SESSION 4A

SOIL-BORNE PATHOGENS AND PESTS OF CEREALS: THE CHANGING SCENE

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INVITED PAPERS 4A-1 to 4A-5

CHANGE AND DECAY - THE SOCIOLOGY OF CEREAL FOOT ROTS.

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ABSTRACT

Technological advances, economic conditions, political changes, and the pressures of public opinion have all influenced agricultural practices over the past 15 years. The paper examines the way in which the changes so induced have affected the incidence and severity of cereal soil-borne diseases and our attitudes to their control.

1971-1981 YEARS OF EXPANSION

In 1971 UK farmers produced some 13 000 000 tonnes of cereals - 59% of the country's total needs. By 1981 they were producing 19 621 000 tonnes - equivalent to 103% of our national needs. We still had to import maize, sorghum and hard milling wheat but, for the first time, our exports of cereals (4 718 000t) exceeded our imports (4 197 000t). Three changes contributed to this increased production. Firstly, there was a slight increase in the area of cereals - from 3 702 000 ha 1971 to 3 979 000 ha in 1981 (MAFF June Returns). Secondly, there was a swing from spring cereals to higher-yielding, autumn-sown crops - with winter wheat replacing spring barley as the most widely-grown cereal (Table 1). Thirdly, and most importantly, there were very substantial increases in the per hectare yields of all types of cereals (Table 2).

TABLE 1

Percentage of cereal area devoted to each crop (Leech and Chalmers 1985).

	Wheat		Barley		Oats		Other
	winter	spring	winter	spring	winter	spring	cereals
1975	29	3	6	54	2	3	3
1981	54	1	28	14	2	0	1

TABLE 2

Average Cereal yields of 350 farms in the eastern counties 1970 - 1981 (Murphy 1986)

	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981
W wheat	4.11	4.66	4.50	4.31	5.26	4.46	3.92	5.22	5.65	5.64	6.42	6.29
S barley	3.13	3.35	4.13	4.02	3.72	3.29	3.47	4.20	4.13	3.90	4.42	4.22
W barley	-	-	-	4.24	4.45	3.79	4.11	4.50	4.76	4.78	5.31	4.92

Intensification of winter cropping

The slight increase in the area of cereals was brought about partly by the expansion of the crop onto new land and partly by the intensification of cropping on fields with a history of cereals. Any move from rotational cropping towards more intensive cereal growing inevitably increases the risks of foot rots. Since the 1960's, however, farmers had become increasingly aware of the phenomenon of 'take-all decline' (TAD) and by 1970 continuous spring barley growing was already a familiar feature of the agriculture of many light land areas. By the mid 1970s, continuous wheat growing was established on the well structured chalky boulder clays of the eastern counties. Because of risks of severe take-all on lighter soils ADAS suggested that barley should be grown during years of peak take-all with a return to wheat when TAD had been established.

From the middle of the decade the advent of higher-yielding varieties of winter barley gave a further boost to the change from spring to autumn sowing. Though it generally suffers less severely than wheat, autumn-sown barley is more susceptible to both take-all and eyespot than is the spring-sown crop.

In 1980 a new problem - barley yellow mosaic virus (BaYMV) - was recognised in winter barley. Its vector, Polymyxa graminis, had long been known as an apparently harmless coloniser of the ageing cortical cells of cereal roots. It is tempting to link the sudden appearance of the virus with the contemporaneous expansion of the winter barley acreage, but in the E. Region it was first found in areas with a long history of growing the highly susceptible variety Maris Otter.

The Decline of the Plough

The move towards the intensive growing of winter cereals had been encouraged by the increasing popularity of minimal cultivation and, later, of direct drilling techniques which overcame the autumn labour peak associated with ploughing. The use of paraquat, a bipyridylium herbicide developed in the late 1950s, on cereal stubbles after harvest made it possible to achieve an effective control of volunteer plants and annual

weeