but at the same rate when applied as a foliar treatment it caused more rapid injury. Hays and Wax (1975) studied the responses of different cultivars of soybean to bentazone. They found that differences in absorption and metabolism resulted in a tenfold greater concentration of bentazone in the treated leaf of susceptible cultivars than in those of tolerant ones.

Dinoseb:

Dinoseb is used as a post-emergence herbicide to control annual broadleaved weeds in cereals, flax and peas.

Dinoseb, according to the work of several investigators reviewed by Ashton and Crafts (1981) and Fedtke (1982), inhibits RNA and protein synthesis, lipid synthesis (which in turn alters membrane structure), photosynthesis and respiration. It uncouples oxidative phosphorylation and reduces ion uptake. The susceptibility of plants to dinoseb, as a pre-emergence treatment, is correlated with the size

Structure:



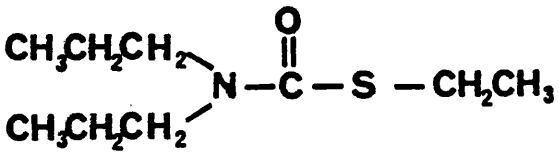
2-*sec*-butyl-4,6-dinitrophenol.

of the seed, large seeded plants are generally more tolerant to dinoseb than small seeded ones. The acute symptoms of dinoseb injury are that green tissues turn brown and desiccate. If the dose is sublethal the plants turn a dull grey colour and stop growing.

EPTC:

EPTC is a soil applied herbicide, used to control annual broadleaved weeds and grasses in many crops such as bean, potato, cotton and lucerne. Because of its relatively high volatility it should be incorporated into the soil. Its water solubility and vapour pressure are 3.75×10^{2} mg ℓ^{-1} (20^{o}C) and 4.55 Pa (35^{o}C) respectively.

Structure:



S-ethyl-dipropylthiocarbamate

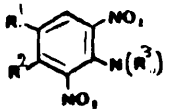
According to Fedtke (1982), EPTC inhibits the growth of germinating seedlings with the shoot being more affected than the root. It affects cell elongation rather than cell division. It also affects a variety of plant processes including photosynthesis, respiration, lipid synthesis, protein synthesis, gibberellic acid formation and nucleic acid metabolism. The inhibition of lipid synthesis appears to be most significant in relation to phytotoxicity. Differences in absorption and metabolism are considered the main factor of the selectivity of this herbicide.

Dinitroaniline Herbicides:

(i) Chemical and Physical Properties

Dinitroaniline herbicides are yellow to orange solids of relatively high vapour pressure and very low water solubility and basicity. Table 2.2 shows the chemical and physical properties of some of them. The subject was reviewed by Helling (1976a, 1976b), Weber and Monaco (1972) and Ellis and Norton (1976). Because most of

Table 2.2 Chemical and physical properties of some of dinitroaniline herbicides



Trade Name	Common Name	Chemical name	R^1	R^2	R^3	Vapour Pressure in Pa (25-30 °C)	Water Solubility (mgL^{-1})
Balan	Benfluralin	N-Butyl-N-ethyl-α-α-α-trifluoro-2,6-dinitro-p-toluidine	CF_3	H	C_2H_5-N-C_4H_9	0.0052	0.5
Amex "A-820"	Butralin	N-sec-Butyl-4-tertbutyl-2,6-dinitroaniline	$-C-(CH_3)_3$	H	H-N-$\overset{CH_3}{\overset{\vert}{C}}HC_2H_5$	-	1.0
Cobex	Dinitramine	N',N'-Diethyl-2,6-dinitro-4-trifluoromethyl-m-phenylenediamine	CF_3	NH_2	C_2H_5-N-C_2H_5	0.00048	1.0
Paarlan	Isopropalin	4-Isopropyl-2,6-dinitro-N,N-dipropylaniline	$-iso-C_3H_7$	H	C_3H_7-N-C_3H_7	0.0019	0.11
Surflan	Oryzalin	3,5-Dinitro-N^4,N^4-dipropylsulphanilamide	NH_2SO_2	H	C_3H_7-N-C_3H_7	<0.00019	2.5
Treflan	Trifluralin	α-α-α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	CF_3	H	C_3H_7-N-C_3H_7	0.0152	0.3-0.5
Planavin	Nitralin	4-Methylsulphonyl-2,6-dinitro-N,N-dipropylaniline	SO_2CH_3	H	C_3H_7-N-C_3H_7	0.0002	0.6
Basalin	Fluchloralin	N-(2-Chloroethyl)-2,6-dinitro-N-propyl-4-(trifluoromethyl)aniline	CF_3	H	C_3H_7-N-C_2H_4Cl	0.0008	1.0
Tolban	Profluralin	N-(Cyclopropylmethyl)-α,α,α-trifluoro-2,6-dinitro-N-propyl-p-toluidine	CF_3	H	$\triangleright$-CH_2-N-C_3H_7	0.0092	0.1

them are relatively volatile, and incidentally undergo photodecomposition when exposed to sun light they should be incorporated into the soil less than 24 hours from application. Very little or no leaching of dinitroaniline herbicides normally occurs because of their low water solubilities and high adsorption. Most of them are less soluble than 1 $mg \cdot \ell^{-1}$ with the exception of oryzalin (2.5) and prosulfalin (5.6) and they are strongly adsorbed to the soil constituents, with organic matter providing the most important site.

(ii) Mode of Action

Feeny (1966) found that trifluralin did not inhibit germination of oat; it had no effect on oxygen uptake by excised oat root and the formation of root and coleoptile was not affected. However, the elongation of both organs was greatly reduced. Hacskaylo and Amato (1968) found similarly that the growth of root and shoot of cotton and maize was inhibited by trifluralin. They also found that the cells of the extreme tip of the root were small, dense and many were multinucleate. They concluded that trifluralin prevents cell division and cell wall formation. Parka (1976) reported that dinitroanilines do not inhibit seed germination, but exert their phytotoxic effect during and immediately after germination. Parka and Soper (1977), Ashton and Crafts (1981) and Fedtke (1982), in their reviews, reported the conclusion of several investigators that dinitroanilines inhibit cell division and wall formation, interact with the microtubular system and alter the chemical composition and several biochemical processes in the plant including changes in sugar, amino acid and nucleic acid contents, and inhibition of photo-

synthesis, RNA synthesis, protein synthesis, lipid synthesis and oxidative phosphorylation.

(iii) Uptake and Morphological Effects

Dinitroaniline herbicides are absorbed by the hypocotyl hook of dicotyledons and via the first internode of monocotyledons. Standifer and Thomas (1965) found that *Sorghum halepense* seedlings were killed when the first internode passed through trifluralin-treated soil indicating absorption by this organ. When Knake *et al.* (1967) germinated *Setaria viridis* in systems that exposed either the root, shoot or both to 1 mg kg^{-1} trifluralin during the elongation period, they found that shoot exposure inhibited shoot growth completely, whereas root exposure had essentially no effect on shoot growth. Barrentine and Warren (1971b) found that the coleoptilar node and hypocotyl hook of sorghum and cucumber (respectively) were the most sensitive sites when localized treatments of trifluralin and nitralin were applied to the shoot zones. Parker (1966) demonstrated that trifluralin was absorbed by both root and shoot of sorghum and concluded that since 0.065 mg ℓ^{-1} of trifluralin was required to inhibit root growth by 50% compared with 2.7 mg ℓ^{-1} for 50% inhibition of shoot growth, root absorption is more effective than shoot absorption. Reviews of several studies (Parka and Soper, 1977 and Ashton and Crafts, 1981) suggest that dinitroaniline herbicides are absorbed by both root and shoot, but there is no or very little translocation from root to shoot or vice versa.

Inhibition of lateral root development and swelling of the tip are

typical root symptoms of dinitroaniline herbicides, whereas symptoms in the shoot are characterized by stunted growth, development of dark green colour, swelling and brittleness of the stem or hypocotyl and a leathery appearance of the cotyledons. Ashton and Crafts (1981) and Parka and Soper (1977), after reviewing the work of several investigators, concluded that dinitroaniline herbicides inhibit the root growth of the susceptible plants and this is usually accompanied by an increase in diameter, swelling of the tip and inhibition of lateral root development. Effects of these herbicides on the shoot include irregular thickening of hypocotyl, inhibition of coleoptile elongation, stunted growth and brittleness of the stem.

(iv) Phytotoxicity

Table 2.2 shows the structure of some dinitroaniline herbicides. The substituent on the R^1 position has a major effect on the bioactivity, the highest activity being associated with the trifluoromethyl substituents. Sulphonyl analogues are of intermediate activity and the alkyl analogues are the least active (Gentner, 1966; Murray *et al.*, 1973). Substituents on the aniline nitrogen, R^3, also affect the bioactivity, Gentner (1966) found that the activity was greatest when R_3 contained a total of six symmetrically arranged aliphatic carbon atoms. In greenhouse experiments (Jordan *et al.*, 1978), cotton tap root length was reduced by dinitroaniline herbicides at the normal rate in the order: dinitramine > profluralin > trifluralin > fluchloralin > pendimethalin > butralin. Pritchard and Stobbe (1980) studied the phytotoxicity of dinitroanilines to oat (*Avena sativa*), *Sorghum bicolor* and *Sorghum sudanense* in different soils under

growth chamber conditions. They found that the phytotoxicity of these herbicides was in the order: dinitramine > trifluralin > profluralin = fluchloralin. In field experiments over three years, the growth reductions caused by dinitroaniline herbicides to lucerne, at rate equivalent to 1.7 kg/ha trifluralin (that is twice the recommended rate) were nitralin (49%), fluchloralin (45%), trifluralin (26%), profluralin (19%), benfluralin (9%) and butralin (3%) (Fawcett and Harvey, 1978).

Barrentine and Warren (1971a) conducted experiments using petri dishes and sand culture to compare the phytotoxicity of trifluralin and nitralin to several plant species. They found that trifluralin was more toxic than nitralin to shoots while nitralin was more toxic than trifluralin to roots. They attributed the phytotoxicity of trifluralin to greater absorption via the shoot. In other experiments, Barrentine and Warren (1971b) found that the rates of entry, uptake and translocation of ^{14}C-trifluralin were greater than those of ^{14}C-nitralin in sorghum and cucumber shoots. In green house studies, Harvey and Jacques (1977) compared the phytotoxicity of different dinitroaniline herbicides to pea. They grew the plants in washed silica sand containing 10^{-5}M herbicide and found that trifluralin and dinitramine were most phytotoxic while butralin was the least. Similar results were obtained by Stollar and Wax (1977) who also studied the phytotoxicity, under field conditions, of different dinitroaniline herbicides to sorghum, *Setaria faberi*, *Abutilon theophrasti*, *Datura stramonium*, *Ipomoea purpurea*, *Ipomoea hedercea*, *Amaranthus retroflexus* and *Chenopodium album*. They found that dinitramine and trifluralin were the most toxic to all these species

while butralin was the least toxic. In studies under field conditions, Harvey (1973a) had demonstrated considerable variation in the effectiveness of 12 dinitroaniline herbicides in controlling *Setaria fabri* and *Abutilon theophrasti* in soybean. There were also differences in the phytotoxicity of these herbicides to soybean, the most phytotoxic was dinitramine, while butralin was the least. However, under glasshouse and laboratory conditions, dinitramine was the most phytotoxic to each species, trifluralin and dinitramine inhibited soybean shoot growth most while oryzalin and dinitramine were most effective on root growth (Harvey, 1973b).

Vapour of both dinitramine and trifluralin was found to inhibit shoot and/or root of germinating oat and pea (Jacques and Harvey, 1979a, 1979b) and *Setaria* spp. (Jordan *et al.*, 1979). They also found that oryzalin and nitralin had no effects on the plants through vapour activity and concluded that the vapour phytotoxicity of dinitroaniline is correlated with the rate of herbicide volatilization.

B. Factors Affecting the Activity of Soil-Applied Herbicides

As already stated on page 14, at least part of the weed control strategy for fenugreek is likely to involve pre-emergence herbicides, that is, compounds which are applied to the soil. There are many factors that affect the activity of soil-applied herbicides. The two most important ones are adsorption and rainfall. They control availability of herbicides to plants by affecting concentration in the soil solution, distribution and decomposition processes.

b) Adsorption

Adsorption of herbicides by soil has been described and reviewed by Hamaker and Thompson (1972), Hance (1976, 1980), Hartley and Graham-Bryce (1980) and Calvet (1980).

The intermolecular interactions involved include:

1. Van der Waals-London forces:

These are electrostatic interactions between atoms and molecules which arise from fluctuations in electron distribution. These fluctuations produce dipoles which cause attractions and repulsions between atoms and molecules. This type of bonding is very weak.

2. Hydrophobic bonding:

Water molecules form a cage of H-bonded clusters around an introduced hydrocarbon. If the hydrocarbon is adsorbed, the water is displaced and reverts to its normal structureless liquid state so increasing the entropy of the system. Thus adsorption by Van der Waals-London forces is reinforced by the entropy change.

3. Charge transfer and Hydrogen bonding :

Any system XH-Y, in which the XH bond has some polarity and the Y atom some basicity may be capable of forming hydrogen bonds, but this depends on the electronegativity of X. The possibility of a herbicide being adsorbed by this means depends on the strength of the hydrogen bond with the adsorbent compared with the hydrogen bond with water. Normally the hydrogen bond with water is stronger than hydrogen bonds with an adsorbent, but it is suggested that hydrogen bonded water bridges between adsorbate and surface may play a role in adsorption.

Hydrogen bonding is a special case of the general phenemenon of charge-transfer complex formation which involves partial overlap of the molecular orbitals of donor and acceptor molecules and partial exchange of electron density.Thus, the formation of a charge-transfer complex involves formation of resonance structures involving ionic forms of donor and acceptor.

4. Ligand-exchange:

Adsorption by this process involves replacement of one or more ligands by the adsorbent molecules, therefore the adsorbent molecule must be the stronger chelating agent.

5. Ion exchange:

This involves the adsorption of ionic herbicides at the negative and positive sites of the soil which are capable of ion exchange.

6. Chemisorption:

This process involves chemical bond formation between the adsorbate molecule and the adsorbent.

Clay minerals and organic matter are both negatively charged and can act as ion exchangers. Clay surfaces are rich in hydroxyl groups and are more hydrophilic than organic matter which tends to be aromatic in structure and hydrophobic. Most soil-applied herbicides are aromatic and have relatively low water solubility. Although many of the mechanisms mentioned above are suggested for the adsorption of certain herbicides, probably the most important process is hydrophobic bonding and the soil organic matter appears to be the most important component.

All soil-applied herbicides are adsorbed to some extent and their herbicidal activities are reduced in direct proportion to the amount adsorbed. In general, adsorption retards leaching, affects uptake, volatilization and decomposition.

Factors which determine the extent of adsorption include the amount of organic matter and clay, temperature, soil moisture, pH,

salts and the inherent properties of the herbicide. There is usually, but not always, a high positive correlation between adsorption and soil organic matter. The amount of a herbicide required to produce a specific level of phytotoxicity is often proportional to the amount of organic matter in the soil, so the soil organic matter content is sometimes used as a guide to advise the farmer of the appropriate application rate. This correlation has been shown to occur in the glasshouse and field for the activity of alachlor, atrazine and trifluralin on oat and soybean (Rahman, 1976; Rahman *et al.*, 1978; Harrison and Weber, 1975; Harrison *et al.*, 1976), in the growth chamber for the activity of trifluralin with wild oat (Moyer, 1979) and for a range of dinitroaniline herbicides (Pritchard and Stobbe, 1980). However, Hance *et al.* (1968) found that the activity of lenacil, simazine, linuron or prometryne against turnip and ryegrass was correlated with soil organic matter content in the glasshouse but not in the field. They attributed this to climatic factors.

b) Rainfall

Rainfall has a major influence on the activity of soil-applied herbicides. The subject has been reviewed by Upchurch (1972) and Walker (1980).

Rainfall is required to bring the herbicide into solution from its formulation. It is also required for redistribution and movement of the herbicide into the soil where interactions may occur with germinating weeds and where photolysis and volatilization are reduced. Movement of herbicides within the soil profile is affected by percolation

of water from rain, the greater the quantity of water entering the soil the more rapid is the leaching rate of the herbicide, but this is important only for herbicides which are relatively soluble in water and not significantly adsorbed by the soil as most soil acting herbicides are retained in the top few centimetres. Rain also influences the performance of the herbicides through its effect on soil moisture. Since herbicides are taken up by weeds from the soil solution so an adequate soil moisture content is necessary for the herbicide activity. A number of herbicides have been shown to be more phytotoxic (in pot experiments) in moist compared with dry soils (Walker, 1980).

C. Effect of Herbicides on Nodulation of Legumes

In view of the widespread use of herbicides on legumes for weed control, attention is being focussed on the possible effects of these chemicals on nodule formation and nitrogen fixation. The effect of herbicides on Rhizobia and nodulation in culture media was reviewed by Anderson(1978). Rhizobium strains which grow faster than others are generally more resistant to herbicides. Pyrazon, for example, has been shown to inhibit the growth of fast-growing strains of *Rhizobium meliloti*, *R. trifolii* and *R. leguminosarum* at concentrations more than 1000 mg ℓ^{-1}. However, it inhibited slow-growing strains of *Rhizobium lupini* and *R. japonicum* at rates 100 - 500 mg ℓ^{-1}. The phenoxy acids 2,4-DB and MCPB inhibited fast growing strains of *Rhizobium meliloti*, *R. trifolii* and *R. leguminosarum* at concentrations exceeding 1000mg ℓ^{-1} and slow-growing strains of *R. lupini* and *R. japonicum* at 100 - 500 mg ℓ^{-1}. However, the growth of *R. meliloti*, *R. trifolii*, *R. leguminosarum* and *R. phaseoli* was inhibited by 5 - 300 mgℓ^{-1}

of MCPA, MCPB or 2,4-D. The differences in the rates of application of phenoxy acids needed for inhibition of growth in culture was explained by the suggestion that the sensitivity of Rhizobia to herbicides may be more a property of the strain and not the species of nodule bacteria. Diuron, linuron, dinoseb acetate and a mixture of propham plus diuron were toxic at several hundred mg kg^{-1} to fast growing strains of *Rhizobium* spp. tested, and to slow-growing strains at 100 mg kg^{-1}. At normal field rate equivalents, neither atrazine nor simazine were found to be toxic to *Rhizobium* spp. tested. These results indicate that herbicides can harm Rhizobia, but a very high concentration is needed for this effect. Since the concentration of herbicides in the soil solution is negligible compared with the concentrations used in these studies,the effects of herbicides on nodulation, in the field, seem to be indirect as a result of crop damage.

The effects of some herbicides on Rhizobia and nodulation of legumes in the soil was also reviewed by Anderson (1978). In general, the most toxic herbicides at relatively higher rates than normally recommended were substituted phenols and pyrazone while the least toxic were dalapon, simazine and prometryne. The anilines (trifluralin and nitralin) and carbamates were listed under those that appeared to have a large negative effect on Rhizobia and nodulation and most of the triazines were considered to be inhibitory even at the recommended field rates.

At 0.72 and 0.86 kg/ha, trifluralin did not affect the nodulation of soybean (Giardini *et al.*, 1979; Massariol and Lam-Sanchez, 1974).

However, at 0.8 and 2.5 kg/ha it was found to thin plant stands, reduce plant dry matter, reduce nodulation and nitrogen content of soybean (Chebotar, 1979; Paromenskaya *et al.*, 1979). They also found that the penetration of Rhizobia into the plant was inhibited and the enzyme activity of nodules and symbiosis was disrupted. However, by seed formation time, nitrogen fixation had recovered and nitrogen nutrition had improved resulting in increased yield. Baltazar and Brotonegro (1979) reported that trifluralin at 2 kg/ha did not inhibit nodulation or nitrogen fixation of soybean when applied 10 days before sowing, reduced nitrogen fixation when applied 5 days before sowing and inhibited both processes when applied at sowing. Under field conditions, decrease in nodulation of birdsfoot trefoil by 2,4-DB and dalapon, alone or in mixture, at 1.125 and 4.0 lb per acre, respectively, was caused by the injurious effect of the herbicides on plant vigour and root growth (Garcia and Jordan, 1969). The effects of trifluralin and carbetamide, under field conditions, on the growth and nodulation of broad red clover *(Trifolium pratense)*, white clover *(T. repens)*, suckling clover *(T. dubium)* and *Lotus pedunculatus* were studied by Brock (1972). He found that trifluralin at 1 and 2 kg/ha and carbetamide at 2 kg/ha reduced nodule number per plant, and total dry weight per plant. He concluded that, since nodule/unit dry weight was not affected and nodule/ plantand root dry weight were positively correlated, neither of the herbicides had any direct effect on the Rhizobia population. A similar conclusion was drawn by Peter and Benzbiba (1979) who found that benfluralin at 1.12 kg/ha and profluralin at 0.56, 0.84 and 1.12 kg/ha, under glasshouse conditions, reduced nodule number and nitrogen fixation of lucerne and red clover. Nutman (1948) has shown that nodule number and

extent of lateral root growth are positively correlated.

Greaves *et al.* (1978) conducted four glasshouse pot experiments to assess the side effects of alloxydim-sodium on the growth, nodulation and nitrogen fixation of pea (*P.sativum*). The results illustrated the difficulties of designing experiments to assess such side effects of herbicides on legumes. There was a lack of reproduceability of repeated experiments and high variations. They concluded that the plant weight and yield are the most reliable indicators of pesticide side effects. Greaves *et al.* (1980) recommended that the measurement of plant growth over time and plant yield give the best estimates of healthy nodulation.

D. Safeners as a New Concept in Chemical Weed Control

For a herbicide to be of use it must be selective. Selectivity can be increased by the timing of the herbicide application, the placement of the herbicide or by using physical barriers. The various aspects of herbicide selectivity have been reviewed by Holly (1976). Contact pre-emergence herbicides, such as paraquat, can only be selective as a result of timing of application so that only the weeds are treated. The placement of the herbicide in the soil in relation to the sowing depth of the crop can be used to obtain selectivity, for example the use of di-allate to control *Avena fatua* in wheat and barley and the use of trifluralin in wheat and barley to control *Setaria viridis*. In these cases, shallow incorporation of the herbicides and deeper sowing of the crops achieve selectivity as the mesocotyl or internode of the weed moves the coleoptile node upwards into the

treated soil at a very early growth stage. The corresponding zones of wheat and barley remain close to the seed so that the sensitive sites remain below the treated soil for a longer period (tolerance increases with age). If herbicide uptake is by the main root system, selectivity may be obtained by deep sowing of the crop. In this case the herbicide may reach the roots of the weeds but not those of the crop, for example the selective weed control in peas by simazines. Another example of the selectivity achieved through the placement of the herbicide is the use of EPTC in cotton to control *Cyprus rotundus*. EPTC is applied in two bands within the soil on both sides of the drill row of cotton. The movement of the herbicide from these sub-surface layers gives good control of the weed without any effect on the marginally tolerant cotton. The use of an adsorptive barrier, usually charcoal, is another way of improving selectivity. In this case the crop seeds are coated with adsorptive material, plant material (e.g. seedlings in case of transplanting) is dipped in the adsorptive material or the adsorptive material is placed as a layer above the crop seed. Examples include the use of propham in beet and simazine in many crops.

Chemicals which protect the crop against herbicides, known as safeners, are a relatively new concept in weed control. They give an opportunity to control weeds which are biologically similar to the crop and also may allow expensive selective herbicides to be replaced by cheaper less selective ones.

Hoffman, the father of herbicide safeners, in 1947 observed the antagonistic effect of (2,4,6-trichlorophenoxy)acetic acid against

2,4-D in tomato *(Lycopersicon esculentum)*. More significantly, he recognized from his observation the possibility of using non-herbicidal compounds to reduce crop injury from moderately selective herbicides. By 1962, Hoffman clearly established and introduced the concept of herbicide safeners and reported several compounds that would protect wheat against barban damage. In 1969, as a result of Hoffman's work, his employer, the Gulf Oil Chemical Co. announced NA (naphthalene-1,8-dicarboxylic anhydride) as a safener against injury by the thiocarbamate herbicides to maize (*Zea* mais). In 1972, the Stauffer Co. introduced their safener R25788 (N,N-diallyl-2,2-dichloroacetamide). Since then new safeners have been developed. These include cyoxymetrinil (Ciba-Geigy); MON4606 (Monsanto); and M32988 (Gulf Oil Chemical Co.). NA; R25788; and cyoxymetrinil are now commercially available. Table 2.3 gives the common and chemical names and the structures of these safeners.

The use of safeners and their chemistry and mode of action, have been reviewed by Blair *et al.* (1976), Pallos and Casida (1978), Stephenson and Ezra (1982) and Parker (1983).

NA will protect both crop and weed (Chang *et al.*, 1973) and hence must be applied to the crop as a seed treatment. It is mainly used to protect maize against thiocarbamate herbicides. However, protection to other crops against a number of herbicides by this safener has been reported. These included: maize against alachlor; metolachlor; perfluidone; barban; diclofop-methyl; and cisanilide; rice against EPTC; molinate; alachlor, meto-

Table 2.3 Chemical names and structures of some of the safeners

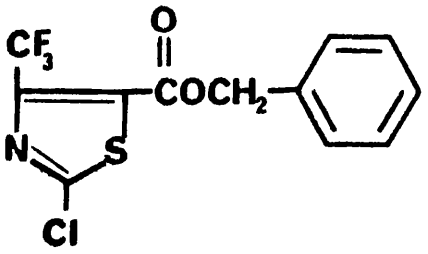
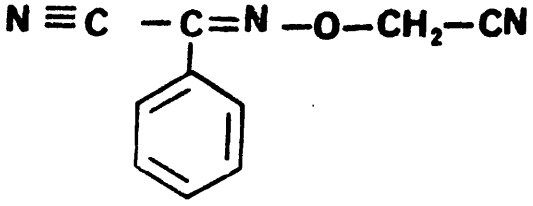
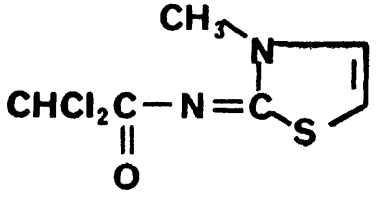
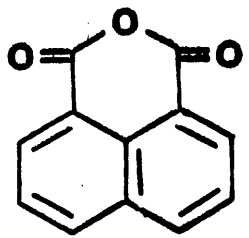
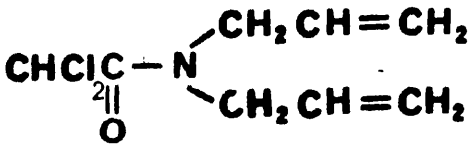
Common or code name	Structure	Chemical name
MON4606		Benzyl 2-chloro-4-(trifluoromethyl)-thiazole-5-carboxylate
Cyoxymetrinil		(Z)-Cyanomethoxyimino(phenyl)acetonitrile
M32988		2,2-Dichloro-N-(3-methyl-4-thiazolin-2-ylidene) acetamide

Table 2.3 continued

Common or Code name	Structure	Chemical name
NA		Naphthalene-1,8-dicarboxylic anhydride
R25788		N,N-Diallyl-2,2-dichloroacetamide

lachlor; and butachlor; oat against di-allate; tri-allate; alachlor; and barban- wheat against tri-allate; barban; and chlorosulfuron; barley against chlorosulfuron; broad bean against EPTC; and cotton against cisanilide.

Unlike NA, R25788 does not protect the weeds. Maize and *Setaria viridis* (weed) were grown in quartz and nutrient culture (Stephenson and Chang, 1978) and were equally exposed to a toxic level of EPTC with or without R25788. They found that R25788 provided complete protection for maize from EPTC injury but did not protect *Setaria viridis*. Similar results were obtained under glasshouse conditions (Chang *et al.*, 1972) and under laboratory and field conditions (Burt, 1976a, 1976b).

Because of high specificity and selectivity of R25788 to any plant (crop and weed), it has the merit that it can be applied in a number of different ways: (1) as a seed treatment; (2) as a pre-planting soil incorporated treatment with the herbicide (mixed together in the spray tank - tank-mix), (3) as a broadcast application to the soil or (4) in a combined formulation of herbicide with safener as in e.g. Eradicane which is EPTC plus R25788.

R25788 is commonly used to protect maize against thiocarbamates (all methods of application mentioned above) and barban (all methods). R25788 has also been shown to protect: sorghum against EPTC (1,2) (Chang *et al.*, 1972); rice against molinate and butachlor (1,2) (Parker and Dean, 1976); wheat against

tri-allate (1) (Blair, 1979); and broad bean against EPTC (1) (Blair, 1979).

In agreement with the view of Blair *et al.* (1976) and Stephenson and Chang (1978) there seem to be few examples of NA or R25788 safening broadleaved crops against thiocarbamate herbicides. They concluded that the safening activity of NA and R25788 is primarily restricted to grasses and that it may relate to some morphological or physiological characteristic common in many grasses, but present in few broadleaved plants.

Studies on the mode of action of safeners, principally NA and R25788, reveal the following: R25788 does not prevent EPTC injury to maize by preventing EPTC uptake. This has been shown underglasshouse and laboratory conditions (Stephenson *et al.*, 1978). EPTC injury to maize was reduced when the seedlings were exposed to injurious concentrations of EPTC for two days and then treated with R25788. However, recent investigations (as reviewed by Stephenson and Ezra, 1982) suggest that R25788 does not inhiibit passive uptake and apoplastic movement of EPTC but reduces its uptake by living cells in the symplast. Stephenson *et al.* (1978) concluded that NA and R25788 either prevented activation or enhanced deactivation of the herbicide within the plant. Another theory (Stephenson *et al.*, 1978, 1979), based on structure-activity relationships, suggests that compounds similar in structure to the thiocarbamate herbicides can be highly active as safeners against these herbicides by acting as competitive inhibitors at the sites of herbicide action. Wilkinson and

Smith (1975) suggest that NA and R25788 reverse the inhibition of fatty acid synthesis by herbicides. This suggestion is supported by Ezra *et al.* (1982) who have observed antagonistic effects of EPTC and R25788 on lipid biosynthesis in maize cell suspension cultures. Lay and Casida (1976, 1978) consider that R25788 induces higher levels of glutathione and glutathione-S-transferase which prevent toxic accumulation of EPTC-sulphoxide (the toxic metabolite of EPTC) by carbamylation. Others (reviewed by Parker, 1983) consider that EPTC-sulphoxide is not as toxic as EPTC itself, but EPTC-sulphone (another metabolite of EPTC) is the most toxic and concluded that EPTC-sulphone, and not EPTC-sulphoxide, forms a complex with glutathione.

Of the other safeners, as yet there is very little information: MON4606 has been introduced to protect grain sorghum against acetanilides, mainly alachlor and acetachlor. Brinker *et al.* (1982) found that MON4606 was very effective against alachlor as seed dressings and in furrow treatment (field experiments). MON4606 gave good protection to sorghum against 2 kg/ha of alachlor without any significant effect on weeds. In his review, Parker (1983) reported that cyoxymetrinil was found to protect sorghum against acetanilides and rice against metolachlor.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

CHAPTER 3

GENERAL MATERIALS AND METHODS

a) Description of the Soils

The soil used for pot experiments was Begbroke North (Sandy Loam) which was obtained from The Weed Research Organization's (W.R.O., Oxford) farm. The soil was air dried and sieved through 10 mm screen. The properties of this soil, as determined by The Agricultural Development and Advisory Service (ADAS, Reading) are summarized in Table 3.1. The field experiments were established on the same type of soil at W.R.O.'s farm. For adsorption and selectivity ratio experiments, the soils used were, Begbroke North, a sandy soil from W.R.O., Allan soil, a clay soil from Bath and an organic Fen soil. The mechanical analyses of these soils are given in Table 3.2. These soils were air dried and ground to 1 mm for adsorption experiments or sieved through a 10 mm screen for selectivity ratio experiments.

b) Herbicide Formulations

The herbicides used and their trade names and formulations are given in Table 3.3. Herbicides were applied as the commercial formulation in water. Doses were given in terms of active ingredients which had been calculated in terms of product at spraying.

c) Liquid Feed

The liquid feed, Vytel Spray, was used in field experiments and it was supplied by Murphy Microfeed Limited, Lymm, U.K. and its analysis is as follows:

Nitrogen (N)	18.9%
Phosphorus pentoxide (P_2O_5)	14.0%
Potassium oxide (K_2O)	7.0%
Magnesium	321 mg kg^{-1}
Iron	105 mg kg^{-1}
Manganese	156 mg kg^{-1}
Copper	30 mg kg^{-1}
Molybdenum	22 mg kg^{-1}
Boron	13 mg kg^{-1}
Cobalt	8 mg kg^{-1}

d) Glasshouse Conditions

In pot experiments, plants were raised under a 14 hour day length. Day temperature was 22 ± 5°C and night temperature was 18 ± 5°C. Humidity was 50 - 70% and natural light was supplemented by artificial light. However, during summer time no heating nor supplementary light were used and the glasshouse conditions varied with the weather outside.

e) General Spraying Techniques

Spraying of the herbicides was done with an Oxford Precision Sprayer. It was equipped with two (Pot Experiments) or four (Field Experiments) Tee-Jet 8002 nozzles which have a tapered edge spray pattern to ensure overall uniform coverage. The nozzles were attached, 50 cm apart, to a boom. The sprayer was operated at a pressure of 103 KPa.

In some pot experiments, a laboratory pot sprayer was used. The sprayer was operated at a pressure of 207 KPa and delivered the spray through a Spraying System fan jet moving at a constant speed 45 cm above the pots (soil surface).

f) Scoring of the Phytotoxicity Symptoms

Surviving plants were scored for symptoms of herbicidal effects and vigour on a 0 - 7 scale (Richardson, 1979) as shown in Table 3.4.

g) Methods of Analysis

1. Protein Content Assay

A semi-micro kjeldahl method, which has been described by Byast *et al.* (1977), was used.

(i) Digestion Mixture

Selenium metal powder, 2 g, was dissolved in 500 ml concentrated sulphuric acid by heating. The digestion mixture was allowed to cool and then was transferred into a dispenser. A sample, 50 mg, of finely ground seed of fenugreek was put into a 16x150 mm rimless thick walled pyrex test tube. The tubes, containing the samples, were placed in an aluminium block which was drilled with 56 holes, each 9 cm deep and 16.5 mm in diameter, and had a central thermometer well. The digestion mixture, 2 ml, was added to each tube. Then the aluminium block with its tubes was placed on a hot plate capable of heating the block up to 330^{o}C. When the temperature of the block reached 150^{o}C, hydrogen peroxide solution (concentrated), 2 drops,

was allowed to run down the inside of the tube. Then a further 0.5 ml hydrogen peroxide was added to each tube. The temperature of the block was then raised to 330°C and maintained at that temperature until the digestion was completed (3 - 3½ hours). The tubes were allowed to cool and the contents of each diluted to 100 ml with de-ionized water. Blank samples were also included in the digestion procedure. Samples were subjected to analysis of N content in the auto-analyser and the results were multiplied by 6.25 to give the protein content.

(ii) Preparation of Standards

A 1000 mg ℓ^{-1} N stock solution was prepared by drying ammonium sulphate at 105°C for two hours and then 4.7162 g dissolved in de-ionized water to 1 litre. Working standards of 5, 10, 15, 20, 25 and 30 mg ℓ^{-1} N were prepared from the stock solution by dilution. To each standard, concentrated sulphuric acid, 2 ml, was added and followed by de-ionized water to 100 ml.

(iii) Auto-analyser

The reagents were prepared as follows:

Sodium phenate:

200 ml de-ionized water was added to 250 g phenol in a beaker and warmed to dissolve. When cool, the solution was transferred quantitatively to a 1 litre graduated flask. Sodium hydroxide, 135 g, was dissolved in 500 ml de-ionized water, the solution then cooled and added to the phenol solution. After mixing and cooling the solution was made up to 1 litre using de-ionized water.

Sodium hypochlorite:

A concentrated sodium hypochlorite was diluted to contain 5 - 7% available chlorine.

A Technicon MK1 Auto-analyser was used. The reagents were run through the instrument for 30 minutes. The colorimeter and the recorder were warmed up for 10 minutes. The sample tray was loaded with the standard first, followed by two water wash samples and then the test samples. A water wash sample was placed after every 10 test samples and a standard after every 40 samples. Four samples from each treatment were prepared and the samples were randomly arranged in the tray.

(iv) Calculation of the Results

From the standards a calibration curve was constructed, peak heights were plotted against the concentrations. The unknown samples were measured against the calibration curve. The results were then calculated as follows:

$$\%N = \frac{R \times 100}{10 \times W} \times \frac{V}{100} = \frac{R.V}{10W}$$

where,

R = $mg\ell^{-1}$ N in solution of the sample (from graph)

W = Weight of sample (in mg.)

V = Volume of the digestion solution after dilution (in ml).

% Protein = %N x 6.25

2. Monohydroxysapogenin Assay

A method established in the Pharmacognosy Group's Laboratories of the University of Bath was used.

(i) Hydrolysis

Hydrochloric acid (2N), 100 ml, was added to 2 g of whole seed in a 250 ml conical flask and boiled for 2 hours over a bunsen burner under a water reflux. Rapid cooling of the samples was achieved by placing the flasks in running cold water. The samples were filtered to collect the acid-insoluble material. The filter paper was rinsed first with distilled water and then with 10% ammonium hydroxide solution. Ammonia was used to make the samples alkaline. The filter paper with its content was placed on a petri dish and oven-dried at 60°C overnight.

(ii) Extraction

The filter paper and contents were placed in a soxhlet thimble and the monohydroxysapogenin extracted with chloroform in a soxhlet apparatus for 24 hours. The solvent was removed on a rotary evaporator and using a Pasteur pipette and Analar chloroform the sapogenin was quantitatively transferred to a 5 ml volumetric flask and the solution made up to volume. For the GLC assay 60 µl of this solution was used.

(iii) Silylation

The chloroform solution, 60 µl, was placed in a 2 ml screw cap vial and 20 µl 5α-cholestan-3β-ol solution (6.25 mg ml^{-1} in chloroform) was added. Cholestan-3β-ol was used as an internal standard. The mixture was evaporated in a vacuum oven at 80°C and the

residue redissolved in 400 µl ethyl acetate. Each sample was silylated by the addition of 100 µl BSTFA + TMCS(N,O-bis-(trimethyl)-trifluoroacetamide plus 1% trimethylchlorosilane)Regisil Reagent. The sample was then left in the oven for two hours at 80°C prior to GLC analysis.

(iv) GLC Conditions

A Sigma 3 Gas Liquid Chromatograph (Perkin Elmer, Ltd.) fitted with a flame ionization detector was used. A 1 m glass column of internal diameter 1.75 mm was packed with Chromosorb-G (80 - 100 mesh) coated with 2.5% OV-17 Stationary phase. The oven temperature was 280°C and the injector and detector temperatures were both 300°C. Nitrogen flow rate was 20 ml $minute^{-1}$, hydrogen 159 KPa and air 179 KPa. The instrument was fitted with a Perkin Elmer AS100 autosampler injecting 1.5 µl. The samples were arranged randomly. Two samples from each treatment were prepared and each sample was injected twice.

(v) Calculation of the Results

Using monohydroxysapogenin (free from dihydroxysapogenin) and isolated from fenugreek seed, a series of standards were prepared (as described above), injected and a calibration curve constructed. The ratios of peak height for monohydroxysapogenin to that for the internal standard were plotted against monohydroxysapogenin concentrations. The unknown samples were measured against the calibration curve. The percentage monohydroxysapogenin in the plant material was calculated as follows:

$$\% \text{ Monohydroxysapogenin} = \frac{R.V.}{v.M} \times 100$$

where,

R = Ratio of monohydroxysapogenin peak height to that for the internal standard (from the graph)

V = Total volume of chloroform solution in ml.

v = Volume in µl of V used to prepare derivative.

M = Weight of plant material (in g) on a moisture-free basis.

Table 3.1. The properties of soils used for pot and field experiments

	pH	% Clay (< 2 µm)	% Fine Silt (2-20 µm)	% Medium Silt (20-50µm)	% Coarse Silt (50-100 µm)	% Fine Sand (100-250 µm)	% Medium Sand (250-500 µm)	% Coarse Sand (500 µm)	K (µg/g)	Mg (µg/g)	P (µg/g)	Boron (µg/g)	Organic Matter (%)
Begbroke North Pot Experiments	7.2	14	9	9	4	15	35	14	195	47	51		4.1
Sandy Loam Field Experiment 1981.	5.8	14	11	13	5	18	29	10	131	45	37	1.12	2.5
Sandy Loam Field Experiment 1982	6.6	15	11	11	6	17	31	9	203	59	38	1.60	4.1

Table 3.2. Mechanical analyses of the soil (adsorption and selectivity experiments)

Name of Soil	Type of Soil	Organic Matter (%)	pH	% Clay	% Silt			% Sand		
				0.2μm	2-20 (μm)	20-50 (μm)	50-100 (μm)	100-250 (μm)	250-500 (μm)	500 (μm)
Begbroke N.	Sandy	4.1	7.2	14	9	9	4	15	35	14
Allan	Clay	4.3	7.5	24.9	21.3	30.7	13.4	3.8	4.3	1.6
Fen	Organic	18.1	6.6	14	15	27	4	14	17	9.0

Table 3.3. Herbicide Formulations

Common Name	Trade Name	Formulation
Aziprotryne	Brasoran	50% powder
Benfluralin	Balan	18% liquid
Bentazone	Basagran	48% liquid
Bentazone with MCPB	Basagran MCPB	40% liquid
Butralin	Amex A820	48% liquid
Chlorthal-dimethyl with methazole	Delozin S	75% powder
Dinitramine	Cobex	24% liquid
Dinoseb (Amine Salts)	Supersevtox	18.5% liquid
Diquat	Reglone	40% liquid
EPTC	Eptam 6E	72% liquid
Isopropalin	Paarlan	72% liquid
MCPB (sodium salt)	Tropotox	40% liquid
MCPB with MCPA (Na salt)	Tropotox Plus	30% liquid
MCPA/MCPB + Cyanazine	Trifolex-Tra + Fortrol	25% + 50% liquids
Metamitron	Goltix	70% powder
Methazole	Probe	75% powder
Nitrofen	Tok E	25% liquid
Oryzalin	Surflan	75% powder
Trifluralin	Treflan	48% liquid

Table 3.4. The scale used for scoring the phytoxicity of herbicides to fenugreek.

Score	Plant Vigour	As a % of control
0	Completely dead	0
1	Moribund, but not all tissues dead	14
2	Alive, with some green tissue, but unlikely to make much further growth	29
3	Very stunted, but apparently still making some growth	43
4	Considerable inhibition of growth	57
5	Readily distinguishable inhibition of growth	71
6	Some detectable adverse effect as compared with control, colour difference, morphological abnormality, epinasty or very slight reduction in growth	85
7	Indistinguishable from control	100

CHAPTER FOUR

GENERAL EVALUATION OF HERBICIDES

CHAPTER 4

General Evaluation of the Herbicides

1. Pot Experiments

a) Methods

Two experiments were conducted in the glasshouse to evaluate the tolerance of four varieties of fenugreek to different herbicides. The varieties were Paul, Barbara, Margaret and RH4351. The seed was obtained from The National Seed Development Organization, Cambridge. In Experiment 1, metamitron, methazole, nitrofen, chlorthal-dimethyl plus methazole, aziprotryne and trifluralin were tested as pre-emergence treatments. In Experiment 2, metamitron, MCPB, bentazone plus MCPB, MCPA plus MCPB, MCPA plus MCPB plus cyanazine and dinoseb were tested as post-emergence treatments. These herbicides were chosen on the basis of the work of Richardson (1979) and the preliminary work of Hardman's group.

i) Sowing and Spraying.

Plastic pots 10 cm in diameter and 12 cm deep were filled with Begbroke North Soil. Before sowing, the fenugreek seed was inoculated with *Rhizobium meliloti,* 2012. This was obtained from the Rothamsted Rhizobium collection. The liquid culture was applied by shaking with batches of 100 seeds in a polyethylene bag. The seeds were then dried away from light and heat. The seeds were dusted with 0.5% of their weight of benomyl (methyl 1-(butylcarbamoyl) 2-benzimadazolecarbamate) and five seeds were sown per pot, 2 cm deep using a dibber.

Spraying of the herbicides was done with an Oxford Precision Sprayer which was calibrated to deliver 200 or 300 ℓ/ha for post- and pre-emergence treatments, respectively. The pre-emergence herbicides (Experiment 1) were applied on the day of sowing, December 10, 1980. For trifluralin, which was incorporated, the spray was applied to a 2.5 cm layer of soil in a tray. The soilwas then put in a polyethylene bag and mixed thoroughly. Pots were then filled with untreated soil to within 5 cm of the top and then a known amount of the trifluralin-treated soil was added to each pot to give a layer 2.5 cm deep of the required concentration. About ten days before applying the post-emergence herbicides, plants were thinned to 2 per pot. The herbicides were applied (Experiment 2) when the plants had 3 trifoliate leaves, which was about 3 weeks after sowing.

Herbicides were applied as the commercial formulations in distilled water. Doses are given in terms of active ingredients (Tables 4.1 and 4.4). Plants were raised under glasshouse conditions (see Chapter 3), and the experiments had a split-plot design with fenugreek varieties as the main plots and herbicide treatments as the sub-plots.

ii) Assessment

Surviving plants were scored periodically for symptoms of herbicide effects and vigour on a 0 - 7 scale (Richardson, 1979), where 0 = all plants were dead and 7 = no effect (Table 3.4) and the results were converted to percentages for presentation

(see Chapter 3). This assessment was made after 10, 20 and 30 days for Experiment 1;and 2, 10 and 20 days from spraying of the herbicides for Experiment 2. Six weeks after sowing (Experiment 1) or 7 weeks (Experiment 2), the aerial parts of the plants were cut at the soil surface, weighed, put into paper bags and dried at 105°C for 24 hours and dry weights recorded. All results were subjected to an analysis of variance.

b) Results

i) The Tolerance of Fenugreek to Pre-emergence Herbicides

Plants treated with aziprotryne germinated and started into growth but their dry weight at the cotyledon stage indicated that this herbicidewas very injurious to fenugreek.

In general, as shown in Table 4.1, slight and negligible effects of pre-emergence herbicides on plant vigour were observed. However, metamitron at 10 kg/ha (normal rate of use is 3 - 5 kg/ha) and trifluralin at 2 kg/ha affected the plant vigour of all varieties tested, variety Barbara was the most affected. Apart from metamitron at 10 kg/ha and aziprotryne at all rates, other pre-emergence herbicides had no significant effect on the shoot fresh weights of fenugreek (Table 4.2). Metamitron at 10 kg/ha and trifluralin at 2 kg/ha significantly reduced shoot dry weight of fenugreek (Table 4.3).

The different response of fenugreek varieties to herbicides was significant only for shoot dry weight (Table 4.3). Variety

Table 4.1 Effect of pre-emergence herbicides on plant vigour*

Herbicides	Rate kg/ha	Fenugreek varieties											
		P			B			M			R		
		10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Metamitron	2.5	100	100	100	100	100	100	100	100	96	100	100	100
	5.6	96	100	89	84	71	64	96	89	89	100	96	93
	10.0	78	54	47	50	22	18	61	22	18	64	48	43
Methazole	0.75	100	100	100	100	100	100	100	100	100	100	100	96
	1.50	96	96	92	86	89	85	100	96	92	92	96	92
	3.0	96	85	92	92	81	85	100	96	96	92	92	92
Aziprotryne	1.0	0	0	0	0	0	0	0	0	0	0	0	0
	2.0	0	0	0	0	0	0	0	0	0	0	0	0
	4.0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrofen	1.5	96	96	96	100	100	100	96	100	100	100	100	100
	3.0	95	85	96	96	89	85	92	92	92	92	100	100
	6.0	85	85	81	89	85	85	92	81	89	92	82	82
Chlorthal-dimethyl/Methazole	3.25	96	100	96	100	100	92	96	100	96	96	100	92
(Delozin-S)	4.50	96	96	96	100	100	96	100	100	96	100	100	100
	9.0	61	92	85	96	92	85	100	100	96	92	96	92
Trifluralin	0.5	100	96	96	100	92	89	100	96	96	100	100	96
	1.0	100	96	89	93	92	85	100	96	96	96	100	89
	2.0	100	85	68	71	78	71	96	89	81	96	93	85
Control (untreated)	0.0	100	100	100	100	100	100	100	100	100	100	100	100

* See Table 3.4 (Chapter 3).
P = Paul B = Barbara M = Margaret R = RH4351

Table 4.2. Effect of pre-emergence herbicides on shoot fresh weights of fenugreek (g/pot)

Herbicide	Rate kg/ha	Fenugreek varieties				Herbicide means
		P	B	M	R	
Metamitron	2.5	1.16	1.45	1.98	1.86	1.61
	5.0	1.30	0.93	1.66	1.38	0.30
	10.0	0.19	0.12	0.15	0.30	0.19*
Methazole	0.75	1.4	2.02	1.98	1.13	1.63
	1.5	1.19	1.18	1.82	2.39	1.65
	3.0	0.92	0.84	2.28	1.40	1.36
Aziprotryne	1.0	0	0	0	0	0 *
	2.0	0	0	0	0	0 *
	4.0	0	0	0	0	0 *
Nitrofen	1.5	1.26	2.10	2.0	1.94	1.83
	3.0	1.08	0.87	1.84	2.25	1.51
	6.0	1.02	1.62	1.93	1.31	1.47
Chlorthal-dimethyl +	2.25	1.32	1.78	1.60	2.13	1.71
Methazole	4.5	2.63	1.86	1.63	2.58	2.03
	9.0	0.64	1.02	2.90	1.56	1.53
Trifluralin	0.5	1.21	0.87	2.12	1.93	1.53
	1.0	1.36	1.31	1.72	1.12	1.39
	2.0	0.82	0.66	1.86	1.21	1.14
Control (untreated)	-	1.05	1.66	2.25	1.62	1.65
Variety mean		1.12	1.27	1.86	1.83	

P = Paul B = Barbara M = Margaret R = RH4351

LSDs:; = 0.43 for herbicide means (significant at P = 0.05)
= 0.23 for variety means (significant P = 0.05)
= Not significant for herbicide x variety means

* Significantly less than the control.

Table 4.3. Effect of pre-emergence herbicies on shoot dry weights of fenugreek (g/pot)

Herbicide	Rate kg/ha	Fenugreek varieties P	B	M	R	Herbicide means
Metamitron	2.5	0.12	0.14	0.22	0.24	0.18
	5.0	0.14	0.13	0.20	0.17	0.16
	10.0	0.04	0.03*	0.13*	0.13*	0.08*
Methazole	0.75	0.16	0.21	0.25	0.17	0.20
	1.5	0.13	0.16	0.23	0.24	0.19
	3.0	0.09	0.12	0.27	0.16	0.16
Aziprotryne	1.0	0.0*	0.0*	0.0*	0.0*	0.0*
	2.0	0.0*	0.0*	0.0*	0.0*	0.0*
	4.0	0.0*	0.0*	0.0*	0.0*	0.0*
Nitrofen	1.5	0.14	0.25	0.23	0.22	0.21
	3.0	0.13	0.12	0.24	0.26	0.19
	6.0	0.12	0.18	0.28	0.16	0.19
Chlorthal-dimethyl +	2.25	0.15	0.21	0.22	0.29	0.23
Methazole	4.5	0.20	0.21	0.22	0.29	0.23
	9.0	0.08	0.11*	0.34	0.17	0.18
Trifluralin	0.5	0.12	0.10*	0.19	0.25	0.17
	1.0	0.15	0.10*	0.19	0.25	0.17
	2.0	0.10	0.09*	0.25	0.16	0.15*
Control (untreated)		0.11	0.21	0.27	0.23	0.21
Variety means		0.12	0.15	0.23	0.20	

P = Paul B = Barbara M = Margaret R = RH4351

LSD = 0.05 for herbicide means (significant)
= 0.022 for variety means (significant)
= 0.097 for herbicide x variety means (significant at P = 0.05)

* Significantly less than the control.

Barbara was the most affected by trifluralin and chlorthal-dimethyl plus methazole. Varieties Paul, Margaret and RH4351 were more tolerant to these herbicides.

iii) The tolerance of fenugreek to post-emergence herbicides

Table 4.4 shows the effect of herbicides on plant vigour. MCPB at 3.0 and 6.0 kg/ha, bentazone/MCPB at all rates and MCPA/MCPB at all rates adversely affected vigour. Plants were very stunted, their stems were thickened and with cracked epidermis and leaves were rolled and chlorotic. The symptoms of herbicide injury were more pronounced at the high rates of the herbicides. Metamitron at all rates had no effect on plant vigour (Paul, Margaret and RH4351), but variety Barbara seemed to be very susceptible to this herbicide, specially at 5 and 10 kg/ha. The mixture of MCPA/MCPB + cyanazine was generally safe, but varieties Paul and Barbara were seriously affected by the highest rate. Dinoseb, at all rates, caused chlorosis to the plants, though they recovered.

Metamitron at 10 kg/ha, MCPB at 3.0 and 6.0 kg/ha, bentazone/MCPB at 3.0 and 6.0 kg/ha, MCPA and MCPB at 2.0 and 4.0 kg/ha, the mixture of MCPA/MCPB/cyanazine at the highest rate and dinoseb at the highest rate were found to have adverse effects on the growth of fenugreek. These were indicated by the significant reduction of shoot fresh and/or dry weights (Tables 4.5 and 4.6). The differences in response of fenugreek varieties to herbicides was significant for shoot fresh weights only (Table 4.6). Varieties Barbara and RH4351 showed less tolerance to MCPA/MCPB,

Table 4.4 Effect of post-emergence herbicides on plant vigour*

Herbicides	Rate kg/ha	Varieties P 2 Days	P 10 days	P 20 days	B 2 days	B 10 days	B 20 days	M 2 days	M 10 days	M 20 days	R 2 days	R 10 days	R 20 days
Metamitron	2.5	100	100	100	100	100	100	100	100	100	100	100	100
	5.0	100	100	82	96	89	57	100	100	100	96	100	100
	10.0	100	96	75	100	82	39	100	96	78	96	92	92
MCPB	1.5	92	89	93	96	78	92	92	92	96	96	92	96
	3.0	85	78	78	85	81	78	85	78	81	89	78	81
	6.0	81	67	68	81	67	71	85	71	53	81	71	71
Bentazone/MCPB	1.5	92	89	89	92	78	82	92	92	92	92	89	96
	3.0	85	67	64	85	64	60	89	75	75	89	75	75
	6.0	81	56	39	75	46	11	85	56	39	78	50	29
MCPA/MCPB	1.0	85	78	85	89	81	85	85	85	85	85	78	88
	2.0	85	78	78	89	78	82	85	81	81	85	81	81
	4.0	81	67	68	85	78	71	81	71	71	81	71	71
MCPA/MCPB +	0.25+0.75	100	100	100	100	100	100	100	100	100	100	100	100
cyanazine	0.5+0.75	100	100	93	100	100	93	100	100	100	100	100	89
	1.0+0.75	89	85	54	92	86	32	96	96	96	96	96	85
Dinoseb	0.925	100	89	89	96	85	81	100	89	92	96	89	92
	1.85	96	85	72	89	78	85	92	85	89	100	85	92
	3.70	85	78	78	78	52	53	85	78	81	89	78	81
Untreated control	0.0	100	100	100	100	100	100	100	100	100	100	100	100

* See Table 3.4 (General Methods)
P = Paul B = Barbara M = Margaret R = RH4351

Table 4.5 Effect of post-emergence herbicides on shoot fresh weights of fenugreek (g/pot)

Herbicides	Rate kg/ha	Varieties P	B	M	R	Herbicide means
Metamitron	2.5	0.96	0.99	1.68	1.19	1.21
	5.0	0.80	0.60*	1.10	1.23	0.93
	10.0	0.48	0.28*	1.00	0.99	0.69*
MCPB	1.5	0.75	0.90	1.15	1.16	0.99
	3.0	0.58	0.86	1.25	0.79*	0.87
	6.0	0.50	0.65*	0.65*	0.55*	0.59*
Bentazone/MCPB	1.5	0.73	0.71*	1.41	0.98	0.96
	3.0	0.60	0.46*	0.75*	0.79*	0.65
	6.0	0.32	0.16*	0.33*	0.24*	0.26*
MCPA/MCPB	1.0	0.74	1.0	1.21	1.07	1.01
	2.0	0.73	0.71*	0.81	1.01	0.82
	4.0	0.52	0.52*	1.11	0.73*	0.72
MCPA/MCPB/	0.25+0.75	1.28	1.30	1.54	0.79*	1.23
cyanazine	0.5+0.75	0.83	1.15	1.26	0.90	1.04
	1.0+0.75	0.50	0.26*	1.00	0.78*	0.64*
Dinoseb	0.925	1.07	1.11	1.36	1.12	1.17
	1.85	0.94	0.70*	1.33	0.96	0.98
	3.70	0.78	0.52*	0.89	0.81*	0.75*
Untreated control	-	0.67	1.20	1.18	1.33	1.10
Variety means		0.73	0.74	1.11	0.92	

LSDs = 0.39 for herbicide means (significant at P = 0.05)
= 0.10 for variety means (significant at P = 0.05)
= 0.42 for herbicide x variety means (significant at P = 0.05)

P = Paul B = Barbara M = Margaret R = RH4351 * significantly less than the control.

Table 4.6. Effect of post-emergence herbicides on shoot dry weight of fenugreek (g/pot)

Herbicides	Rate kg/ha	Varieties				Herbicide means
		P	B	M	R	
Metamitron	2.5	0.26	0.27	0.40	0.29	0.31
	5.0	0.20	0.16	0.30	0.33	0.26
	10.0	0.15	0.13	0.26	0.24	0.20*
MCPB	1.5	0.22	0.28	0.34	0.33	0.29
	3.0	0.14	0.25	0.30	0.23	0.23*
	6.0	0.14	0.22	0.19	0.17	0.18*
Bentazone/MCPB	1.5	0.20	0.21	0.33	0.24	0.25
	3.0	0.17	0.16	0.22	0.19	0.19*
	6.0	0.10	0.07	0.11	0.11	0.10*
MCPA/MCPB	1.0	0.20	0.28	0.31	0.27	0.27
	2.0	0.18	0.25	0.22	0.26	0.23*
	4.0	0.14	0.19	0.27	0.25	0.21*
MCPA/MCPB/	0.25+0.75	0.29	0.35	0.37	0.25	0.31
cyanazine	0.5+0.75	0.21	0.27	0.33	0.23	0.26
	1.0+0.75	0.13	0.11	0.26	0.20	0.18*
Dinoseb	0.925	0.28	0.33	0.36	0.20	0.18
	1.85	0.24	0.20	0.34	0.26	0.26
	3.70	0.22	0.18	0.27	0.24	0.23*
Untreated control	-	0.21	0.32	0.33	0.34	0.30
Variety means		0.19	0.22	0.29	0.25	

LSDs = 0.066 for herbicides means (significant at P = 0.05) * Significantly less than the control
= 0.022 for variety means (significant at P = 0.05)
(not significant for herbicide x variety means)

P = Paul B = Barbara M. = Margaret R = RH4351.

MCPA/MCPB/cyanazine or dinoseb than varieties Paul and Margaret. Variety Barbara was very susceptible to metamitron.

Generally speaking, MCPB, MCPB/MCPA, bentazone/MCPB were the most injurious treatments to fenugreek whereas metamitron, MCPA/MCPB/cyanazine and dinoseb treatments seemed to be reasonably safe.

c) Discussion

Fenugreek showed good tolerance to pre-emergence herbicides except aziprotryne which killed the plants at the cotyledon stage. Chlorthal-dimethyl plus methazole, methazole, metamitron, trifluralin and nitrofen proved safe to fenugreek; even at twice normal rates of use there was no or very little effect. Nitrofen was reported to be weak against weeds (Richardson, 1979), hence it is unlikely to be useful in the field and for this reason it was not included in the field experiment.

The tolerance of fenugreek to post-emergence herbicides was not as good as its tolerance to the pre-emergence ones. MCPB, MCPA/MCPB, bentazone + MCPB were very injurious to fenugreek at the high rates, but they showed some selectivity at the lower rates. Since they are very effective against broadleaved weeds, this selectivity might be high enough in the field to be useful. Metamitron, MCPA/MCPB/Cyanazine, and dinoseb were good enough for further investigation in the field. Bentazone, although not tested in the pot experiments, was included in the field experiment as a comparison with bentazone + MCPB.

2. Field Experiment,1981

a) Methods

This experiment was conducted at The Weed Research Organization, Oxford, for further evaluation of the herbicides under field conditions. Plots were established on sandy loam soil (see Chapter 3).

(i) Sowing and Application of Herbicides

A rough seed bed was prepared, 250 kg/ha of fertilizer was applied to give 18 units N, 48 units K and 48 units P and plots were rotavated to incorporate the fertilizer and to prepare a fine seed bed. Before sowing, the seeds were inoculated with *Rhizobium meliloti*, 2012 and then dressed with benomyl in the manner already described. Only varieties Paul, Barbara and Margaret were tested. Variety RH4351 was excluded because there was no seed available. Trifluralin was applied and incorporated in the soil on April 6, 1981, before sowing on the next day. Fenugreek seeds were drilled 2 cm deep at 20 - 23 kg/ha, depending on the variety, in rows 14 cm apart. This seed rate was intended to give 80 plants/m^2. Individual plots measured 2 m (one drill width) by 6 m. Each plot had 14 rows. Pre-emergence herbicides, other than trifluralin, were applied on April 8, 1981, and post-emergence ones were applied on May 29, 1981 when the fenugreek was at the 5 - 7 true leaf stage. In all cases, an Oxford Precision Sprayer was used and it was calibrated to deliver 225 ℓ/ha. The experiment had 21 treatments (Table 4.7) randomized in a complete block design with 4 replicates. The experiment was netted against rabbits, slug pellets were spread over the plots, mouse bait was put around each plot and banging ropes

were used to repel birds. After crop emergence all plots were sprayed with Hostathion(triazophos) at a rate of 850 ml product per ha in 625 litre of water to control weevils. Benomyl at 0.275 kg/ha was applied twice, at early flowering and at the pod setting. Liquid feed (Vytel spray) containing NPK and trace elements (see General Materials and Methods), at 1.125 ℓ/ha was applied 10 and 15 weeks after sowing.

ii) Assessment

Visual scoring of phytotoxicity was made 12 weeks after sowing and observations were made periodically. Scoring was based on a 0 - 7 scale as described in Table 3.4. During the course of the experiment, 2 green harvests were taken after 10 and 20 weeks of sowing. Each plot had 14 rows and after rejecting the outer row from each side, the next two rows from the left or right hand side were harvested for the first or second green harvest, respectively. Plants were cut at soil level, transferred into paper bags or tin trays and dried at 105^{o}C for 24 hours. A plant stand count was done at the same time as the first green harvest. Every plant was counted regardless of its condition or vigour. No assessment on the effect of the herbicides on weed control was made. However, observations were made during the course of the experiment. Control plots were handweeded twice, 4 and 8 weeks after sowing. During the weeding operations, some observations were made on weed control.

iii) Harvesting and threshing of the crop

The final harvest was done on September 20, 1981, the 6 central rows from each plot were harvested. An Allan Mayfield Cutter Bar was used to cut the plants at soil level and plants were collected in hessian sacks and left to dry under cover at air temperature. After one month the plants were threshed by a stationary machine. Seeds were cleaned from plant debris, soil particles and weed seeds. Seed yield was then obtained and converted to g/m^2 of plot for the statistical analysis. A composite sample from each treatment was taken for protein and diosgenin assays.

b) Results

i) Plant Vigour

Pre-emergence trifluralin,metamitron and methazole were found to affect fenugreek plant vigour (Table 4.7), but at low rates the effect was very slight. However, all plants recovered and the phytotoxicity symptoms disappeared later on.

The post-emergence herbicides, dinoseb, the mixture of MCPA/MCPB plus cyanazine and bentazone were injurious to fenugreek. Metamitron, MCPB and bentazone/MCPB affected plant vigour at their high rates only (Table 4.7).

ii) Plant Stands

Trifluralin at 3 kg/ha, metamitron at 9 kg/ha and

Table 4.7. Effect of pre- and post-emergence herbicides on plant vigour*

Treatments	Rate kg/ha	P	B	M
Trifluralin (pre-em)	1.0	86	100	89
	3.0	54	68	64
Metamitron "	3.0	93	100	100
	9.0	54	71	54
"Delozin S"+ "	4.5	100	100	100
"	13.5	71	93	93
Methazole "	1.5	86	100	100
"	4.5	68	68	75
Dinoseb (post-em)	1.5	43	36	50
	4.5	0	0	0
Metamitron "	3.0	89	89	89
	9.0	82	85	85
MCPB "	2.5	78	89	85
	7.5	64	54	71
MCPA/MCPB + cyanazine	0.5+0.75	61	57	64
(post-em)	1.5+2.25	23	14	19
Bentazone (post-em)	1.0	68	71	75
	3.0	29	19	33
Bentazone/MCPB (post-em)	1.0	80	85	85
	3.0	57	57	71
Handweeded control	-	100	100	100

* See Table 3.4 (Chapter 3)

P = Paul B = Barbara M = Margaret

+ Chlorthal-dimethyl plus Methazole

methazole at 4.5 kg/ha significantly reduced plant stand of fenugreek (Table 4.8). Of the post-emergence herbicides, only dinoseb at 4.5 kg/ha and the mixture of MCPA/MCPB plus cyanazine at 1.5 plus 2.25 reduced fenugreek plant stand. Statistically, there was no significant difference between the response of fenugreek varieties to the herbicides (Table 4.8), but the data indicated that variety Paul was not affected by 9 kg/ha metamitron and Margaret was not affected by 3 kg/ha trifluralin (Table 4.8).

iii) First Green Harvest

Table 4.9 shows the effect of herbicides on dry weights of fenugreek. Trifluralin, metamitron and methazole, at high rates, were found to affect the growth of fenugreek. They reduced the shoot dry weights and the reduction was highly significant. Dinoseb, the mixture of MCPA/MCPB + cyanazine and bentazone were found to reduce the dry weights of fenugreek. The reduction was highly significant even at the low rates. Bentazone/MCPB and MCPB were less injurious to fenugreek and reduced the growth at the high rates only. Metamitron as a post-emergence treatment had no effect on the growth of fenugreek. Variety Margaret showed more tolerance to 3 kg/ha trifluralin, 4.5 kg/ha methazole, 1 kg/ha bentazone and 7.5 kg/ha MCPB than varieties Paul and Barbara, but it showed less tolerance to 9 kg/ha metamitron (Pre-emergence) than the other two varieties.

Table 4.8. Effect of pre-emergence and post-emergence herbicides on fenugreek plant stands (Plants/m^2)

Treatment	Rate kg/ha	Varieties P	B	M	Means
Trifluralin (pre-em)	1.0	93	71.7	122.7	95.8
	3.0	67.5	55.2	102.5	75.1*
Metamitron "	3.0	97	91	107.2	98.4
	9.0	95	68	92.2	85.1*
"Delozin S" "	4.5	97	81.2	126	101.4
	13.5	93.5	74.7	108.7	92.3
Methazole "	1.5	92.5	67.5	116.2	92.1
	4.5	60.7	30.5	84.0	58.4*
Dinoseb (post-em)	1.5	76.2	65.5	98.5	80.1
	4.5	0.0	0.0	0.0	0 .0*
Metamitron "	3.0	92.7	79.2	125.7	99.2
	9.0	76	89.7	136	100.6
MCPB "	2.5	84.2	80	124.2	96.2
	7.5	93.5	72.0	111.2	92.2
MCPA/MCPB +	0.5+0.75	89.7	78.5	105.7	91.3
cyanazine "	1.5+2.25	56.5	66.7	65.8	63.0*
Bentazone "	1.0	83.7	81.2	122	95.7
	3.0	78.5	74.2	112.7	88.5
Bentazone/MCPB "	1.0	87.2	77.2	123.5	96.0
	3.0	98.7	88.5	120.7	102.7
Handweeded control	-	98.7	80.7	121.2	100.2
Varieties means		81.5	70.2	106.1	

LSDs = 14.7 for treatment means } (Significant at
= 5.54 for variety means } P = 0.05)
(Not significant for treatment x variety means)

P = Paul
B = Barbara
M = Margaret

* Significantly less than the control

Table 4.9. Effect of pre- and post-emergence herbicides on shoot dry weights of fenugreek (first green harvest)

Treatments	Rate kg/ha	Dry weight yield g/m^2 P	B	M	Means
Trifluralin (pre-em)	1.0	51.9	65.8	82.7	66.8
	3.0	26.5*	40.0*	49.4	38.9*
Metamitron "	3.0	76.5	82.0	99.2	85.9
	9.0	53.0	56.8	44.4*	51.4*
"Delozin S" "	4.5	61.6	75.0	88.5	75.2
	13.5	62.3	63.2	84.3	69.9
Methazole "	1.5	65.3	63.2	84.3	69.9
	4.5	34.0*	22.3*	58.8	38.3*
Dinoseb(amine) (post-em)	1.5	29.9*	30.6*	42.9*	34.4*
	4.5	0.0 *	0.0*	0.0*	0.0*
Metamitron "	3.0	48.8	70.7	95.0	71.5
	9.0	50.6	67.1	68.6	62.1
MCPB (Na salt) "	2.5	47.6	59.0	63.6	56.7
	7.5	39.8*	33.5*	62.8	45.4*
MCPA/MCPB (Na salt	0.5+0.75	35.3*	31.5*	39.9*	35.6*
+ Cyanazine (post-em)	1.5+2.25	25.4*	16.1*	16.7*	19.4*
Bentazone "	1.0	33.3*	45.8*	61.0	46.71*
	3.0	24.0*	27.6*	35.9*	29.2*
Bentazone/MCPB "	1.0	41.7*	54.3	72.0	56.0
	3.0	45.7	46.5	59.0	50.4*
Hand weeded control	0.0	62.6	65.6	68.3	65.5
Variety means		43.6	48.9	60.4	

LSDs = 11.2 for treatment means (significant at P = 0.05)
= 4.2 for variety means (significant at P = 0.05)
= 19.5 for treatment x variety means (significant at P = 0.05)

P = Paul

B = Barbara

M = Margaret

* Significantly less than the control

iv) Second Green Harvest

None of the pre-emergence herbicideswas found to reduce fenugreek dry weights (Table 4.10). Although there were damaging effects at the beginning of the growth, they disappeared and the plants recovered from the herbicide injury.

Variety Margaret showed good tolerance to post-emergence herbicides, the dry weights were statistically similar to that of the control, but dinoseb at 4.5 kg/ha killed all the plants. Variety Barbara was very susceptible to the post-emergence herbicides. The herbicides to which it showed good tolerance were bentazone/MCPB and metamitron. However, at the low rate, metamitron reduced the dry weight. Bentazone and dinoseb were quite injurious to varieties Barbara and Paul. Variety Paul showed better tolerance to post-emergence herbicide than variety Barbara.

v) Seed Yield

Table 4.11 shows the effect of herbicides on fenugreek seed yield. Seed yield of all varieties was not affected by pre-emergence herbicides.

Dinoseb at 4.5 kg/ha, MCPB at 2.5 kg/ha and bentazone at 1.0 kg/ha statistically reduced the seed yield of variety Margaret. All post-emergence herbicides, except bentazone at high rate reduced the seed yield of variety Barbara. Only metamitron at high rates and bentazone/MCPB at both rates did not affect the seed yield of variety Paul.

Table 4.10 Effect of pre- and post-emergence herbicides on shoot dry weights of fenugreek (Second green harvest)

Treatments	Rate kg/ha	Dry weight in g/m^2+ P	B	M
Trifluralin (pre-em)	1.0	2.286	2.235	2.496
	3.0	2.131	2.358	2.447
Metamitron "	3.0	2.433	2.394	2.492
	9.0	2.549	2.494	2.578
"Delozin S" "	4.5	2.393	2.336	2.569
	13.5	2.517	2.454	2.551
Methazole "	1.5	2.382	2.265	2.516
	4.5	2.432	2.467	2.606
Dinoseb (post-em)	1.5	1.897*	1.957*	2.304
	4.5	0.0 *	0.0 *	0.0 *
Metamitron "	3.0	1.94*	2.048*	2.264
	9.0	2.187	2.139	2.200
MCPB "	2.5	2.120	2.043*	2.213
	7.5	2.024	2.077*	2.420
MCPA/MCPB + cyanozine	0.5+0.75	1.922*	2.049*	2.188
(post-em)	1.5+2.25	1.986*	1.215*	2.117
Bentazone "	1.0	1.874*	1.884*	2.458
	3.0	1.702*	1.417*	2.129
Bentazone/MCPB	1.0	2.125	2.018*	2.295
	3.0	2.063	2.275	2.231
Handweeded control	-	2.269	2.319	2.319
LSD at (P = 0.05)	-	0.301	0.238	0.282

+ Data were transferred into log 10

P = Paul
B = Barbara
M = Margaret

* Significantly less than the control.

Table 4.11 Effect of pre- and post-emergence herbicides on fenugreek seed yield

Treatments	Rate kg/ha	Seed yield in g/plot+ P	B	M
Trifluralin (pre-em)	1.0	2.465	2.554	2.761
	3.0	2.527	2.588	2.725
Metamitron "	3.0	2.568	2.654	2.692
	9.0	2.668	2.684	2.845
"Delazin S" "	4.5	2.537	2.590	2.778
	13.5	2.563	2.686	2.772
Methazole "	1.5	2.546	2.625	2.727
	4.5	2.578	2.613	2.795
Dinoseb (post-em)	1.5	2.059*	1.988*	2.622
	4.5	0.0 *	0.0 *	0.0 *
Metamitron "	3.0	1.972*	2.198*	2.511
	9.0	2.374	2.285*	2.453
MCPB "	2.5	2.187*	2.247*	2.249*
	7.5	2.222*	2.286*	2.656
MCPA/MCPB + cyanazine	0.5+0.75	2.180*	2.130*	2.422
(post-em)	1.5+2.25	2.173*	1.360*	2.538
Bentazone	1.0	2.017*	1.920*	2.739
	3.0	1.811*	1.251*	2.321*
Bentazone/MCPB "	1.0	2.320	2.252*	2.619
	3.0	2.031*	2.461	2.491
Handweeded control	-	2.456	2.573	2.679
LSD at P = 0.05	-	0.227	0.228	0.289

+ Data were transferred into log 10 (plot size was $3.4m^2$)

P = Paul

B = Barbara

M = Margaret

* Significantly less than the control.

c) Discussion

In general, pre-emergence herbicides were very safe in fenugreek. Trifluralin at 3 kg/ha, metamitron at 9 kg/ha (pre-emergence) and methazole at 4.5 kg/ha, at an early stage of fenugreek development, were slightly toxic. However, the plants recovered and there was no effect on forage (second green harvest) or seed yields.

Most of the post-emergence herbicides tested were very damaging. The first green harvest was done early, before weed competition would have occurred and so was a clear indication of the phytotoxicity of these herbicides. Dinoseb, bentazone and the mixture of MCPA + MCPB + cyanazine were very injurious to fenugreek. Since dinoseb and the mixture of MCPA + MCPB + cyanazine were well tolerated by fenugreek in the glasshouse experiments, the results in the field were disappointing. It is probable that this was mostly due to increased uptake of herbicides in the field where the climatic conditions may have affected the amount of wax on the leaves as well as damaging the leaves mechanically. In pot experiments the amount of cyanazine in the mixture (MCPA + MCPB + cyanazine) was kept constant (0.75), but in the field this was increased to 2.25 kg/ha. So, the phytotoxicity of this mixture at the high rate is likely to be due to cyanazine, which has been reported to be very toxic to fenugreek (Richardson, 1979).

Fenugreek was very sensitive to the weed competition; the weeds present in the field are listed in Table 4.12. All pre-emergence herbicides gave very good weed control and this resulted in higher yield (forage and seed) than the handweeded control. However, trifluralin was very weak against *Capsella bursa-pastoris* and chlorthal-dimethyl + methazole did not control *Fumaria officinalis*.

Table 4.12 List of weeds present at the sites of field experiments (1981, 1982)

Scientific Name	Common Name
Anthemis spp.	Mayweed
Capsella bursa-pastoris	Shepherd's Purse
Chenopodium album	Fat hen
Convolvulus arvensis	Field bindweek
Fumaria officinalis	Fumitory
Lolium spp.	Ryegrass
Matricaria spp.	Mayweed
Polygonum aviculare	Knotgrass
Stellaria media	Chickweed
Urtica dioica	Stinging nettle
Veronica sp.	Speedwell
Sonchus sp.	Thistle
Senecio vulgaris	Groundsel
Sinapsis arvensis	Charlock
Sisymbrium officinale	Hedge mustard
Papaver rhoeas	Corn poppy
Aegopodium podagraria	Ground elder
Silene alba	White campion

The best weed control was obtained with metamitron and methazole. The post-emergence herbicides did not give good weed control. This may be due to late application which was delayed until the crop had developed the third trifoliate leaf. Also the prevailing wet conditions at that stage caused further delay. However, bentazone plus MCPB gave satisfactory weed control and bentazone eliminated all broadleaved weeds, but it was very weak against grasses. In general the highest yields were given by treatments which effectively reduced the weed competition which was also illustrated by the higher yield at the high rates of some herbicides, than at the lower rates.

Metamitron and chlorthal-dimethyl + methazole are rather expensive (the 1983 prices are £8.75, £70, and £53* per ha for trifluralin, metamitron and Delozin-S respectively). Methazole thinned the plant stand, but this did not reduce the yield of fenugreek (forage and seed). Probably this was due to elimination of weeds and because those plants which survived the treatments recovered very quickly. With the wide spacing resulting from the elimination of weeds and thinning of the crop, the growth was vigorous and resulted in increased yield. Unfortunately, the future of methazole is doubtful and the company may cease its production (Richardson, pers. comm.). Hence of the herbicides investigated for fenugreek only trifluralin is likely to be useful. It gave satisfactory weed control and it is cheap, but where resistant weed species are expected a post-emergence herbicide might be needed as a supplement. Dinitroanilines, to which trifluralin belongs, are likely to be safe in fenugreek. In the next experiments this group was investigated further.

* 1981 price, (not available in 1983).

CHAPTER FIVE

STUDIES WITH DINITROANILINE HERBICIDES

CHAPTER 5

Studies with Dinitroaniline Herbicides

1. Relative Phytotoxicity Experiment

a) Methods

This first experiment included 5 different dinitroaniline herbicides chosen after preliminary experiments. Begbroke North Soil was treated with several concentrations of trifluralin, oryzalin, isopropalin, dinitramine or butralin (Table 5.1). Each 5 kg of soil was laid on a polyethylene sheet in a 2.5 cm layer and sprayed with 50 ml of the herbicide suspension to give the required concentration in mg kg^{-1}. An aerosol sprayer was used for this purpose. Immediately after spraying, the soil was put into a polyethylene bag and mixed thoroughly. Pots were filled with the herbicide-treated soil, 1 kg per pot, and five seeds of fenugreek (variety Margaret) were sown in each pot at a depth of 2 cm using a dibber. Before sowing, the seeds were inoculated with *Rhizobium meliloti*, 2012 and then coated with benomyl as described previously. Sowing was done on January 10, 1982. Pots were first given an overhead watering and then watered regularly at an interval of 2 days. After emergence, seedlings were thinned to 2 plants per pot. The experiment had a randomized block design with four replicates and the plants were raised under the glass-house conditions described before (see Chapter 3). Six weeks after sowing, shoots were cut at the soil surface and the roots were washed free of soil particles. Shoot and root dry weights were obtained as described before. To compare the phyto-

toxicity of the different herbicides, 2 ED_{50}s (for shoot and root) for each herbicide were obtained. The ED_{50} is the herbicide concentration required to inhibit plant growth by 50% as compared to the untreated plants. The ED_{50} was derived by plotting the dry weights of the shoot or the root, as a percentage of the untreated control plants, against the logarithm of the herbicide concentration. The antilogarithm of the point on the concentration axis that corresponded with the point of interaction of the curve and the 50% dry weight level gave an estimate of the ED_{50} (Murray *et al.*, 1973).

b) Results and Discussion

Table 5.1 shows the effect of dinitroaniline herbicides on shoot and root dry weights of fenugreek. Dinitramine up to 0.75, oryzalin up to 1.0 trifluralin up to 2.0 and butralin up to 6.0 $mgkg^{-1}$ were tolerated by fenugreek. This was indicated by the dry weights of shoot and root which were, statistically, similar to the control. The results with isopropalin were somewhat erratic, but generally this herbicide was safe in fenugreek up to 14 $mgkg^{-1}$. Table 5.2 gives the ED_{50}s, the concentration required to reduce growth by 50%, for shoot and root for each herbicide. On this basis the phytotoxicity of these herbicides to fenugreek in decreasing order was: Dinitramine > oryzalin > trifluralin > butralin > isopropalin.

The ED_{50}s obtained from roots measurement were less than those from shoots for all herbicides. This indicated that the root was more affected by dinitroaniline herbicides than the shoot. Whether these

Table 5.1 Effect of dinitroaniline herbicides on shoot and root dry weights of fenugreek (variety Margaret)

Herbicides	Rate mg kg^{-1}	Dry weight g/pot	
		Shoot	Root
Dinitramine	0.25	0.13	0.08
"	0.50	0.11	0.11
"	0.75	0.14	0.08
"	1.0	0.08*	0.04*
"	1.25	0.08*	0.05*
"	1.50	0.07*	0.03*
"	1.75	0.07*	0.02*
"	2.00	0.06*	0.02*
Oryzalin	0.6	0.14	0.09
"	0.8	0.12	0.10
"	1.0	0.11	0.07
"	1.2	0.09*	0.05*
"	1.4	0.11	0.05*
"	1.6	0.10*	0.04*
"	1.8	0.11	0.05*
"	2.0	0.07*	0.02*
Trifluralin	2.0	0.13	0.08
"	4.0	0.09*	0.04*
"	6.0	0.09*	0.02*
"	8.0	0.08*	0.02*
"	10.0	0.07*	0.02*
"	12.0	0.07*	0.02*
"	14.0	0.07*	0.01*
"	16.0	0.07*	0.005*
Butralin	2.0	0.21	0.12
"	4.0	0.17	0.11
"	6.0	0.11	0.07
"	8.0	0.14	0.04*
"	10.0	0.12	0.05*
"	12.0	0.09*	0.06*
"	14.0	0.10*	0.06*
"	16.0	0.09*	0.04*
Isopropalin	10	0.11	0.06*
"	12	0.1*	0.04
"	14	0.12	0.08
"	16	0.09*	0.04*
"	18	0.09*	0.02*
"	20	0.06*	0.04*
"	22	0.08*	0.02*
"	24	0.07*	0.02*
Untreated control	0.0	0.14	0.09
LSD at P = 0.05		0.03	0.024

* Significantly less than the control.

Table 5.2. Relative phytotoxicity of dinitroaniline herbicides to fenugreek (variety Margaret) (ED_{50}s)

Herbicides	ED_{50}s mg kg^{-1}	
	for shoot	for root
Dinitramine	1.5	1.0
Oryzalin	2.0	1.45
Trifluralin	10.0	3.4
Butralin	>16.0	12.0
Isopropalin	>18.0	11.2

herbicides are less absorbed by the shoot than by the root or the root is more susceptible than the shoot is not clear. Dinitroanilines are absorbed by the hypocotyl hook of dicotyledons and via the first internode of monocotyledons (Standifer and Thomas, 1965; Knake *et al.*, 1967; Barrentine and Warren, 1971b), but Parker (1966) reported that root uptake of trifluralin is more effective than shoot uptake.

2. Site of Uptake

a) Methods

These experiments were carried out to compare the phytotoxicity of dinitroanilines to fenugreek when shoot or root was exposed to them as well as to find the major sites of uptake. Dinitramine, trifluralin, oryzalin and benfluralin were used in this study. The soil (Begbroke North) was sprayed with an aqueous suspension of the herbicide to give 0, 2 and 4 $mgkg^{-1}$ concentrations for each herbicide as already described.

Shoot Exposure

Pots were filled with untreated soil to within 5 cm of the top. The soil was then covered with aluminium foil which had been cut so as to fit in the pot. Two holes, 2 mm in diameter and 2 cm apart , were made in the centre of the aluminium foil. Fenugreek seeds, variety Margaret, were germinated on a moist filter paper. After the emergence of their radicles, 2 such germinated seeds were planted in each pot, their radicles being inserted carefully

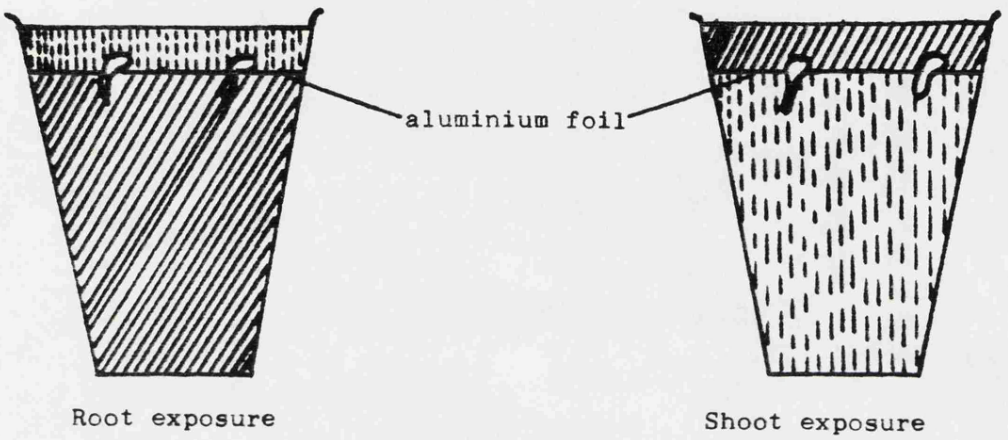


Figure 5.1 Technique used to expose shoot or root to herbicides.

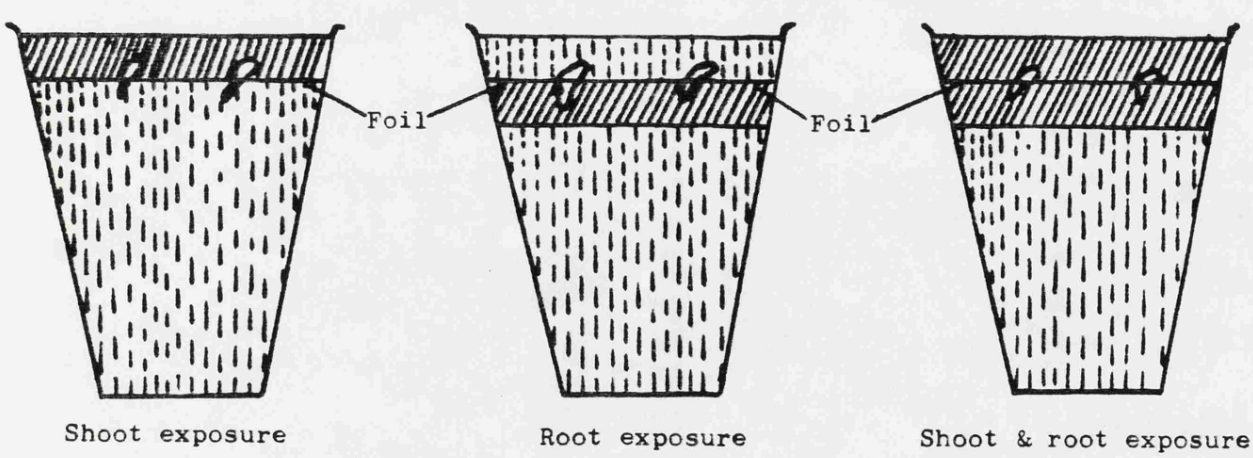


Figure 5.2 Technique used to expose shoot, root or shoot and root to herbicides.

Treated soil Untreated soil Germinating seeds

through the holes. The seeds were then covered with a 2 cm layer of a known amount of herbicide-treated soil. Thus only the shoot was exposed to the herbicide. Figure 5.1 illustrates the technique used.

Root Exposure

The same technique was used except that the treated soil was below the aluminium foil as shown in Figure 5.1. In this case the root, but not the shoot, was exposed to the herbicides.

In another experiment, using the same technique, the shoot, root and shoot and root of fenugreek were exposed to 0, 2, 4 and 6 mg kg^{-1} concentrations of trifluralin (Fig. 5.2). For seed exposure, the seeds were sown in trifluralin-treated soil (the same concentrations) for 48 hours, dug out, washed with water and resown in herbicide-free soil. In order to avoid root absorption, any seed which showed signs of radicle emergence at the washing stage was rejected.

In all cases, pots were stood on the glasshouse bench in a randomized block design with four replicates. Six weeks after sowing, the plants were harvested and shoot and root dry weights were obtained.

b) Results and Discussion

(i) Uptake by root or shoot of fenugreek

The results of the effect of dinitroaniline herbicides on shoot

and root dry weights of fenugreek through shoot or root exposure are summarized in Table 5.3. Benfluralin at 2 and 4 mg kg^{-1} did not affect the growth of shoot and root of fenugreek at any exposure. Through shoot or root exposures, dinitramine and oryzalin reduced the dry weight of shoot and root of fenugreek. However, the reduction of shoot dry weight of the plants treated with 2 $mgkg^{-1}$ oryzalin, through shoot exposure, was not significant. Trifluralin, through shoot exposure did not affect shoot or root dry weight. However, through root exposure, both shoot and root dry weights were reduced. The results indicate that dinitroanilines are absorbed by both shoot and root of fenugreek.

The root seemed to be more susceptible to these herbicides than the shoot. This is supported by the previous results (ED_{50}s) where the root was the most affected. The results also indicate that the root absorbs more herbicide than the shoot. However the root comes in continuous contact with the herbicide-treated soil and this might result in more absorption by the root than by the shoot where the hypocotyle hook comes in contact with the treated soil for a short period (2 - 3 days only).

ii) Uptake By Different Parts of Fenugreek

Table 5.4 shows the dry weight of shoot (a) and root (b) of fenugreek treated with different concentrations of trifluralin. At all rates, trifluralin through seed exposure did not significantly reduce the shoot or root growth of fenugreek. The results indicated that there was no absorption of trifluralin by fenugreek seed or

Table 5.3. Effect of dinitroaniline herbicides on the growth of fenugreek (variety Margaret) through root or shoot exposure.

Herbicides	Rate in mg/kg^{-1}	Dry weights in g/pot			
		Shoot exposure		Root exposure	
		Shoot	Root	Shoot	Root
Dinitramine	2.0	0.203*	0.057*	0.100*	0.027*
	4.0	0.100*	0.023*	0.077*	0.001*
Oryzalin	2.0	0.280	0.107*	0.083*	0.017*
	4.0	0.143*	0.043*	0.660*	0.002*
Trifluralin	2.0	0.353	0.163	0.143*	0.077*
	4.0	0.297	0.147	0.113*	0.063*
Benfluralin	2.0	0.317	0.140	0.217	0.133
	4.0	0.300	0.110	0.227	0.123
Control	0.0	0.373	0.163	0.260	0.123
LSD (P = 0.05)		0.096	0.043	0.060	0.027

* Significantly less than the control

if there was any, it was not enough to affect the growth. The growth of shoot and root were not affected when the shoot was exposed to 2 or 4 $mgkg^{-1}$ trifluralin. However, at 6 $mgkg^{-1}$ the growth of both organs was seriously reduced. Through root exposure, the root growth was significantly reduced by all rates of trifluralin, but the shoot growth was reduced by 4 and 6 mg kg^{-1} trifluralin. The dry weights of shoot and root were reduced when both were exposed to 2, 4 or 6 mg kg^{-1} trifluralin. Through this shoot and root exposure a cumulative effect was observed indicating absorption of this herbicide by both shoot and root.

In the case of trifluralin the shoot and/or the root were exposed to the same amount of trifluralin-treated soil and the root was allowed to pass through the treated soil and then most of the root system grew freely in the untreated soil. The results indicated that root absorption was more effective than shoot absorption.

3. Vapour Phytotoxicity

The object of this experiment was to compare phytotoxicity of dinitroaniline herbicides to fenugreek through shoot absorption of the vapour.

a) Methods

Fenugreek, variety Margaret, was planted in 1 kg capacity pots.

Table 5.4. Effect of trifluralin on the dry weight of shoot (a) and root (b) of fenugreek(variety Margaret) through seed, shoot, root or shoot and root exposure

Treatments	(a) Shoot dry weight in g/pot+			
	Seed exposure	Shoot exposure	Root exposure	Shoot and root exposure
Trifluralin at 0.0 mgkg^{-1}	0.53 bcd	0.57 bc	0.605 bc	0.77 a
Trifluralin at 2.0 mg kg^{-1}	0.42 def	0.638 b	0.568 bc	0.503 cde
Trifluralin at 4.0 mg kg^{-1}	0.40 def	0.610 bc	0.393 efg	0.245 hj
Trifluralin at 6.0 mg kg^{-1}	0.41 def	0.248 ghj	0.305 fgh	0.145 j
	(b) root dry weight in g/pot+			
Trifluralin at 0.0 mg kg^{-1}	0.4075 bcd	0.335 bcd	0.44 bc	0.55 a
Trifluralin at 2.0 mgkg^{-1}	0.3175 d	0.440 bc	0.325 d	0.38 bcd
Trifluralin at 4.0 mg kg^{-1}	0.310 d	0.370 bcd	0.310 d	0.158 e
Trifluralin at 6.0 mg kg^{-1}	0.3375 cd	0.168 e	0.125 e	0.083 e

+ Figures followed by the same letters are not significantly different as determined by Dunncan's multiple range test (P = 0.05).

After emergence (at the cotyledon stage) plants were thinned to 2 seedlings per pot and were exposed to vapour of trifluralin, oryzalin or isopropalin. This was done by using the double pot technique shown in Figure 5.3. Acetone, 1 ml, containing 5 mg of trifluralin, oryzalin or isopropalin was pipetted into a weighing boat. After evaporation of the acetone and crystallization of the herbicides, the weighing boat was stuck by sellotape to the upper pot as shown in Figure 5.3. The upper pot had no holes and the two pots were sellotaped together. The plants were exposed to the herbicides for 3 days before the upper pot was removed and the plants left to grow for 6 weeks. After that they were harvested and shoot dry weights were obtained.

b) Results and Discussion

Figure 5.4 shows the vapour activity of dinitroaniline herbicides. Only trifluralin, at 5 mg/pot, showed vapour activity and significantly reduced fenugreek growth. Isopropalin and oryzalin did not reduce the shoot dry weight presumably because they are less volatile. Jacques and Harvey (1979a,b) and Jordan *et al.* (1979) found that oryzalin and nitralin were less active than trifluralin and dinitramine through vapour and concluded that the vapour phytotoxicity of dinitroanilines is correlated with the rate of herbicide volatilization. Trifluralin is likely to have phytotoxic action in the field through its vapour. Also its favourable rate of volatilization may assist in good distribution and movement within the soil.

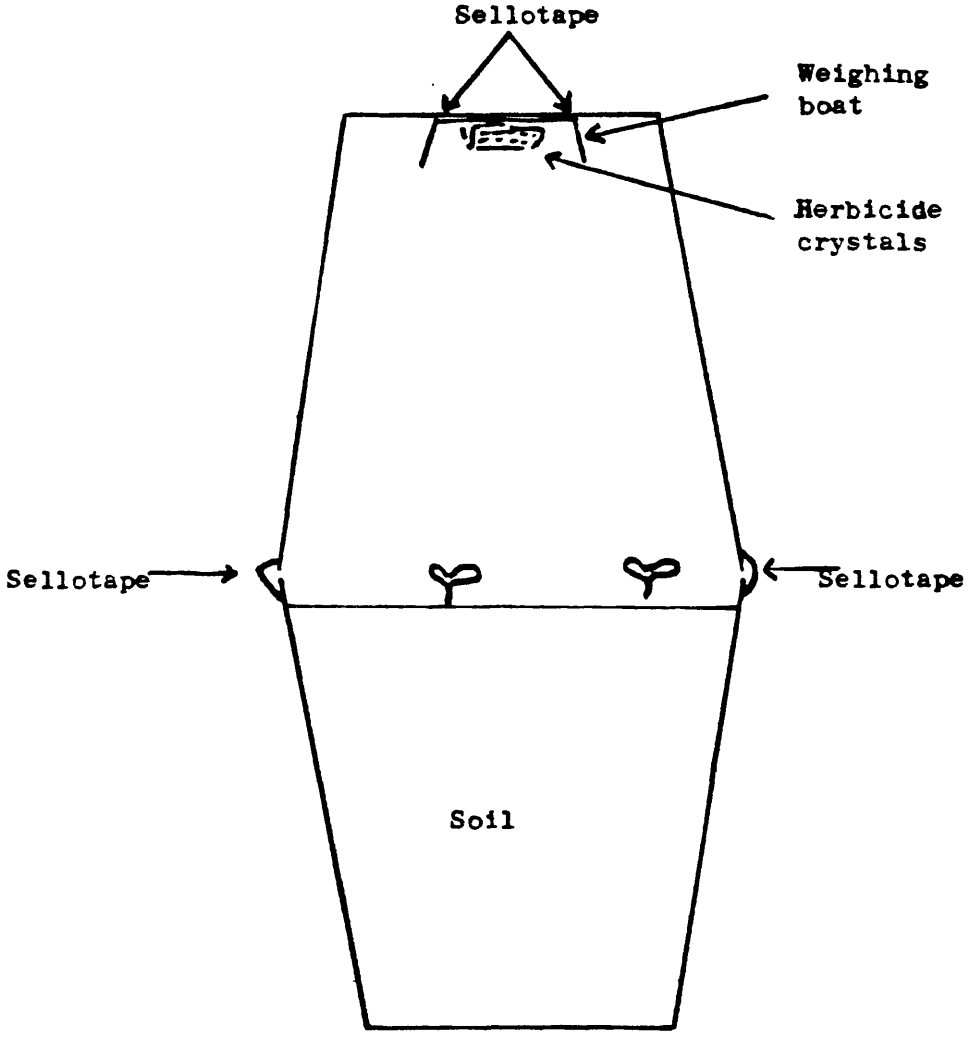
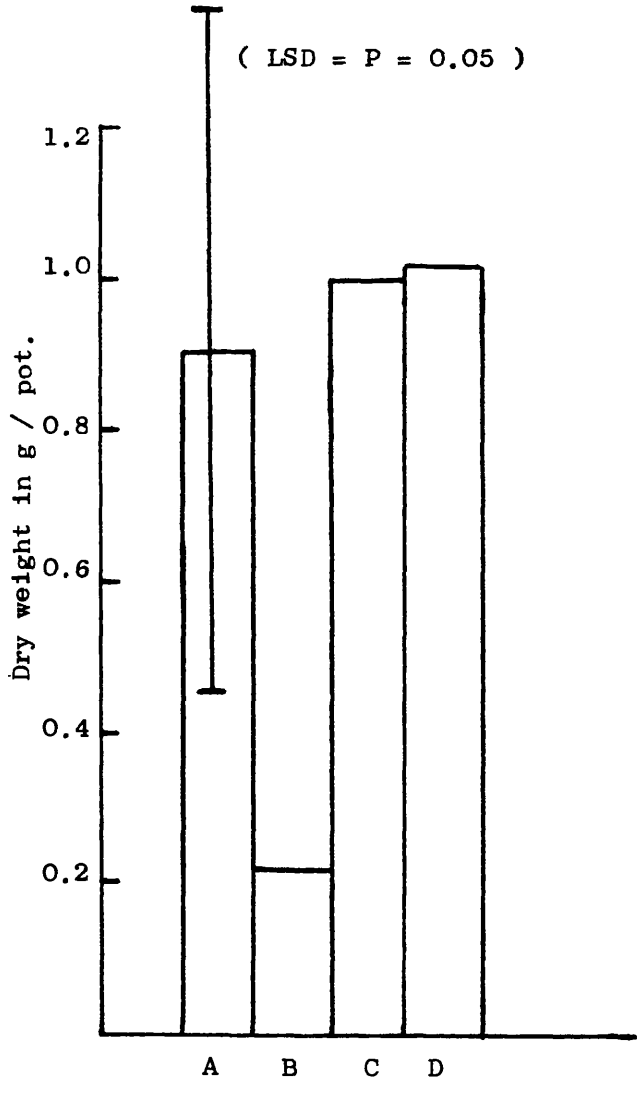


Figure 5.3 Double pot technique used for vapour absorption.

Figure 5.4 Vapour activity of the dinitroanilines on the growth of fenugreek (variety Margaret).



A = Control

B = Trifluralin (5mg / pot)

C = Oryzalin (")

D = Isopropalin (")

4. Field Experiment 1982

a) Methods

The experiment was conducted at The Weed Research Organization, Oxford. Plots were established on sandy loam soil (Table 3.1) near the 1981 experiment site. The objects of this experiment were to evaluate the tolerance of fenugreek to the dinitroaniline herbicides under field conditions, to investigate the effect of these herbicides on protein and diosgenin yields and on root nodulation and to evaluate the value of diquat as a desiccant to enhance ripening. Diquat was chosen on the basis of its success in other legumes and preliminary experiments carried out at Bath (Hardman, unpublished data).

i) Land Preparation and Application of Herbicides

The land was prepared as described for the 1981 Field Experiment except that ground limestone, at a rate of 2 tonne/ha was applied to the rough seed bed as well as fertilizer (at the same rate as in the previous field experiment) and incorporated into the soil with a rotovator. The lime was used so as to raise the soil pH from 6.6 to 7 (actually 7.4). Trifluralin at 1, 2 and 3; oryzalin at 0.75, 1.5 and 2.25 and isopropalin at 1.5, 3 and 4.5 kg/ha were applied on March 24, 1982. The plot size, the sprayer used, and the method of application were the same as in the 1981 Field Experiment. Immediately after application of the herbicides, all plots were rotovated to give incorporation to a normal depth of 5 cm.

ii) Inoculation of the Seeds and Sowing

A *Rhizobium meliloti* inoculum on peat (Pelinoc) with adhesive

(Pelgal), provided by The Nitragin Co., U.S.A., was used. Pelgal solution (47% w/w), 20 ml, was added to 1 kg of seed contained in a bucket. The seeds were mixed with the solution until each seed was coated and then the Pelinoc, 25 g, added with mixing. Then the seeds were dressed with 0.5% of their weight of benomyl. All these operations were carried out in the shade and the seeds were left to dry for 30 minutes. Then the seed (variety Margaret) was drilled as described in the 1981 field experiment on the same day on which the herbicides had been already incorporated into the soil.

iii) Assessment

Scoring of phytotoxicity symptoms, green harvests and general observations were carried out as before. Five plants were selected randomly from each plot for visual estimate of nodulation. This was done 12 weeks after sowing. On July 18, 1982, all plots were handweeded in an attempt to save the crop from weed damage and to facilitate harvesting. During the weeding operation, observations were made of the effect of the herbicides on weed control.

iv) Harvesting of the Crop

On August 9, 1982, half of each plot was desiccated with diquat at a rate of 0.5 kg/ha (a.i.). Ten days later, 50 pods were collected from the desiccated part of each plot for diosgenin assay. On August 26, 1982, the 6 central rows of the undesiccated half of each plot were harvested. Plants were pulled by hand and taken straight to the stationary machine for threshing. Seed yield was obtained and subjected to analysis of variance.

b) Results

(i) Plant Vigour

Trifluralin and oryzalin, especially at high rates, affected plant vigour (Table 5.5). Plants treated with isopropalin showed no symptoms. Plants recovered from the toxicity of trifluralin and oryzalin after two months, but the symptoms of oryzalin at 2.25 kg/ha persisted. In all cases the symptoms of these herbicides on fenugreek were minor and characterized by slight stunted growth and dark green colour of the leaves.

ii) Plant Stand

Table 5.6 shows the effect of dinitroaniline herbicides on plant stand of fenugreek. None of the treatments significantly reduced the number of plants (oryzalin at 2.25 kg/ha thinned fenugreek stand, but statistically this was not significant).

iii) First Green Harvest

Oryzalin at all rates (Table 5.7) reduced fenugreek shoot dry weight. Trifluralin at 2 and 3 kg/ha reduced the growth of fenugreek, but the reduction was not significant. Fenugreek tolerated isopropalin well and the reduced growth of fenugreek at its lowest rate was the result of weed competition (Table 5.7).

iv) Second Green Harvest

Herbicides at all rates reduced the dry weight of fenugreek,

Table 5.5. Effect of dinitroanilines on plant vigour: Field Experiment, 1982. (Fenugreek, variety Margaret).

Treatments	Rate kg/ha	Plant vigour* as% of control
Trifluralin	1.0	100
"	2.0	88.8
"	3.0	81.2
Oryzalin	0.75	89.0
"	1.50	78.0
"	2.25	71.0
Isopropalin	1.50	92.5
"	3.0	100
"	4.5	88.75
Handweeded control	0.0	100

*See Table 3.4 (Chapter 3)

Table 5.6. Effect of dinitroanilines on fenugreek (variety Margaret) plant stand: Field experiment, 1982.

Treatments	Rate kg/ha	Plant/m^2 +
Trifluralin	1.0	62.9
"	2.0	62.2
"	3.0	60.2
Oryzalin	0.75	64.6
"	1.5	66.6
"	2.25	55.5
Isopropalin	1.5	59.2
"	3.0	68.3
"	4.5	60.5
Handweeded control	-	61.8
LSD (P = 0.05)		20.7

+ No significant differences between treatments

Table 5.7 Effect of dinitroaniline on shoot dry weight (First green harvest): Field Experiment, 1982 (Fenugreek, variety Margaret)

Treatments	Rate kg/ha	Dry weight g/m^2
Trifluralin	1.0	67.7
"	2.0	54.7*
"	3.0	58.5
Oryzalin	0.75	48.8*
"	1.50	53.2*
"	2.25	35.7*
Isopropalin	1.5	48.7*
"	3.0	71.5
"	4.5	69.4
Handweeded control	-	78.1
LSD at (P = 0.05)		23.0

* Significantly less than the control

but the reduction by trifluralin and isopropalin at their highest rates was not significant (Table 5.8). The results indicated that weed competition was responsible for the reduction of fenugreek growth.

v) Seed Yield

Table 5.9 shows the effects of dinitroaniline herbicides on fenugreek seed yield. Trifluralin and isopropalin, generally,

Table 5.8 Effect of dinitroaniline on shoot dry weight (Second green harvest): Field Experiment, 1982 (Fenugreek, variety Margaret)

Treatments	Rate kg/ha	Dry weight g/m^2
Trifluralin	1.0	222.9*
"	2.0	236.4*
"	3.0	299.1
Oryzalin	0.75	160.8*
"	1.50	232.0*
"	2.25	219.5*
Isopropalin	1.5	157.9*
"	3.0	184.5*
"	4.5	278.9
Handweeded control	-	345.1
LSD at (P = 0.05)		74.2

* Significantly less than the control

had no effect on the seed yield. The reduction of the yield by isopropalin at the lowest rate seemed to be the result of poor weed control. Plots treated with oryzalin gave the lowest yield. Oryzalin seemed to be too toxic to fenugreek while trifluralin and isopropalin were acceptable.

c) Discussion

Generally speaking, oryzalin was the most phytotoxic to fenugreek whereas isopropalin was the least toxic. The results were

Table 5.9 Effect of dinitroanilines on fenugreek(variety Margaret) seed yield: Field Experiment, 1982

Treatments	Rate kg/ha	Seed yield g/m^2
Trifluralin	1.0	133.2
"	2.0	76.2*
"	3.0	88.9
Oryzalin	0.75	68.3*
"	1.5	79.8*
"	2.25	76.4*
Isopropalin	1.5	81.2*
"	3.0	123.6
"	4.5	102.5
Handweeded control	-	114.2
LSD at (P = 0.05)		30.5

* Significantly less than the control

similar to those obtained from the pot experiments. Trifluralin and isopropalin were well tolerated by fenugreek, but trifluralin showed some phytotoxicity at the highest rate. Although this experiment was carried out for toxicity and not for weed control, during weeding operations observations were made on the effect of these herbicides on weed. Table 4.12 gives the common weeds present at the site. Oryzalin and trifluralin gave good weed control, except for *Capsella bursa-pastoris* and *Matricaria* spp. which were very resistant. The elimination of other weeds by these herbicides left these two weeds without competition and

they became a big problem in fenugreek and were largely responsible for the reduction of fenugreek dry weight (second green harvest). Isopropalin did control all grasses but failed to control most of the broadleaved weeds, for example *Convolvulus arvensis* and *Chenopodium album*. *Capsella bursa-pastoris* and *Matricaria* sp.were not a problem in isopropalin-treated plots and this might be due to the competition from other weeds. However, isopropalin showed an adequate margin of selectivity and its efficiency against weeds was better at the highest rate.

Isopropalin looks to be a promising herbicide for fenugreek. Oryzalin, though was very effective against weeds, but showed less margin of selectivity and may damage the crop. Trifluralin showed some phytotoxicity towards fenugreek at 3 kg/ha (three times the normal field rate), but this did not affect the seed yield.

Regarding weed control, the resistant weeds seemed to be very competitive with fenugreek and they were responsible for the reduction of the growth in trifluralin and isopropalin-treated plots. In the case of isopropalin, it is likely to give better weed control with higher rates. In the forthcoming experiments, the phytotoxicity of these herbicides to different plant species was compared in three different types of soil since the soil constituents may reduce the activity of the herbicides through adsorption.

5. Adsorption Experiments

a) Methods

The adsorption of trifluralin, oryzalin and isopropalin by three different soils was compared. The method used was that of Chassin *et al.* (1981). The soils used were sandy soil (Begbroke North), clay soil (Allan soil) and organic soil (Fen) (Table 3.2).Stock solutions of trifluralin (0.5 mg L^{-1}), isopropalin (0.1 mg ℓ^{-1}) and oryzalin (2.5 mg ℓ^{-1}) were prepared in distilled water. Further dilution of each herbicide was made with distilled water to give a minimum of 6 concentrations of each. Samples of the soil, 10 - 100 mg (depending on the herbicide and on the soil type), were shaken with 10 ml of the herbicide solution in a glass test tube. The tubes were covered with aluminium foil and then with plastic covers. Tests were made to ensure that there was no significant adsorption of the herbicides to the glass or to the foil. After 16 hours shaking, calcium chloride, 5 mg, was added to each tube and then the soil slurries were centrifuged at 2500 rpm for 10 minutes. A sample,5 ml, of the supernatant liquid was taken for analysis. All treatments were carried out in triplicate.

Analysis:

(i) Trifluralin and isopropalin

The supernatant liquid, 5 ml, was shaken vigorously with hexane, 5 ml. The liquids were allowed to separate and 2 ml of the hexane layer was taken for GLC analysis.

(ii) Oryzalin

For measurement by GLC, oryzalin was converted to the dimethyl derivative using dimethyl sulphate (DMS) as described by Sieck *et al.* (1976). To the supernatant, 5 ml, was added 1 ml 10 M sodium hydroxide solution followed by 0.3 ml dimethyl sulphate and 5 ml dioxane. These were shaken together for 20 minutes on a wrist-action shaker. The mixture was transferred to a 100 ml separating funnel and shaken with 20 ml chloroform. After the separation of the liquids, the chloroform layer was passed through sodium sulphate in a filter funnel and collected in a 100 ml conical flask. A second 10 ml chloroform was similarly used. Finally the sodium sulphate filter funnel was washed with 5 ml chloroform. All the chloroform extracts were combined, evaporated to dryness and the residue redissolved in 5 ml hexane for GLC analysis.

GLC conditions : A Pye 104 gas chromatograph, fitted with ^{63}Ni electron capture detector, was used. The column was 1.5 m x 4 mm, i .d. glass, packed with 1.5% XE60 on Chromosorb WHP. The injector temperature was 160°C, column temperature was 190°C for oryzalin and trifluralin, 210°C for isopropalin and the detector temperature was 300°C. The carrier gas was O_2-free nitrogen at 60 ml $minute^{-1}$. Attenuation was 10 x 10^2. In all cases 5 µl was injected from each sample using an automatic injector.

Calculation of the Results

A series of standards of each herbicide were prepared in hexane, oryzalin was derivatized first, and a calibration curve for each herbicide was constructed by plotting the peak heights of the standards against their concentrations on a log-log graph paper. The unknown samples were measured against the calibration curves. The amount adsorbed by the soil was calculated as follows:

$$\text{Amount adsorbed in } \mu g g^{-1} = \frac{C_o - C}{S} \times 10$$

where

C_o = initial concentration in $\mu g\ ml^{-1}$

C = Equilibrium concentration in $\mu g ml^{-1}$ (from the graph)

S = Amount of soil in g

Adsorption isotherms were obtained by plotting, for each herbicide, the amounts adsorbed by each soil against the equilibrium solution concentrations on log-log graph paper assuming the Freundlish adsorption isotherm $\frac{x}{m} = kC^{1/n}$ could be used.

where

$\frac{x}{m}$ is the amount of herbicide adsorbed in $\mu g\ g^{-1}$

C = equilibrium concentration in $\mu g\ ml^{-1}$

K and 1/n are constant

K is given by the value of $\frac{x}{m}$ when C = 1 and can be used for comparisons.

b) Results and Discussions

The k values at C = 1 (the comparison will be different at other concentrations, because the lines are not parallel) of trifluralin (Fig. 5.5) were 3200, 81

and 400, for oryzalin (Fig. 5.6) 2800, 240 and 130 and for isopropalin (Fig. 5.7) 2150, 170 and 210 on organic, clay and sandy soil, respectively.

Generally speaking, the three herbicides were highly adsorbed by organic soil. Clay and sandy soils did adsorb the herbicides, but to a lesser extent than the organic soil. Trifluralin, in general, was adsorbed to the greatest extent and isopropalin to a lesser extent. The effect of clay soil on oryzalin was more pronounced than that on trifluralin or isopropalin. Trifluralin was the least adsorbed on clay soil and oryzalin was the least adsorbed on sandy soil. Trifluralin has been shown to be highly adsorbed by organic soil, but much less so by clay (Scott and Phillips, 1972; Grover, 1974). The higher adsorption of oryzalin by clay is probably associated with the slightly ionizable aminosulphonyl group. In general, bioactivity of a herbicide is closely related to soil texture, so greater rates are often required on organic and clay soils. Phytotoxicity of trifluralin decreases as soil organic matter content increases (Rahman, 1978; Harrison *et al.*, 1976). Variation in soil organic matter content accounted for 94% of the variability in trifluralin dose rates required for 90% reduction in fresh weight of wild oat, whereas clay content had no significant effect (Moyer, 1979). Dinitroaniline herbicides appear to be highly adsorbed by soil constituents with organic matter content being the most important soil factor that affects the bioactivity of these herbicides. Recommendation rate should be adjusted to the amount of organic matter content. The effect of these soil constituents on the bioactivity was investigated in the next experiments.

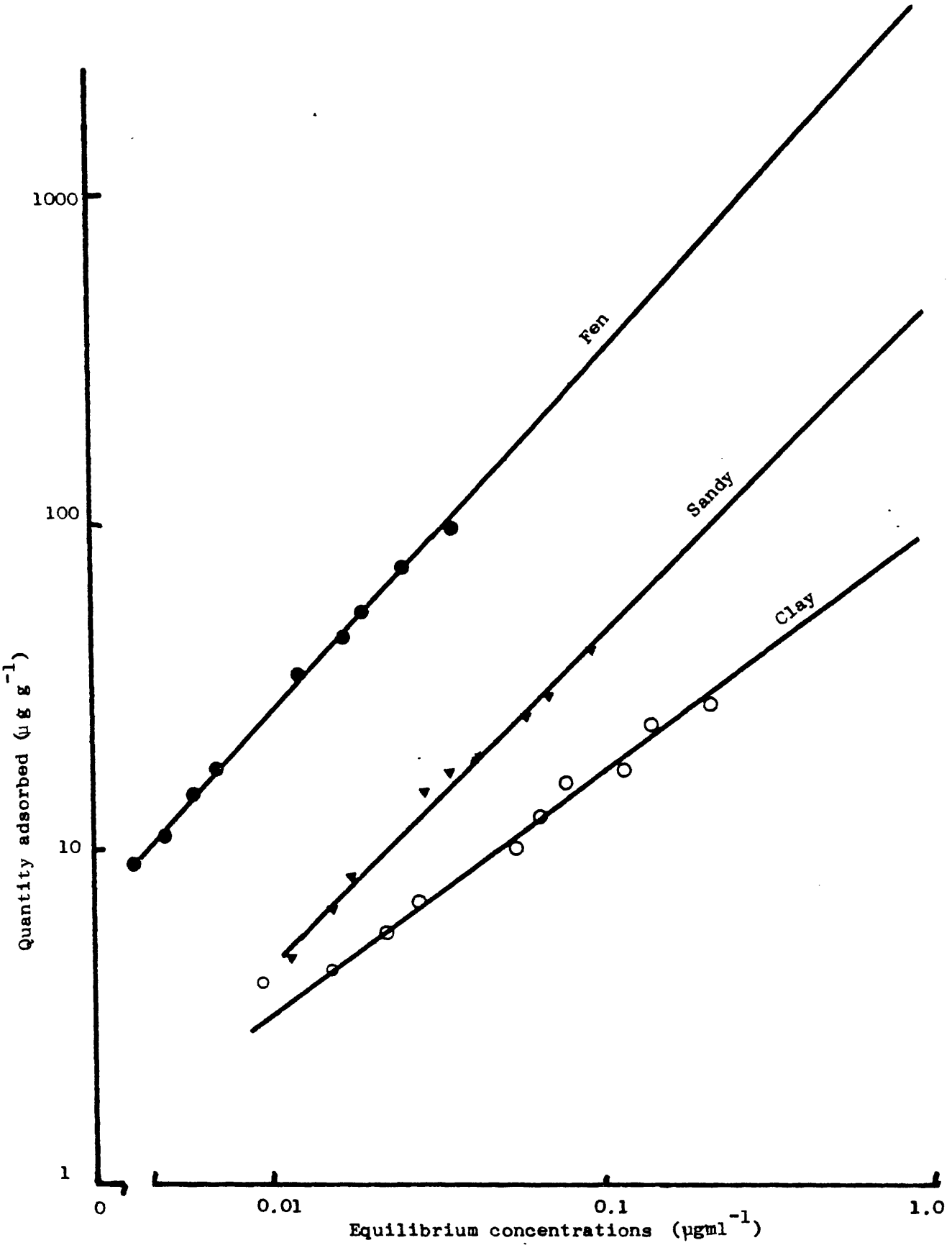


Figure 5.5 Isotherms for trifluralin

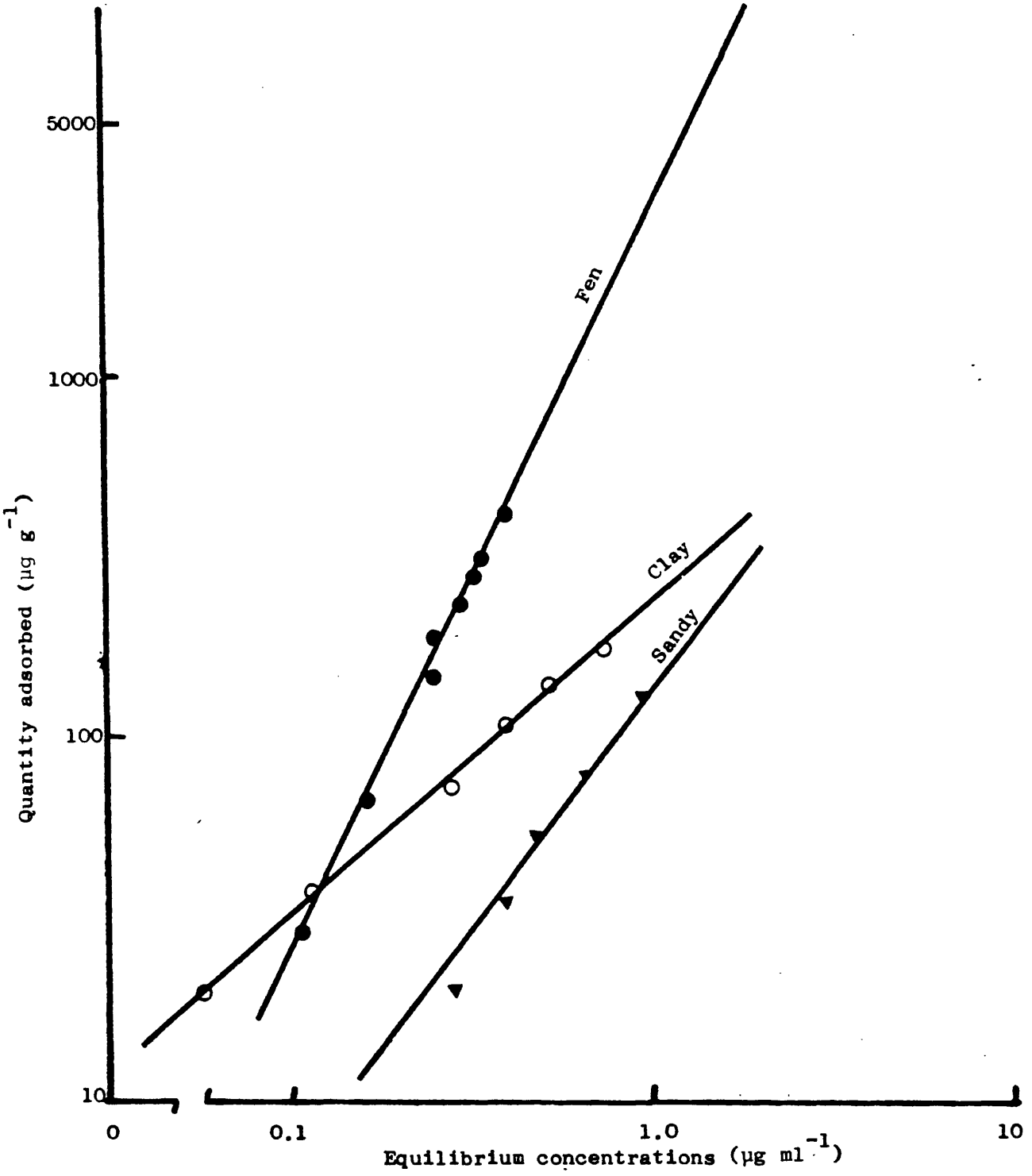


Figure 5.6 Isotherms for oryzalin

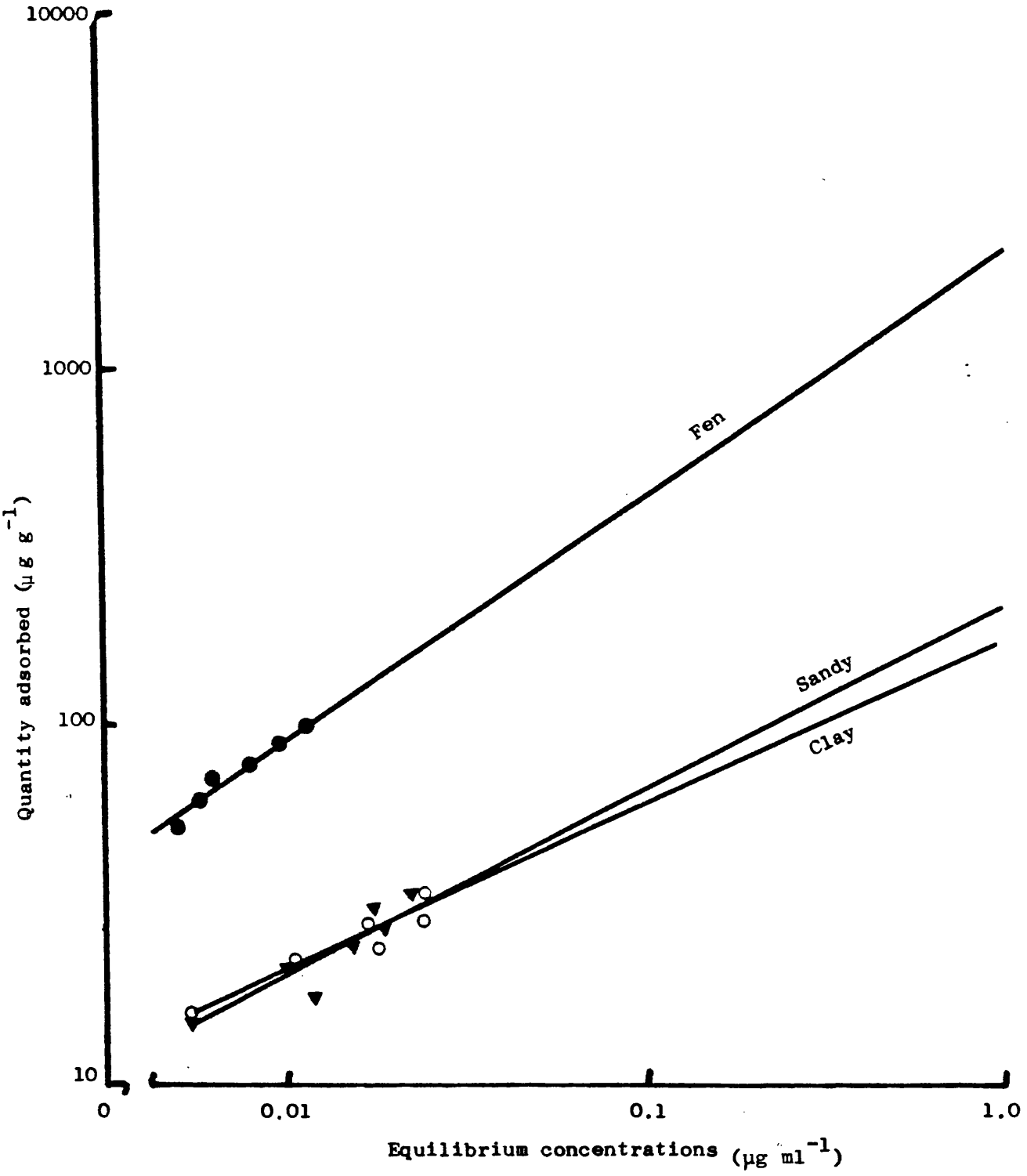


Figure 5.7 Isotherms for isopropalin

6. Selectivity Ratio and Crop Safety

a) Methods

A series of indoor pot experiments was carried out to compare the phytotoxicity of trifluralin, oryzalin and isopropalin to fenugreek with that to four other plant species in 3 different soils. The soils were those that were used for the adsorption experiments. The bioassay plants were fenugreek (variety Margaret), turnip *(Brassica campestris)*, representing cruciferous weeds, *Polygonum aviculare* (Knotgrass), *Lolium anna* (ryegrass) and *Stellaria media* (chickweed). The seeds of turnip, *L. anna* and *S. media* were obtained from The Weed Research Organization, Oxford and that of *P. aviculare* was bought from B and S Weed Seeds Supplies, Nottingham.

i) Spraying and Raising of the Plants

The soils (sandy, clay and organic) were sprayed, using an aerosol sprayer, with several concentrations of trifluralin, oryzalin or isopropalin, and mixed thoroughly in a polyethylene bag as described earlier. The rates of the herbicides were determined on the basis of preliminary experiments. Seeds were planted in 1 kg herbicide-treated soil in 10 cm diameter pots. The depth of sowing was 2 cm for fenugreek and 0.5 cm for the other plant species. In the case of *P. aviculare*, the seeds were mixed with moist sand and stored in a closed polyethylene bag in the refrigerator for six weeks before sowing to stimulate germination. After germination, all plants were thinned to 5, for fenugreek, or to 10 plants/pot for the other species. There were nine herbicide-soil

combinations. Each combination was considered as a separate experiment and set out in a randomized block design with three replicates. These experiments were carried out in the glasshouse during the period between May and August, 1982, so no supplementary heat or light was used.

ii) Harvesting and Calculation of the Results

Six weeks after sowing, the above ground parts (shoot) were cut at the soil level and dry weights were obtained as already described. For each plant species, ED_{50} values, for the nine herbicide-soil combinations were obtained. ED_{50}s were obtained through regression analysis (for those where r =0.90 or more) or as before, through eye-fitting the curve obtained by plotting shoot dry weights, as a percentage of the control, against the logarithm of the herbicide concentrations (for those where r is <0.9). The selectivity ratios (the margin of selectivity between weeds and fenugreek) were obtained by dividing the ED_{50} for each plant species by that for fenugreek.

b) Results and Discussion

Table 5.10 shows the ED_{50} values for each plant species. For all plant species, the ED_{50} values for all herbicides, were higher in organic soil than in sandy or clay soils indicating that the activity of these herbicides is affected by the organic matter content of the soil. The results were comparable to those obtained from the adsorption experiments where the adsorption of these

Table 5.10 ED_{50} values in $mgkg^{-1}$

Plant Species	Trifluralin			Oryzalin			Isopropalin		
	Organic soil	Clay soil	Sandy soil	Organic soil	Clay soil	Sandy soil	Organic soil	Clay soil	Sandy soil
Fenugreek	24.5	>12	6.5	>10	1.64	1.15	>60	>20	>20
Turnip	≤10	2.36	1.7	5.56	0.84	0.83	45.8	5.5	>12
Stellaria media	1.53	0.296	0.383	0.43	<0.063	-	2.20	0.16	>0.8
Polygonum aviculare	1.44	0.135	0.73	0.49	<0.063	0.10	2.6	-	>1.5
Lolium sp.	1.88	0.16	0.45	0.60	0.074	0.13	2.47	0.52	0.78

Table 5.11 Selectivity Ratios ($\frac{\text{Weed } ED_{50}}{\text{Fenugreek } ED_{50}}$)

Plant Species	Trifluralin			Oryzalin			Isopropalin		
	Organic soil	Clay soil	Sandy soil	Organic soil	Clay soil	Sandy soil	Organic soil	Clay soil	Sandy soil
Fenugreek	-	-	-	-	-	-	-	-	-
Turnip	0.4	<0.2	0.26	<0.56	0.512	0.72	<0.76	<0.275	-
Stellaria media	0.06	<0.025	0.06	<0.043	< 0.038	-	< 0.037	<0.008	-
Polygonum aviculare	0.06	<0.011	0.11	<0.049	<0.038	0.087	<0.043	-	<0.075
Lolium sp.	0.07	<0.013	0.07	<0.06	< 0.045	0.113	< 0.04	< 0.026	<0.039

herbicides, by the organic soil, was very high. However, the clay soil which was expected to reduce the activity of oryzalin, compared with the sandy soil, had no effect on the activity of any of the herbicides except on fenugreek and turnip with trifluralin and oryzalin.

The selectivity ratios of these herbicides (weed ED_{50}/fenugreek ED_{50}) were very low (Table 5.11) indicating high tolerance of fenugreek compared with other plant species. However turnip, which had been included to represent the cruciferous weeds, *Capsella bursa-pastoris* and *Matricaria* spp., showed high tolerance to these herbicides resulting in high selectivity ratios. This result confirmed that dinitroanilines are less effective against cruciferae as had been shown already in the Field Experiment, 1982, where *C. bursa-pastoris* and *Matricaria* spp. proved very resistant to these herbicides.

In general, oryzalin was the most toxic herbicide whereas isopropalin was the least. From pot and field experiments, it appears that these three dinitroaniline herbicides can be of use only where there is no resistant weed.

In the 1981 Field Experiment, bentazone as a post-emergence herbicide was very effective against broadleaved weeds including *C. bursa-pastoris* and *Matricaria* spp., but it was injurious to fenugreek and less effective against grasses. Other post-emergence herbicides, including bentazone with MCPB, MCPB, and metamitron, might be of use as a supplement to pre-emergence dinitroaniline herbicides, but might be costly. Another potential pre-planting

incorporated herbicide is EPTC which is very effective against a wide range of broadleaved weeds and grasses (Richardson, pers. comm.), but it is only marginally tolerated by fenugreek (Richardson, 1979). The tolerance of many species to EPTC can be improved by the use of safeners, so their effect with fenugreek was examined.

CHAPTER SIX

STUDIES WITH EPTC AND THE SAFENERS

CHAPTER 6

Studies with EPTC and the Safeners

1. Spray Application of EPTC

a) Methods

The objective of these experiments was to assess the possibility of improving the selectivity of EPTC to fenugreek. Four experiments were carried out under glasshouse conditions to evaluate four different safeners, R25788, NA, MON4606 and M32988. Tables 6.1, 6.2, 6.3 and 6.4 give the treatments for Experiments 1, 2, 3 and 4 respectively (see Table 2.3 for the chemical names).

i) Safeners as Seed Treatments

A 30 $g\ell^{-1}$ solution of methyl cellulose, 0.15 ml, was shaken with 5 g of fenugreek seed in a 100 ml conical flask. The appropriate amount of the safener was added, shaken and allowed to dry. The actual amount of safener retained on the seed was not determined. Loss occurred because of debris created by shaking and by safener adhering to the glass. In the case of MON4606, an aqueous suspension, 0.15 ml, of the safener was mixed with the fenugreek seed, 5 g.

ii) EPTC and R25788 as a Tank-Mix

The appropriate amounts of EPTC and R25788 were mixed in water just prior to spraying on to the soil. R25788 was also

applied as a soil treatment as "Eradicane", a formulation of EPTC with R 25788 in a ratio of 12:1 (herbicide:safener).

iii) Spraying and Raising of the Plants

Plastic trays containing the soil (Begbroke North) were sprayed with EPTC or EPTC + R25788 using a laboratory pot sprayer (Experiments 1 and 2) or using an Oxford Precision sprayer. After spraying, the trays were emptied immediately into polyethylene bags and the herbicide mixed with the soil by shaking the bags. Pots were filled with untreated soil to within 3 cm of the top. Five seeds of fenugreek, variety Barbara (safener-coated seeds or untreated seed) were distributed evenly on the top of the untreated soil (Variety Barbara was chosen for these experiments because of its potential as a forage crop planted with maize). A known amount of the treated soil was added to each pot to cover the seeds 2 cm deep and to give the required concentration. The sowing dates were August 5, September 15, November 10 and December 10, 1982, respectively, for experiments 1, 2, 3 and 4. The pots were stood on the glasshouse bench in a randomized block design with 3 or 4 replicates. Experiment 3 was carried out in duplicate, one set was left till seed production for protein and diosgenin assays. After emergence, the fenugreek plants were thinned to 2 seedlings per pot. Ten days after sowing, 5 ml of liquid culture of *Rhizobium meliloti,* 2012, was added to each pot.

Six weeks after sowing, shoots were cut at the soil surface and roots were washed free of soil particles. A visual estimate

of root nodulation was made and shoot or shoot and root dry weights were obtained. For diosgenin and protein assays, the plants were left until the production of seed and five pods from each plant were collected and composited for each treatment.

b) Results and Discussion

EPTC alone, at all rates (2 - 10 kg/ha) significantly reduced shoot and root dry weights of fenugreek. The higher the rate of EPTC, the higher the reduction of fenugreek dry weight (Tables 6.1 - 6.4). The symptoms of herbicide injury to fenugreek were clearly noticeable and characterized by cupped, necrotic, deformed leaves with twisting and folding shoots of a dark green colour and stunted growth.

As shown in Table 6.1 (Experiment 1), R25788, as a seed treatment, at 0.5% w/w effectively protected fenugreek from injury caused by EPTC (2 and 4 kg/ha), the plant dry weights were, statistically, not significantly different from that of the control.

NA afforded no protection to fenugreek from EPTC injury (Table 6.1) and the further reduction in the dry weights indicated that it was phytotoxic to fenugreek as this resulted in a cumulative effect.

In Experiment 2 (Table 6.2), there was some protection to fenugreek to EPTC by NA. This was indicated by slight increase in shoot dry weights at 0.5 and 1.0% w/w of the safener, but it was lost at the 2% w/w rate of NA. On its own, NA was toxic to

Table 6.1. The effect of EPTC with or without safeners on fenugreek dry weights: Experiment 1

Treatments	Rate of herbicide in kg/ha	Dry weight in g/pot	
		Shoot	Root
1. EPTC	2.0	0.30*	0.11*
2. EPTC + NA at 0.5% w/w (ST)	2.0	0.20*	0.08*
3. EPTC + R25788 at 0.5% w/w (ST)	2.0	0.36	0.15
4. EPTC	4.0	0.25*	0.09*
5. EPTC + NA at 0.5% w/w (ST)	4.0	0.23*	0.08*
6. EPTC + R25788 at 0.5% w/w (ST)	4.0	0.35	0.14
7. Untreated control	0.0	0.47	0.18
LSD at (P = 0.05)		0.12	0.04

ST = Seed treatment

* Significantly less than the control.

Table 6.2. Effect of EPTC, with or without safener, and the safeners, on fenugreek shoot dry weights: Experiment 2

Treatments	Rate of herbicide in kg a.i./ha	Shoot dry weight g/pot
EPTC	2.0	0.59*
EPTC + NA at 0.5% w/w as ST	"	0.70*
EPTC + NA at 1.0% w/w as ST	"	0.66*
EPTC + NA at 2.0% w/w as ST	"	0.55*
EPTC + R25788 at 0.5% w/w as ST	"	0.82*
EPTC + R25788 at 1.0% w/w as ST	"	0.83*
EPTC + R25788 at 2.0% w/w as ST	"	0.97
EPTC	4.0	0.47*
EPTC + NA at 0.5% w/w as ST	"	0.36*
EPTC + NA at 1.0% w/w as ST	"	0.65*
EPTC + NA at 2.0% w/w as ST	"	0.33*
EPTC + R25788 at 0.5% w/w as ST	"	0.52*
EPTC + R25788 at 1.0% w/w as ST	"	0.67*
EPTC + R25788 at 2.0% w/w as ST	"	0.76*
NA at 0.5%	0.0	0.81*
NA at 1.0%	"	0.99
NA at 2.0%	"	0.82*
R25788 at 0.5%	"	1.05
R25788 at 1.0%	"	0.95
R25788 at 2.0%	"	0.96
Untreated control	"	1.18
LSD (P = 0.05)		0.23

ST = Seed treatment
* Significantly less than the control.

fenugreek.

The protection of fenugreek from EPTC injury (Table 6.2) by R25788 (as a seed treatment) was dependent on the rate of the safener. The higher the rate of R25788, the better the the protection, . although the increase of shoot dry weights with increased concentrations of safener, regardless of EPTC rate was not significant. Without the herbicide, R25788 had no significant effect on fenugreek.

In experiment 3 (Table 6.3) R25788 as a seed treatment at all rates (0.5, 1.0 and 2.0% w/w) effectively protected fenugreek from the herbicide injury when EPTC was applied at 2 kg/ha. At 4 kg/ha EPTC, less and not significant protection was afforded by R25788 at 0.5 or 1.0% w/w, but at 2.0% w/w there was significant protection. In the absence of EPTC, R25788 at 2.0% w/w adversely affected the growth of fenugreek.

R25788 (as a tank mix) at 2.0 kg/ha when applied with 2 kg/ha EPTC significantly protected fenugreek (Table 6.3). There was no protection by R25788 at 4 kg/ha when mixed with 2 kg/ha EPTC. However, at 4 kg/ha of EPTC less protection was obtained by R25788 at 2 kg/ha, than at 4 kg/ha. R25788 as a soil treatment, without the herbicide, (at 2 or 4 kg/ha) did not significantly reduce the growth of fenugreek.

MON4606 at 0.25% w/w (seed treatment) significantly reduced herbicide injury when EPTC was used at 4 kg/ha (Table 6.3). However, at

Table 6.3. Effect of EPTC with or without safeners on the growth of fenugreek: Experiment 3

Treatments and Rates	Dry weights in g/pot	
	Root	Shoot
1. EPTC at 2 kg/ha	0.0538*	0.1975
2. EPTC at 2 kg/ha + R25788 0.5% w/w ST	0.0730	0.2550
3. EPTC at 2 kg/ha + R25788 1.0% w/w ST	0.0820	0.3225
4. EPTC at 2 kg/ha + R25788 2.0% w/w ST	0.0668	0.2675
5. EPTC at 2 kg/ha + R25788 2.0 kg/ha TM	0.0696	0.1975
6. EPTC at 2 kg/ha + R25788 4.0 kg/ha TM	0.0543	0.1650*
7. EPTC at 2 kg/ha + MON4606 0.25% w/w ST	0.0500*	0.1425*
8. EPTC at 2 kg/ha + M32988 0.5% w/w ST	0.0638	0.1850
9. EPTC 4 kg/ha	0.0428*	0.1450*
10. EPTC at 4kg/ha + R25788 0.5% w/w ST	0.0500*	0.2075
11. EPTC at 4kg/ha + R25788 1.0% w/w ST	0.0535*	0.1350*
12. EPTC at 4kg/ha + R25788 2.0% w/w ST	0.01713	0.1750
13. EPTC at 4kg/ha + R25788 2.0 kg/ha TM	0.0578	0.1325*
14. EPTC at 4kg/ha + R25788 4.0 kg/ha TM	0.0635	0.1900
15. EPTC at 4 kg/ha + MON4606 0.25% w/w ST	0.0630	0.2350
16. EPTC at 4kg/ha + M32988 0.5% w/w ST	0.0495*	0.1350*
17. R25788 0.5% w/w ST	0.0708	0.21250
18. R25788 1.0% w/w ST	0.1013	0.2950
19. R25788 2.0% w/w ST	0.0498*	0.1375*
20. R25788 2.0 kg/ha TM	0.0710	0.2000
21. R25788 4.0 kg/ha TM	0.0675	0.18250
22. MON4606 0.25% w/w ST	0.0623	0.2000
23. M32988 0.5% w/w ST	0.0645	0.1900
24. Untreated control	0.0793	0.2525
LSD (P = 0.05)	0.0252	0.0852

ST = Seed treatment

TM = Tank Mix

* Significantly less than the control.

the lower rate of EPTC (2 kg/ha) with MON4606 at the same rate (0.25% w/w), further reduction of shoot dry weights occurred. This indicated that at the lower rate of EPTC (2 kg/ha), MON4606 was phytotoxic to fenugreek. However, in the absence of EPTC, MON4606 reduced plant dry weights, but not significantly.

M32988 at 0.5% w/w as a seed treatment protected fenugreek against EPTC injury at 2 kg/ha of the herbicide but not at 4 kg/ha (Table 6.3).

With EPTC at 5 kg/ha in Experiment 4 (Table 6.4), R25788 at all rates, as a seed or soil (in a combined formulation with EPTC, Eradicane) treatment, significantly increased shoot dry weights of fenugreek. However shoot dry weights were statistically lower than that of the control.

With EPTC at 5 kg/ha (Table 6.4), MON4606 afforded the best protection and the shoot dry weight was not significantly different from the control.

At 10 kg/ha of EPTC, none of the safeners significantly protected fenugreek (Table 6.4).

In general the incidence of fenugreek injury from EPTC was effectively reduced by seed treatments with the safeners R25788 and MON4606, with the latter being superior to the former at the higher rates of EPTC.

Table 6.4. Effect of EPTC, with or without safeners, on fenugreek growth: Experiment 4

Treatments and Rates	Shoot dry weights+ g/pot
EPTC 5 kg/ha	0.08 d
EPTC 5 kg/ha + R25788 0.5% w/w as ST	0.125 bc
EPTC 5 kg/ha + R25788 1.0% w/w as ST	0.130 bc
EPTC 5 kg/ha + R25788 2.0% w/w as ST	0.140 bc
EPTC 5 kg/ha + MON4606 0.25% w/w as ST	0.165 ab
EPTC 5 kg/ha + M32988 0.50% w/w as ST	0.115 cd
EPTC 5 kg/ha + R25788 0.416 kg/ha soil treatment$^{\phi}$	0.125 bc
EPTC 10 kg/ha	0.08 d
EPTC 10 kg/ha + R25788 0.5% w/w as ST	0.115 cd
EPTC 10 kg/ha + R25788 1.0% w/w as ST	0.120 cd
EPTC 10 kg/ha + R25788 2.0% w/w as ST	0.1225 cd
EPTC 10 kg/ha + MON4606 0.25% w/w as ST	0.100 cd
EPTC 10 kg/ha + M32988 0.5% w/w as ST	0.120 cd
EPTC 10 kg/ha + M32988 0.5% w/w as ST	0.120 cd
EPTC 10 kg/ha + R25788 0.832 kg/ha seed treatment$^{\phi}$	0.100 cd
Untreated control	0.200 a

+ Figures followed the same letters are not significantly different as determined by Dunncan's multiples range test at P = 0.05

ST = Seed treatment

ϕ = EPTC and R25788 in a combined formulation (12:1) known as "Eradicane".

As shown in plates 6.1, 6.2, 6.3, 6.4 and 6.5, the symptoms of EPTC at 5.0 kg/ha completely disappeared when using MON4606 at 0.25% w/w and to a lesser extent when using R25788 at 2.0% w/w. However, symptoms persist at 10 kg/ha EPTC. The plates show the effect of safeners quite clearly. It appeared that the protection given by R25788 and MON4606 is rate-dependent; this might need further investigation.

Most of the major broad-leaved crops have been tested by various authors (reviewed by Blair *et al.*, 1976) for the possibility of protection by NA or R25788 against many of the common herbicides. Most results have been negative, but R25788 and NA gave moderate protection to field bean from EPTC injury, and to cotton from cisanilide injury, respectively.

MON4606 reduced the growth of fenugreek when used alone or when used with the lower rate of EPTC (2 kg/ha). However the result of this safener and EPTC at 4 - 10 kg/ha indicated that the efficiency of MON4606 for safening fenugreek from EPTC injury is rate dependent. These results(see plates 6.1 - 6.5) justify further work with MON4606. Although higher rates of EPTC (5 - 10 kg/ha) did damage fenugreek treated with R25788 in this pot experiment, it is likely that in the field R25788 would allow fenugreek to tolerate rates of up to 4 kg/ha EPTC, which is an adequate rate for effective weed control.

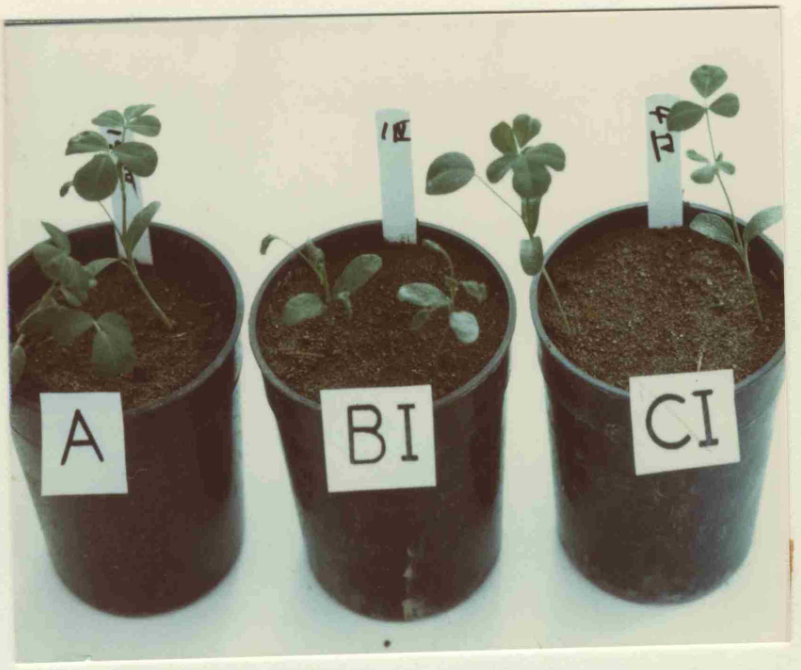
The added selectivity provided by safeners R25788 and possibly MON4606 may allow the use of EPTC for effective weed control in fenugreek alone or in amixed crop, for example with maize. This

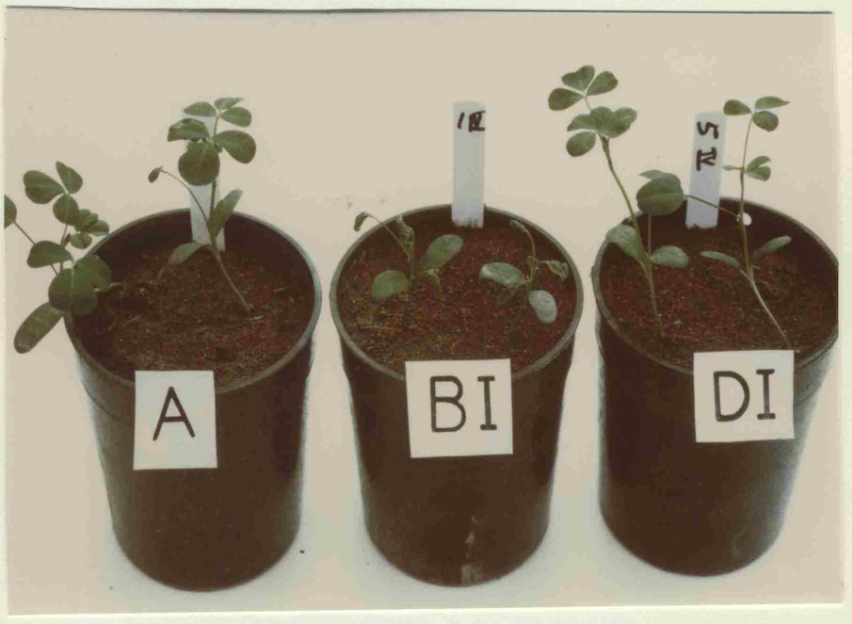
Plates 6.1 - 6.5 Effects of R25788 and MON4606, as seed treatments, in reducing fenugreek injury by EPTC.

Plate 6.1
A = Untreated control
BI = EPTC at 5 kg/ha only
CI = EPTC at 5 kg/ha + R25788 at 2% w/w (seed treatment).

Plate 6.2
A = Untreated control
BI = EPTC at 5 kg/ha only
DI = " " " " + MON4606 at 0.25% w/w (seed treatment).

Plate 6.3
A = Untreated control
BII = EPTC at 10 kg/ha only
CII = " " " " + R25788 at 2% w/w (seed treatment).


A
BI
CI


A
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DI

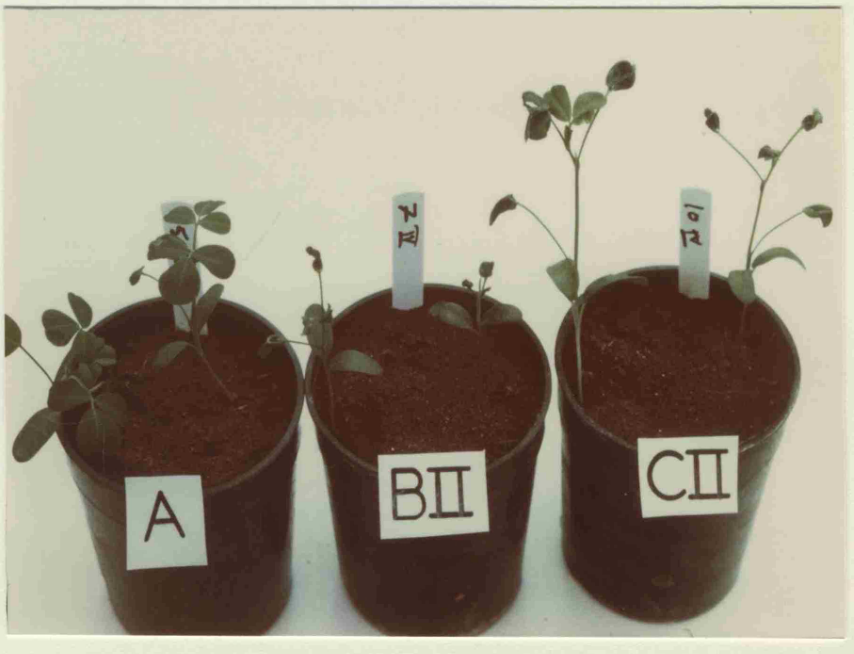
A
BII
CII

Plate 6.4

A = Untreated control
BII = EPTC at 10 kg/ha only
DII = " " " + MON4606 at 2% w/w (seed treatment).

Plate 6.5

A = Untreated control
BI = EPTC at 5 kg/ha only
CI = " " " + R25788 at 2% w/w (seed treatment).
DI = " " " + MON4606 at 0.25% w/w (seed treatment).


A
BII
DII


A
BI
CI
DI

is of importance as fenugreek may then be interplanted with maize for forage purposes,with fenugreek for protein and as an appetizer, as well as providing some nitrogen for the growing maize.

2. EPTC - Coated Seed

a) Introduction

Dawson (1979, 1980, 1981) has been conducting experiments to evaluate the use of EPTC applied to the crop seed for selective weed control. His studies have shown that lucerne seed, for example, can be coated with selected herbicides and planted successfully. His method was tried with fenugreek seed using EPTC in a combined formulation with safener R25788 (Eradicane). The object was to eliminate the early competition of weeds in fenugreek. The experiment was carried out under glasshouse conditions.

b) Methods

i) Coating of the seed

A solution of 30 g/ℓ of methyl cellulose, 0.2 ml, was added to 10 g fenugreek seed (variety Margaret) in a 100 ml conical flask. The seeds were shaken until every seed was coated with the solution. Nitragin's Peat inoculum, 25 mg, was added to the seed, for inoculation and the flask shaken, followed by benomyl at the rate used before and again the flask was shaken. A further 0.4 ml methyl cellulose solution was added and shaken with the inoculated seed. Immediately 5 g gypsum was added and the flask again shaken. The gypsum-coated seeds were dried and 1 µl of acetone, containing 0.0, 0.2 or 0.4 mg "Eradicane" was applied to each seed. The seeds were left to dry before sowing. This method of coating proved to be quite satisfactory for pot experiments if the seed was handled with care. The method would not permit the coating to be

retained in a drilling operation. In his research, Dawson was provided with commercially coated seed and no coating details are given in his publications.

ii) Sowing and Raising of the Plants

Window boxes, measured 90 x 15 x 15 cm, were used. Treated fenugreek seed was sown, at a depth of 2 cm, in rows 10 cm apart and 5 cm between seeds. Each box had 9 rows and each 3 rows were considered as a separate treatment and randomly labelled A, B or C. Before sowing of fenugreek, 50 seeds of rye-grass (treatment B) or turnip (treatment C) were incorporated in the top 0.5 cm layer of soil. Sowing was done on January 20, 1983. The plants were raised under the same glasshouse conditions as already described. Four weeks after sowing, seedlings of ryegrass and turnip were counted and harvested. Two weeks later, fenugreek plants were harvested. In all cases, the plants were cut at the soil surface and shoot dry weights were obtained.

c) Results and Discussion

Table 6.5 shows the effect of Eradicane (EPTC + R25788) on the growth of fenugreek when applied to the seed. In the absence of the weeds (treatment A), the growth of fenugreek was significantly reduced by both rates of the herbicide, but in the presence of ryegrass (treatment B) or turnip (treatment C), the growth was reduced by the higher rate of the herbicide only. However, this was due to the reduction of fenugreek growth in the control treatment by the presence of the weeds. The effect of the herbicide

on weed control is shown in Table 6.6. The 0.2 and 0.4 mg/seed of the herbicide gave 50 and 75% (in terms of population) or >75 and >90% (in terms of dry weight) control of ryegrass, respectively. Turnip was slightly affected by the herbicide at 0.4 mg/seed. However, turnip plants around fenugreek were stunted and deformed. In general, the effect of the herbicide on both turnip and ryegrass was more pronounced around fenugreek plants indicating the higher concentration of the herbicide around the fenugreek. Assuming even distribution of the herbicide resulting from diffusion, which is unlikely to be the case, the herbicide field rate would have been about 0.4 (from 0.2 mg/seed) or 0.8 kg/ha (from 0.4 mg/seed). This rate is too low for effective weed control; the normal field rate is 2 - 4 kg/ha. However 0.4 mg/seed of Eradicane was too toxic for the crop. In the case of lucerne the seed rate allowed 6 kg/ha of EPTC from 0.2 mg per seed (Dawson, 1981). The fenugreek seed rate used in this experiment (200 plants/ m^2) was twice the normal field rate and still the spacing only allowed 0.4 kg/ha of the herbicide to be applied as a seed treatment and even this rate was toxic to fenugreek.

Dawson's method is unlikely to be of importance for large-seeded crops such as fenugreek. For small-seeded crops, the method may allow even distribution of the herbicide from a low concentration on the seed.

Table 6.5. Effect of Eradicane (EPTC + R25788) on fenugreek dry weight when applied to the seed

Treatments	Dry weight in g/treatment			
	A	B	C	Mean
Coated seed (no Eradicane)	0.93	0.83	0.8	0.85
Coated seed + 0.2 mg EPTC as Eradicane	0.63*	0.66	0.6	0.63
Coated seed + 0.4 mg EPTC as Eradicane	0.43*	0.36*	0.3*	0.36*
Untreated control	0.93	0.6	0.6	0.71
		LSD = 0.21		
Mean	0.73	0.61	0.58	LSD 0.12
		LSD = 0.11		

A = Fenugreek B = Fenugreek + Ryegrass C = Fenugreek + turnip

* Significantly less than the control.

Table 6.6 Effect of Eradicane (EPTC + R25788) on weed control when applied to fenugreek seed

Treatments	No. of plants		Dry weight in mg	
	Ryegrass	Turnip	Ryegrass	Turnip
Coated seed only	53.3	36	148.7	1040
Coated seed + 0.2 mg EPTC	21*	30	49.6*	827
Coated seed + 0.4 mg EPTC	13*	28.3	18.3*	563*
Untreated control	52.3	32.6	131.6	893
LSD at P = 0.05	15.4	4.8	17.7	114

*Significantly less than the control.

CHAPTER SEVEN

EFFECTS OF HERBICIDES ON NODULATION AND SEED QUALITY

CHAPTER 7

Effects of Herbicides on Nodulation and Seed Quality

1. Assessment of Nodulation

a) Methods

(i) Field Experiments

In the 1981 Field Experiment, the fenugreek failed to nodulate probably because of the soil acidity. This problem was solved in the 1982 Field Experiment by using lime to raise the pH of the soil to 7 (actually 7.4). Five plants were selected randomly from each plot and nodulation was scored visually on a 0 - 3 scale (Richardson, 1979), where 0 = no nodulation (0%), 1 = very few and ineffective nodulation (33%), 2 = moderate nodulation (66%) and 3 = effective nodulation , similar to the control (100%).

(ii) Pot Experiment

This experiment was conducted in the glasshouse to investigate further the effect of dinitroaniline herbicides on nodulation. Trifluralin at 2, 4 and 6 mg kg^{-1}, oryzalin at 0.5, 1 and 2 mg kg^{-1} and isopropalin at 4, 6 and 8 mg kg^{-1} were incorporated into Begbroke North soil. Spraying of the herbicides was done by an aerosol sprayer as described before. Pots were filled with the herbicide-treated soil and 5 fenugreek seeds (variety Margaret) were sown 2 cm deep, in each pot, using a dibber. Before sowing, the seeds were inoculated with

the strain of *Rhizobium* as described in the Field Experiment, 1982. Sowing was done on October 19, 1982. Plants were raised under the glasshouse conditions as described before.

After 8 weeks, plants were harvested, shoots were cut at the soil level and roots were washed free of soil particles and visually estimated for nodulation as already described. Observation was also made on the size, colour and distribution of nodules. Shoot and root dry weights were obtained.

b) Results and Discussion

Table 7.1 shows the effect of dinitroaniline herbicides on the nodulation of fenugreek (Field Experiment). Trifluralin at 3 kg/ha and oryzalin at 2.25 kg/ha adversely affected the nodulation of fenugreek. A slight effect on nodulation was also observed for the low rates of both these herbicides. Isopropalin had no apparent effect on the nodulation. As shown in Table 7.1, the reduction of nodulation by the dinitroaniline herbicides was associated with reduction in shoot dry weight. This was investigated further in the pot experiment.

Table 7.2 shows the effect of these herbicides on nodulation of fenugreek (Pot Experiment). Trifluralin at 4 and 6 mg kg^{-1} and oryzalin at 1 and 2 mg kg^{-1} reduced the nodulation of fenugreek. Isopropalin at all rates had no effect on the nodulation.

In general, nodulation of fenugreek is mainly on the primary

Table 7.1 Effect of dinitroaniline herbicides on nodulation of fenugreek: Field Experiment, 1982*

Treatments	Rate kg/ha	Nodulation as % of control	Shoot dry weight as % of control +
Trifluralin	1.0	95	87
"	2.0	85	70
"	3.0	68	75
Oryzalin	0.75	98	63
"	1.5	81	68
"	2.25	78	45
Isopropalin	1.5	92	62
"	3.0	93	92
"	4.5	92	89
Handweeded control	0.0	100	100

* Nitragin's strain of *Rhizobium*

+ From table 5.7.

Table 7.2. Effect of dinitroaniline herbicides on the growth and nodulation of fenugreek: Pot Experiment

Treatments	Rate mg kg^{-1}	Dry weight as % of control		Nodulation as % of control
		Shoot	Root	
Trifluralin	2.0	68.7*	95.3	83
"	4.0	49*	55.7*	33
"	6.0	41.3*	30.3*	25
Oryzalin	0.5	100	100	100
"	1.6	77.3	40.7*	66
"	2.0	62*	20*	33
Isopropalin	4.0	88	90.3	100
"	6.0	100	100	100
"	8.0	78.3	92.3	92
Untreated control	0.0	100	100	100
LSD (P = 0.05)		24.9	25	

* Significantly less than the control.

root and to a lesser extent on secondary roots, large and pink in colour (Hardman and Petropoulos, 1975). Isopropalin and control treatments followed this pattern.

In the case of oryzalin (1 and 2 mg kg^{-1}) the nodules were large and pink in colour and on the primary root only; secondary roots were very few, short and deformed.

In the case of trifluralin treatments (4 and 6 mg kg^{-1}), the nodules were mainly on the secondary roots, with few on the primary root which were small in size and white in colour. Those on the secondary roots were also small and some were white and others pink.

It appears that direct effects on the plant are the most important and that nodule number and quality were only affected where there were significant effects on shoot and root dry weights. This suggests nodule effects are most likely due to changes in photosynthesis and photosynthate supply to the root and exudation from the root.

The effect of trifluralin seems to be principally on the young plant in that while nodules on primary roots were markedly affected, nodulation on secondary roots appeared to be recovering.

For oryzalin, it appeared that there was some delay in the main effect since there was a small effect on the primary root nodules while the latter formed secondary roots were badly

affected and devoid of nodules.

In the pot experiment, 1 mg kg^{-1} of a herbicide is equivalent to a field rate of 1.5 - 2 kg/ha (depending on the depth of incorporation). At 2 mg kg^{-1} trifluralin, there was no or only slight effect on nodulation (equivalent to 3 - 4 kg/ha field rate). This suggests that trifluralin is unlikely to have any effect on nodulation in the field since the normal field rate is 0.86 - 1.72 kg/ha. Similarly oryzalin is likely to be safe up to 0.75 kg/ha, but since oryzalin showed less margin of selectivity than isopropalin, or trifluralin, damage to the crop and hence to the nodules from error of application is likely. Isopropalin is the safest herbicide and it showed an adequate margin of selectivity.

EPTC and the safeners (when applied together or separately), as shown in Table 7.3, had no effect on the nodulation of fenugreek.

2. Seed Quality

a) Methods (see Chapter 3)

b) Results and Discussion

(i) Field Experiment, 1981:

Table 7.4 shows the effects of pre-emergence herbicides on protein content of fenugreek seed. There was no effect by these herbicides on protein content of varieties Barbara and

Table 7.3. Effect of EPTC with or without safeners on the nodulation of fenugreek

Treatments	Nodulation as % of control
EPTC at 2 kg/ha	100
" + R25788 0.5% w/w ST*	100
" + R25788 1.0% w/w ST	100
" + R25788 2.0% w/w ST	100
" + R25788 2 kg/ha TM+	100
" + R25788 4 kg/ha TM	92
" + MON4606 0.25% w/w ST	100
" +M32988 0.5% w/w ST	100
EPTC at 4 kg/ha	92
" + R25788 0.5% w/w ST	100
" + R25788 1.0% w/w ST	92
" + R25788 2.0% w/w ST	100
" + R25788 2 kg/ha TM	100
" + R25788 4 kg/ha TM	92
" + MON4606 0.25% w/w ST	92
" + M32988 0.5% w/w ST	92
R25788 0.5% w/w SD	100
" 1.0% w/w SD	100
" 2.0% w/w SD	100
" 2 kg/ha TM	100
" 4 kg/ha TM	100
MON4606 0.25% w/w SD	100
M32988 0.5% w/w SD	92
Untreated control	100

* ST = Seed Treatment

+ TM = Tank mix

Table 7.4 Effect of pre-emergence herbicides on the yield of protein from the seed of fenugreek: Field Experiment, 1981.

Treatments	Rate kg/ha	% of protein in the seed		
		Paul	Barbara	Margaret
Trifluralin	1.0	21.1	20.4	23.8
"	3.0	21.0	22.6	20.4
Metamitron	3.0	20.5	21.2	22.6
"	9.0	21.5	24.1	20.6
Chlorthal-dimethyl- + Methazole	4.5	18.8*	19.8	21.7
"	13.5	17.8*	22.8	20.8
Methazole	1.5	20.3	22.5	20.9
"	4.5	20.3	21.9	22.9
Handweeded control	-	21.7	21.3	21.3
LSD at (P = 0.05)	-	1.5	2.6	1.1

*Significantly less than the control.

Margaret. Chlorthal-dimethyl plus methazole reduced the protein content of variety Paul. The protein content for all varieties was lower than that of the seed sown (35% for Barbara, 32% for Margaret and 29% for Paul), and this was probably due to the failure of the fenugreek to nodulate because of soil acidity (pH was 5.8).

As shown in Table 7.5, none of the herbicides affected diosgenin yield. The diosgenin yield of the fenugreek seed used is usually 1.2 - 1.5%.Compared with this value, the diosgenin yields shown in Table 7.5 arequite high. It appears that there is a negative association between protein content and diosgenin yield, when the plants have failed to nodulate.

In general, Paul is the first to ripen, followed by Margaret, and then Barbara. The presence of weeds delayed the ripening of the seed. The crop was harvested and stored at air temperature, under cover, to accelerate ripening. This resulted in fungal attack with variety Paul the most affected and variety Barbara was the least affected.

(ii) Field Experiment, 1982

As shown in Table 7.6, all treatments,with the exception of isopropalin at 4.5 kg/ha, reduced the protein content of fenugreek seed (variety Margaret). Two main factors might be responsible for this reduction. The first is the reduction of nodule number and quality as a result of the

Table 7.5. Effect of pre-emergence herbicides on the yield of diosgenin from the seed of fenugreek: Field Experiment, 1981. (Low pH and no nodulation).

Treatments	Rate kg/ha	% of diosgenin in the seed* Paul	Barbara	Margaret
Trifluralin	1.0	1.7*	1.74*	1.84*
"	3.0	1.66*	1.67*	1.76*
Metamitron	3.0	1.77*	1.76*	1.81*
"	9.0	1.77*	1.63*	1.64*
Chlorthal-dimethyl + Methazole	4.5	1.78*	1.71*	1.82*
"	13.5	1.77*	1.75*	1.82*
Methazole	1.5	1.82*	1.58*	1.84*
"	4.5	1.7*	1.61*	1.81*
Handweeded control	-	1.84	1.68	1.87
LSD (P = 0.05)	-	0.176	0.146	0.188

* No significance differences between treatments.

phytotoxic action of the herbicides on the crop (Table 7.1). The second factor is the weed competition for major elements including N, and hence reduction in the shoot and/or root growth of the crop. Both factors are likely to be involved in the reduction of protein content by trifluralin and oryzalin.

In the case of isopropalin, the reduction in protein at the two lower rates of application is likely to be due to the weed competition only since this herbicide had no phytotoxic action on fenugreek and it was less effective against weeds than the other two herbicides. This view is supported by the result with this herbicide at the highest rate when better weed control was obtained and this resulted in high protein content.

In general, the percentage of protein in the seed was high and this was mainly due to effective nodulation in soil at pH 7.4 compared with that in the 1981 Field Experiment (pH of the soil was 5.8).

Regarding the effect of these herbicides on diosgenin yield (Table 7.6), there were no significant differences between all treatments. However, desiccation of the control plants resulted in an increase in diosgenin yield. This might be due to accumulation of diosgenin in the seed resulting from the

Table 7.6. Effect of dinitroaniline herbicides on protein and diosgenin contents of the seed of fenugreek: Field Experiment, 1982 (variety Margaret).

Treatments	Rate kg/ha	% Protein	% Diosgenin+	
			Non-desiccated	Desiccated
Trifluralin	1.0	27.8*	1.34	1.28
"	2.0	27.2*	1.40	1.43
"	3.0	27.7*	1.38	1.47
Oryzalin	0.75	29.5*	1.32	1.40
"	1.50	29.4*	1.32	1.34
"	2.25	29.6*	1.39	1.37
Isopropalin	1.5	29.6*	1.24	1.36
"	3.0	28.5*	1.28	1.39
"	4.5	31.5	1.35	1.37
Handweeded control	-	32.7	1.18	1.35
LSD (P = 0.05)	-	1.82		

+ No significant differences between treatments

* Significantly less than the control

sudden changes in the metabolism of the green parts as the control plants were quite green at the time of application of the desiccant.

Early sowing, a good summer and the application of the desiccant resulted in good ripening of the seed and hence no or slight fungal attack in the desiccated or undesiccated plants, respectively.

(iii) Pot Experiment: EPTC with Safeners

As shown in Table 7.7, neither EPTC nor the three safeners alone and with EPTC had a significant effect on seed protein content of fenugreek (variety Barbara). EPTC alone and the three safeners alone did not significantly reduce the yield of diosgenin from the seed (Table 7.7). However, significant reduction occurred with EPTC at 2 kg/ha, when used with each of the safeners R25788 (at 2% w/w, seed treatment and 2 kg/ha, tank mix) and MON4606 (at 0.25%, seed treatment). With 4 kg/ha EPTC reduction in diosgenin yield occurred only with the safener R25788 (at 2% w/w, seed treatment). Safener M32988 alone and with EPTC did not affect protein content nor diosgenin yield but M32988 (at 0.5% w/w, seed treatment) failed to protect the fenugreek against EPTC at 4 kg/ha (see Table 6.3, p. 132).

There is no report in the literature on the effect of EPTC with safeners on diosgenin yield. The need to take account of the effect of herbicide treatments on the yield of a commercially important constituent, such as diosgenin is thus stressed.

Table 7.7 Effect of EPTC with or without safeners on protein and diosgenin contents of the seed of fenugreek variety Barbara: Pot Experiment.

Treatments	% Protein +	% Diosgenin
EPTC at 2 kg/ha	29.6	1.13
" + R25788 at 0.5% w/w ST	31.5	1.34
" +R25788 at 1.0% w/w ST	32.6	1.29
" + R25788 at 2.0% w/w ST	29.5	1.01*
" + R25788 at 2 kg/ha TM	32.2	1.01*
" + R25788 at 4 kg/ha TM	29.8	1.25
" + MON4606 at 0.25% w/w ST	30.9	1.09*
" + M32988 at 0.5% w/w ST	31.4	1.21
EPTC at 4 kg/ha	32.0	1.21
" + R25788 at 0.5% w/w ST	35.7	1.34
" + R25788 at 1.0% w/w ST	34.8	1.18
" + R25788 at 2.0% w/w ST	32.2	0.98*
" + R25788 at 2 kg/ha TM	30.8	1.14
" + R25788 at 4 kg/ha TM	29.7	1.14
" + MON4606 at 0.25% w/w ST	30.7	1.14
" + M32988 at 0.5% w/w ST	30.3	1.17
R25788 at 0.5% w/w ST	32.6	1.20
" 1.0% w/w ST	30.3	1.23
" 2.0% w/w ST	33.5	1.13
" 2 kg/ha TM	30.0	1.23
" 4 kg/ha TM	34.1	1.22
MON4606 at 0.25% w/w ST	34.5	1.30
M32988 at 0.5% w/w ST	35.5	1.19
Untreated control	32.6	1.26
LSD at P = 0.05	5.23	0.147

+ No significant differences between treatments
ST = Seed treatment
TM = Tank mix
* Significantly less than the control.

CHAPTER EIGHT

SUMMARY AND CONCLUSIONS

Chapter 8

Summary and Conclusions

Fenugreek tolerated metamitron; "Delozin-S", i.e. chlorthal-dimethyl plus methazole; and trifluralin as pre-emergence treatments, both in the glasshouse and in the field. In the field, they selectively controlled a broad spectrum of weeds including grasses and broadleaved weeds. However, "Delozin-S" was inactive on *Fumaria officinalis* and trifluralin was inactive on *Capsella bursa-pastoris*. Methazole (alone) controlled all these weeds, thinned fenugreek stand in the field but the yield was unaffected. However, there is some doubt about the future availability of methazole and therefore of "Delozin-S" also. Metamitron and "Delozin-S", which proved safe in fenugreek and gave good weed control, are rather expensive:

	Price per ha	
	October 1981	June 1983
Trifluralin	£11.80	£8.75
Metamitron	£77.70	£70.00
"Delozin-S"	£52.90	No longer available

Post-emergence herbicides which were safe to fenugreek in the glasshouse and in the field, included metamitron; MCPB; and bentazone plus MCPB. The slow establishment of fenugreek makes the use of post-emergence herbicides alone unsuitable. Fenugreek tolerated such herbicides at the 3 - 5 trifoliate leaf stage, but by that time the weeds would normally have already become too vigorous to be controlled. Bentazone, and

bentazone plus MCPB proved very effective against broadleaved weeds. Bentazone on its own would need a higher rate which reduces the margin of selectivity towards fenugreek and so it is unlikely to be useful. Metamitron is expensive. Bentazone plus MCPB, and MCPB, as post-emergence treatments, are likely to be of use as supplements to pre-emergence herbicides to control resistant weeds and/or late emerging weeds.

Trifluralin, as a pre-planting soil incorporated treatment gave satisfactory results. Further work was carried out with other dinitroaniline herbicides. In the glasshouse fenugreek showed good tolerance to a number of them, namely: trifluralin and benfluralin (trifluoro analogues), isopropalin and butralin (alkyl analogues) and to a lesser extent oryzalin (sulfonyl analogue). Dinitramine (a trifluoro 2,5-dinitroaniline) was very toxic to fenugreek. Trifluralin, isopropalin and oryzalin were selected for further evaluation and oryzalin proved to be the most toxic to fenugreek and isopropalin the least toxic.

Results from absorption experiments indicate that dinitroaniline herbicides are absorbed by both shoot and root of fenugreek, but root absorption appears to be more effective than shoot absorption.

In general, trifluralin, oryzalin and isopropalin were highly adsorbed by organic soil and to a lesser extent by clay and sandy soils. Soil organic matter content is the most important soil component to govern the activity of these

herbicides. Trifluralin was the most affected by soil organic matter content whereas isopropalin was the least affected. For the appropriate application rate of these herbicides, soil organic matter content must be taken into account; 4 to 6 times the normal field rate is required for effective weed control by these herbicides in soils containing about 18% organic matter compared with soils containing 2 - 4% organic matter.

The tolerance of fenugreek to trifluralin, oryzalin and isopropalin under field conditions followed the same pattern as in the pot experiments, where oryzalin was the most toxic and isopropalin was the least. Both isopropalin and trifluralin showed adequate safety margins towards fenugreek. However, these herbicides were inactive against *Capsella bursa-pastoris* and *Matricaria* spp. Results from pot experiments indicated that trifluralin, oryzalin and isopropalin can selectively control *Stellaria media*, *Polygonum aviculare* and *Lolium* spp., but turnip, which had been included to represent cruciferous weeds, *Capsella bursa-pastoris* and *Matricaria* spp., proved very resistant to these herbicides.

The trifluoro and alkyl analogues of the dinitroaniline herbicides, would be expected to be safe to fenugreek and to control most annual grasses and some broadleaved weeds. Oryzalin (sulfonyl analogue) showed less safety margin of selectivity towards fenugreek than isopropalin or trifluralin; other sulfonyl analogues are most likely to have similar activity.

EPTC is very effective against a wide range of broadleaved weeds and grasses including *Capsella bursa-pastoris* and *Matricaria* spp. However, it is only marginally tolerated by fenugreek. Safeners were used to improve the selectivity of this potential herbicide to fenugreek. NA (naphthalene-1,8-dicarboxylic anhydride) and M32988 (2,2-dichloro-N-(3-methyl-4-thiazoline-2-ylidene) acetamide) failed to protect fenugreek from the injury caused by 4 kg/ha EPTC, and NA was toxic to the crop. The results with MON4606 (benzyl-2-chloro-4-(trifluoromethyl)-thiazole-5-carboxylate) and EPTC at 4 - 10 kg/ha indicated that the efficiency of this compound for safening fenugreek from EPTC injury is rate dependent. The recommended rate of use of this safener (MON4606) is 0.125 - 0.25% w/w of the seed. At 0.25% w/w, MON4606 afforded significant protection for fenugreek from 4 and 5 kg/ha EPTC, but at the same rate of the safener and only 2 kg/ha EPTC, the growth of fenugreek was retarded. Using MON4606 at 0.0625 - 0.125% w/w is likely to give better protection for fenugreek from EPTC injury caused by the lower rate of the herbicide. These results justify further work with MON4606 and EPTC.

Although higher rates of EPTC did damage fenugreek treated with R25788 (N,N-diallyl-2,2-dichloroacetamide) in the pot experiments, it is likely that in the field R25788 would allow fenugreek to tolerate rates up to 4 kg/ha EPTC, which is an adequate rate for effective weed control.

"Eradicane", which is EPTC + R25788 in a combined formulation, was used for studying the effectiveness of the herbicides on weed control when applied to the crop seed. The method was

very effective when EPTC was applied to lucerne seed (Dawson, 1980, 1981). Because this seed is small and the seed rate high, the method gave an even distribution of the herbicide in the soil from a very low concentration on the seed. In the case of the larger seeded fenugreek and other row crops, a high concentration of the herbicide on the seed is needed to provide sufficient herbicide for weed control. However, a high rate is likely to damage the crop as proved the case when Eradicane was used as a seed dressing for fenugreek. For Dawson's method to be effective, high tolerance of the crop to a specific herbicide is necessary as well as high seed rate to ensure even distribution of the herbicide.

The effects of all the herbicides used in this work on nodulation of fenugreek have been examined. In general, nodule numbers and quality were only affected when there was a significant effect on shoot or root growth. This was particularly true in the case of the dinitroaniline herbicides, where trifluralin and oryzalin reduced nodule number and quality as a result of their effects on the growth of fenugreek. Richardson (1979) reported that nodule effects correspond to effects on other plant parts. Similarly Naryana and Jain (1978) found that post-emergence applications of alachlor to fenugreek reduced growth and nodule numbers. Nutman (1948) has shown that nodule number and extent of lateral root growth are positively correlated in red clover. Since the dinitro-anilines inhibit lateral root development, the reduction in the nodule numbers and quality by trifluralin and oryzalin

appeared to be as a result of reduced growth of fenugreek. However, the reduction of the growth and nodule numbers of fenugreek by trifluralin and oryzalin was caused by 4 and 1 mg kg^{-1}, respectively. The dose 1 mg kg^{-1} is equivalent to 1.5 - 2.0 kg/ha. The normal field rates of these herbicides are 0.86 - 1.72 kg/ha. So trifluralin is unlikely to have such effects, but oryzalin would be expected to reduce the growth and nodulation of fenugreek. Isopropalin showed the best safety margin but the poorest weed control.

Trifluralin, oryzalin and isopropalin reduced protein content of the seed of fenugreek. However, the reduction was associated with the reduction of the growth and nodulation of fenugreek caused by weed competition and/or elevated rates of the herbicides. A negative correlation was observed between abnormally low protein content and disogenin yield when no nodulation occurred at low pH of soil.

Using diquat as a desiccant, fungal attack which is usually associated with late ripening in the U.K., was effectively reduced. Seeds from desiccated plants yielded more diosgenin than that from non-desiccated ones.

Trifluralin and isopropalin, incorporated with the soil before sowing, were safe to fenugreek. These two herbicides may be expected to control most annual grasses and some broad-leaved weeds. A post-emergence herbicide, for example MCPB or bentazone plus MCPB, may be needed where there are resistant

weeds. With the safener R25788 (as a seed treatment), EPTC is likely to give effective weed control without any significant effect on fenugreek. Further work with safener MON4606 is needed to establish the effectiveness of this safener in reducing EPTC injury to fenugreek. In the case of land heavily infested with weeds, the pre-emergence herbicide metamitron, though expensive, is the herbicide of choice.

REFERENCES

REFERENCES

Anderson, J.R. (1978).

Pesticide effects on non-target soil micro-organisms. In Pesticide Microbiology (I.R. Hill and S.J.L. Wright, Eds.), pp. 313-534, Academic Press, London.

Ashton, F.M. and A.S. Crafts (1981).

Mode of Action of Herbicides, 2nd Edition, WIley Inter-Science, New York, 525 pp.

Baltazar, A.M. and S. Brotonegro (1979).

Effect of trifluralin, glyphosate and U-44,078 on nodulation and nitrogen fixation of soybean *(Glycine max.* L.). *Philipp, J. Weed Sci. 6,* 69-80.

Barrentine, W.L. and G.F. Warren (1971a).

Differential phytotoxicity of trifluralin and nitralin. *Weed Sci. 19,* (1), 31-37.

Barrentine, W.L. and G.F. Warren (1971b).

Shoot zone activity of trifluralin and nitralin. *Weed Sci. 19,* (1), 37-41.

Blair, A.M., C. Parker and L. Kassasian (1976).

Herbicide protectants and antidotes. A review. *PANS 22,* 65-74.

Blair, A.M. (1979).

The interaction of protectants with EPTC on field bean and tri-allate on wheat. *Ann. Applied Biol. 92* (1), 105-111.

Boger, P., B. Beese and R. Miller (1977).

Long term effects of herbicides on the photosynthetic apparatus. II. Investigation on bentazone inhibition. *Weed Res. 17* (1), 61-67.

Bovey, R.W. (1980).
Physiological effects of phenoxy herbicides in higher plants, 217-238. In
The Science of 2,4,5-T and Associated Phenoxy Herbicides (R.W. Bovey and A. Young, Eds.), John Wiley and Sons, 462 pp.

Briggs, G.G., R.H. Bromilow and A.A. Evans (1982).
Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley.
Pestic. Sci. 13, (5), 495-504.

Brinker, R., D. Schafer and R. Radke (1982).
The effectiveness of MON4606 as seed safener against alachlor and acetochlor in grain sorghum.
Proc. 1982 British Crop Protection Conference - Weeds Vol. 2, 469-473.

Brock, J.L. (1972).
Effect of the herbicides trifluralin and carbetamide on nodulation growth of legume seedlings.
Weed Res. 12, 155-163.

Burt, G.W. (1976a).
Factors affecting thiocarbamate injury to corn. I. Temperature and Soil Moisture.
Weed Sci. 24, (3), 319-321.

Burt, G.W. (1976b).
Factors affecting thiocarbamate injury to corn. II. Soil incorporation, seed placement, cultivars, leaching and breakdown.
Weed Sci. 24, (3), 327-330.

Byast, T.H., E.G. Cotterill and R.J. Hance (1977).

Methods of analysis for herbicide residues.

Techn. Report, ARC Weed Res. Organ. (2nd edition), 15, pp.73.

Calvet, R. (1980).

Adsorption-desorption phenomena. In Interactions between Herbicides and the Soil (R.J. Hance, ed.).

pp. 1-30, Academic Press, London.

Chang, F.Y., J.D. Bandeen and G.R. Stephenson (1972).

A selective antidote for prevention of EPTC injury in corn.

Can. J. Plant Sci. 52, 707-714.

Chang, F.Y., G.R. Stephenson and J.D. Bandeen (1973).

Comparative effects of three EPTC antidotes.

Weed Sci. 21, (4), 292-295.

Chang, F.Y., G.R. Stephenson, G.W. Anderson and J.D. Bandeen (1974).

Control of wild oats in oats with barban plus antidote.

Weed Sci. 22, (6), 546-548.

Chassin, P., R. Calvet and M. Terace (1981).

Adsorption de l'atrazine et due chlortoluron par les acides humiques. Proc. EWRS Symp. Theory and Practice of the Use of Soil Applied Herbicides, 1981, 10-17.

Chebotar, N.I. (1979).

The influence of herbicides on the interaction between nodule bacteria and soybean plants in the northern zone of the Moldavian, S.S.R. Byulleten Vsesoyuznogo Nauchnoissled-ouatel'skogo Instituta Sel'skokhzyais-tvennor Mikrobiologii 32, 99-100.

Coppen, J.J.W. (1979).

Steroids: From plants to pills - the changing picture.

Trop. Sci. 21, 125-141.

Dawson, J.H. (1979).

EPTC applied to crop seeds for selective weed control.

Abstracts of 1979 Meeting of the *Weed Sci. Soc. America,* 31-32.

Dawson, J.H. (1980).

Herbicide-coated seed looks good.

Furrow 85, (3), 17.

Dawson, J.H. (1981).

Selective weed control with EPTC-treated seed of alfalfa *(Medicago sativa).*

Weed Sci. 29, (1), 105-110.

Ellis, J.F. and J.A. Norton (1976).

Factors affecting the biological activity of dinitro-aniline herbicides.

Supplement to the Proceedings of the North East Weed Sci. Soc. 52-73.

Ezra, G., H.M. Flowers and J. Gressel (1982).

Rapid multilevel interactions of a thiocarbamate herbicide protectants at the level of uptake into maize cells in culture.

Pestic.Bioch.Physio.17,(1) 48-58

Fawcett, R.S. and R.G. Harvey (1978).

Field comparison of seven dinitroaniline herbicides for alfalfa *(Medicago sativa)* seedling establishment.

Weed Sci. 26 (2), 123-127.

Fedtke, C. (1982).

Biochemistry and Physiology of Herbicide Action, Eds. Springer-Verlag, Berlin (1982), 202 pp.

Feeny, R.W. (1966).

Effect of trifluralin on the growth of oat seedlings, and respiration of excised oat root.

Proceedings North East Weed Control Conference 20, 595-603.

Fletcher, W.W. (1956).

Effect of hormone herbicides on the growth of *Rhizobium trifolii*.

Nature 177, 1244.

Frear, D.S. (1976).

The benzoic acid herbicides. In, Herbicides Chemistry, Degradation and Mode of Action (P.C. Kearney and D.D. Kaufman, Eds.) pp. 541-608 (Vol. 2), Marcel Dekker Inc., New York.

Gad, A.M. and M.A. El-Mahadi (1972a).

Effect of nitralin on two annual grassy weeds and some vegetable, field and forage crops.

Desert Inst. Bull., Egypt, 22 (2), 393-405 .

Gad, A.M. and M.A. El-Mahadi (1972b).

Effect of the local herbicide, M15, and its residue on darnel and some vegetable and field crops.

Desert Inst. Bull., Egypt 22 (2), 407-419.

Garcia, M.N. and D.C. Jordan (1969).

Action of 2,4-DB and dalapon on the symbiotic properties of *Lotus corniculatus* (Birdsfoot trefoil).

Plant and Soil. 30, 317-334.

Gentner, W.A. (1966).

Herbicidal properties of trifluralin analogues.

Weeds 14, 176-178.

Ghosal, S., R.S. Srivastava, D.C. Chatterjee and S.K. Dutta (1974).

Fenugreekine, a new steroidal sapogenin-peptide of *Trigonella foenumgraecum*.

Phytochemistry 13, 2247-2251.

Giardini, A.R., E.S. Lopes and R. Deuber (1979).

Influence of herbicides on nodulation of soybean *(Glycine max.* L.)

Planta Daninha 2, (1), 21-32.

Greaves, M.P., L.A. Lochart and W.G. Richardson, (1978).

Measurement of herbicides effects on nitrogen fixation by legumes.

Proceedings British Crop Protection Conference - Weeds, Vol. 2, 581-585.

Greaves, M.P., N.J. Poole, K.H. Domsch, G. Jagnow and W. Verstraete (1980).

Recommended tests for assessing the side effects of pesticides on the soil microflora.

Tech. Rep., ARC/Weed Res. Organ., 59, 15 pp.

Grover, R. (1974).

Adsorption and desorption of trifluralin, tri-allate and di-allate by various adsorbents.

Weed Sci. 22, 405-408.

Hack, H. and R.R. Schmidt (1976).

Use of metamitron in weed control systems in sugar beet

Proceedings 1976 British Crop Protection Conference Weeds
Vol. 1, 197-204.

Hacskaylo, J. and V.A. Amato (1968).
Effect of trifluralin on roots of corn and cotton.
Weed Sci. 16, 513-515.

Hamaker, J.W. and J.M. Thompson (1972).
Adsorption. In
Organic Chemicals in the Soil Environment (C.A.I. Goring
and J.W. Hamaker, Eds.), pp. 49-143, Dekker, New York.

Hamdoun, A.M. and K.B. El-Tigani (1977).
Weed control problems in The Sudan.
PANS 23, (2), 190-194.

Hance, R.J., S.D. Hocombe and J. Holroyd (1968).
The phytotoxicity of some herbicides in field and pot
experiments in relation to soil properties.
Weed Res. 8, 136-144.

Hance, R.J. (1976).
Some interactions between pesticides and the soil.
Sintesi dei seminari tenuti presso il Laboratorio
dal 30 Novembre al 3 Dicembre 1976.

Hance, R.J. (1980).
Herbicides and the soil.
Chemistry in Britain 16, (3), 128-156.

Hardman, R. (1969).
Pharmaceutical products from plant steroids.
Trop. Sci. 11, 196-228.

Hardman, R. (1974).
Steroid plants in the production of contraceptives.
J. Mond. Pharm. 17, (1), 60-80.

Hardman, R. and G.A Petropoulos (1975).

The response of *Trigonella foenum-graecum* (Fenugreek) to field inoculation with *Rhizobium meliloti,* 2012.

Planta Medica 27, (1), 53-57.

Hardman, R., J. Kosugi and R.T. Parfitt (1980).

Isolation and characterization of a furostanol glycoside from fenugreek.

Phytochemistry 19, 698-700.

Hardman, R. (1982).

From Mexican yam to the development of sex steroids.

MIMS Magazine 42, 43, 46 and 47.

Harrison, G.W., J. B. Weber and J.V. Baird (1976).

Herbicides phytotoxicity as affected by selected properties of North Carolina soils.

Weed Sci. 24, (1), 120-125.

Harrison, G.W. and J.B. Weber (1975).

Comparative phytotoxicity of five herbicides in ten North Carolina soils.

Proceedings South Weed Sci Soc., 28, 283-291.

Hartley, G.S. and I.J. Graham-Bryce (1980).

Physical Principals of Pesticides Behaviour, Eds.

Vol. (1), pp. 11-110, Academic Press, London.

Harvey, R.G. (1973a).

Field comparison of twelve dinitroaniline herbicides.

Weed Sci. 21, 512-516.

Harvey, R.G. (1973b).

Relative phytotoxicity of dinitroaniline herbicides.

Weed Sci. 21, 517-521.

Harvey, R.G. and G.L. Jacques (1977).

Dinitroaniline herbicides for weed control in peas.

Weed Sci. 25, (3), 256-259.

Hassan, H.I. (1976).

The Sudan. In

World Atlas of Agriculture (pp 624-639) Vol. IV.

Africa, I.G.D.A. Italy, 761 pp.

Hays, R.M. and L.M. Wax (1975).

Differential intraspecific responses of soybean cultivars

to bentazone.

Weed Sci. 23, (6), 516-521.

Helling, C.S. (1976a).

Dinitroaniline herbicides in soils.

J. Envir. Quality 5, (1), 1-15.

Helling, C.S. (1976b).

Chemical and physical properties of the dinitroaniline

herbicides.

Supplement of the Proceedings North

East Weed Sci. Soc., 44-51.

Hilton, J.L. and M.N. Christiansen (1972).

Lipid contribution to selective action of trifluralin.

Weed Sci. 20, 290-294.

Hoffman, O.L. (1962).

Chemical seed treatments as herbicide antidotes.

Weeds 10, 322-323.

Hoffman, O.L. (1969).

Chemical antidotes for EPTC on corn.

Weed Sci. Soc. America. Abstract 12.

Holly, K. (1976).

Selectivity in relation to formulation and application methods. In,

Herbicides: Physiology, Biochemistry, Ecology. Vol. 2, (L.J. Audus, Ed.), pp. 249-277, Academid Press, London and New York.

Jacques, G.L. and R.G. Harvey (1979a).

Vapour absorption and translocation of dinitroaniline herbicides in oat *(Avena sativa)* and peas *(Pisum sativum)*.

Weed Sci. 27, (4), 371-374.

Jacques, G.L. and R.G. Harvey (1979b).

Dinitroaniline herbicides phytotoxicity as influenced by soil moisture and herbicide vaporization.

Weed Sci. 27, (5), 536-539.

Jordan, T.N., R.S. Baker and W.L. Barrentine (1978).

Comparative toxicity of several dinitroaniline herbicides.

Weed Sci. 26, (1), 72-75.

Jordan, D.C. and M.M. Garcia (1969).

Interaction between 2,4-DB and the root nodule bacteria of *Lotus corniculatus*.

Plant and Soil 30, (3), 360-372.

Keeler, R.F., S. Young and D. Brown (1976).

Spina bifida exencephly and cranial bleb produced in hamsters by the solanum alkaloid solasodine.

Chem. Patho. Pharmacol. 13, (4), 723-730.

King, J .M. and C.M. Knott (1979).

Weed control in peas (pp. 14-18).

U.K. Processors' and Growers' Research Organization, Ann. Report 1978 (1979), 41pp.

Knake, E.L., A.P. Appleby and W.R. Furtick (1967).
Soil incorporated and site of uptake of pre-emergence herbicides.
Weeds 15, 228-232.

Kumar, S., S.K. Pahwa, K. Promila and H.R. Shrma (1981).
Effect of simazine and prometryne on the growth and nodulation of Chick pea *(Cicer arietinum*, L.)
J. Agric. Sci. 97, (3), 663-668.

Lay, M.M. and J.E. Casida (1976).
Dichloroacetamide antidotes enhance thiocarbamate sulfoxide detoxification by elevating corn root glutathione content and glutathione-S-transferase activity.
Pest. Biochem. Phys . 6, 442-456.

Lay, M.M. and J.E. Casida (1978).
Involvement of glutathione and glutathione-S-transferase in the action of dichloroacetamide antidotes for thiocarbamate herbicides. In,
Chemistry and Action of Herbicide Antidotes.
(Pallos, F.M. and J.E. Casida, Eds.), pp. 151-160.
Academic Press, New York, 171 pp.

Massariol, A.A. and A. Lam-Sanchez (1974).
Effect of 5 herbicides on nodulation, weed control and yield of soybean.
Cientifica 1, (1), 18-23.

Mine, A. and S. Matsunaka (1975).
Mode of action of bentazone: Effect on photosynthesis.
Pest. Biochem. Physio. 5, (5), 444-450.

Morris, D.B., P.A. Birch and P.W. Rose (1976).

The development of metamitron, a highly selective and versatile herbicide, for use on sugar beet in the U.K. Proceedings 1976 British Crop Protection Conference - Weeds, Vol. 1, 189-196.

Morris, D.B., P.A. Birch and N.K. Rowley (1978).
Sugar beet weed control programmes based upon metamitron. Proceedings 1978 British Crop Protection Conference - Weeds Vol. 1, 277-284.

Moyer, J.R. (1979).
Soil organic matter, moisture and temperature: Effects on wild oat control with trifluralin.
Cana. J. Plant Sci. 59, 763-768.

Murray,D.S., P.W. Santelman and H.A.L. Greer (1973).
Differential phytotoxicity of several dinitroaniline herbicides.
Agrono. J. 65, 34-36.

Narayana, H.S. and P.S. Jain (1978).
Effect of post-emergence application of Lasso and nitrofen on growth and nodulation in *Trigonella foenum graecum,* L.
Geobios 5, (5), 193-196.

Nayar, S.L. (1955).
Vegetable insecticides.
Bull. National Inst. Sci. (India) 4, 137-145.

Oliver, L.R. and R.E. Frans (1968).
Inhibition of cotton and soybean roots from incorporated trifluralin and persistance in soil.
Weed Sci. 16, 197-203.

Pallos, F.M. and J.E. Casida (1978).

Chemistry and Action of Herbicide Antidotes. Academic Press, New York, London, 171 pp.

Paraka, S.J. (1976).

Mode of action of the dinitroaniline herbicides. Supplement of the Proceedings *North East Weed Sci. Soc.* 42-43.

Parka, S.J. and O.F. Soper (1977).

The physiology and Mode of action of the dinitroaniline herbicides. *Weed Sci. 25,* (1), 79-87.

Parker, C. (1966).

The importance of shoot entry in the action of herbicides applied to the soil. *Weeds 14*, 117-121.

Parker, C. and M.L. Dean (1976).

Control of wild rice in rice. *Pestic. Sci. 7,* 403-416.

Parker, C. (1982).

Further studies with herbicide safeners on rice and maize. Proceedings 1982 British Crop Protection Conference - Weeds Vol. 2, 475-482.

Parker, C. (1983).

Herbicide antidotes: A review. *Pestic. Sci. 14,* (1), 40-48.

Paromenskaya, L.N., V.L. Samoshkin and N.Z. Tolkachev (1979).

The influence of treflan (trifluralin) on the soybean - nodular bacteria symbiosis. Byulleten' Vsesoyuznogo Nauchnoissledouatel's kogo

Instituta Sel'skokhzyaistvennor Mikrobiologii 32, 97-98.

Peter , E.J. and M. Benzbiba (1979).
Effects of herbicides on nitrogen fixation of alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*).
Weed Sci. 27, (1), 18-21.

Petropoulos, G.A. (1973).
Agronomic, Genetic and Chemical Studies of *Trigonella foenum graecum* L.
PhD Thesis, (1973), University of Bath.

Pritchard, M.K. and E.M. Stobbe (1980).
Persistence and phytotoxicity of dinitroaniline herbicides in Manitoba soils.
Cana. J. Plant Sci. 60, (1), 5-11.

Rahman, A . (1976).
Effect of soil organic matter on the phytotoxicity of soil applied herbicides (Glasshouse Studies).
New Zealand J. Exp. Agric. 4, (1), 85-88.

Rahman, A., C.B. Dyson and B. Burney (1978).
Effect of soil organic matter on the phytotoxicity of soil applied herbicides (Field Studies).
New Zealand J. Exp. Agric. 6, (1), 69-75.

Retzlaff, G. and R. Hamm (1976).
The relationship between CO_2 assimilation and the metabolism in wheat plants .
Weed Res. 16, (4), 263-266.

Richardson, W.G. (1979).
The tolerance of fenugreek (*Trigonella foenum graecum* L.)

to various herbicides.

Techn. Report, ARC, Weed Res. Organ. 58, pp. 31.

Richardson, W.G., M.L. Dean and C. Parker (1976).

The activity and pre-emergence selectivity of some recently developed herbicides: metamitron, HOE 22870, HOE 23408, RH 2915, RP 20630.

Techn. Report. ARC, Weed Res. Organ. 38 pp.

Richardson, W.G. and C. Parker (1978).

The activity and post-emergence selectivity of some recently developed herbicides: NP 48, RH 5205 and pyridate.

Techn. Report, ARC, Weed Res. Organ. 48, pp. 38.

Schmidt, R.R., W. Draber, L. Eve and H. Timmler (1975).

Herbicidal activity and selectivity of new 3-alkyl-4-amino-6-aryl-1,2,4-triazine-5-one.

Pestic. Sci. 6, 239-244.

Schmidt, R.R. and C. Fedtke (1977).

Metamitron activity in tolerant and susceptible plants.

Pestic. Sci. 8, (6), 611-617.

Scott, H.D. and R.E. Phillips (1972).

Diffusion of selected herbicides in soils.

Soil Sci. Soc. Amer. Proc. 36, 714-719.

Sieck, R.F., W.S. Johnson, A.F. Cockerill, D.N.B. Mallen, D.J. Osborne and S.J. Barton (1976).

Gas chromatographic analysis of oryzalin residues in agricultural crops and soil.

J. Agric. Food Chem. 24, (3), 617-620.

Standifer, L.C. Jr. and C.H. Thomas (1965).

Response of johnsongrass to soil incorporated herbicides.

Weed Sci. 13, 302-306.

Stephenson, G.R. and F.Y. Chang (1978).
Comparative activity and selectivity of herbicide antidotes. In,
Chemistry and Action of Herbicide Antidotes (Pallos, F.M. and J.E. Casida, Eds.), pp. 35-61, Academic Press , New York, London, 171 pp.

Stephenson, G.R., N.J. Bunce, R.I. Makowski, M.B. Bergsma and J.C. Curry (1979).
Structure-activity relationship of antidotes to thiocarbamate herbicides in corn.
J. Agric. Food Chem. 27, 543-547.

Stephenson, G.R. and C. Ezra (1982).
The mode of action of herbicide safeners.
Proceedings 1982 British Crop Protection Conference - Weeds.
Vol. 2, 451-459.

Stoller, E.W. and L.M. Wax (1977).
Persistence and activity of dinitroaniline herbicides in soil.
J. Env. Quality 6, (2), 124-127.

Tewari, M.N. and D. Balasimha (1976).
The influence of substituted ureas and uracils on growth, sugar content and peroxidase activity in fenugreek *(Trigonella foenum graecum).*
Indian J. Plant Physio. 19 (2), 217-219.

Tewari, M.N. D. Balasimha and C. Ram (1976).
Biochemical changes in the germinating seeds of *Trigonella foenum graecum.* L. in relation to S-triazine herbicides.
Biologia Plantarum (PRAHA) 18, (4), 268-272.

Upchurch, R.P. (1972).

Herbicides and plant growth regulators : Influence of chemical properties, plants, edaphic factors and climatic factors on action and fate. In Organic Chemicals in the Soil Environment (C.A. Goring and J.W. Hamaker, Eds.), pp. 481-496. Dekker. New York.

Van Oorschot, J.L.P. and P.H. Vanleeuwen (1979).

Recovery from inhibition of photosynthesis by metamitron in various plant species.

Weed Res. 19, (1), 63-67.

Walker, A. (1980).

Activity and Selectivity in the field. In, Interaction between Herbicides, and the Soil (R.J. Hance, Ed.), pp. 203-222, Academic Press, London.

Weber, J.B. and T.J. Monaco (1972).

Review of the chemical and physical properties of the substituted dinitroaniline herbicides.

Proceedings South Weed Sci. Soc. 25, 31-37.

Wilkinson, R.E. and A.E. Smith (1975).

Reversal of EPTC induced fatty acid synthesis inhibition.

Weed Sci. 23, 90-92.

Yu-Sing, Bai and Zhou He-Ting (1982).

The effect of suppressing cotton aphid population by interplanting *Trigonella foenum-graecum*.

Acta Entom. Sinica 25, 350.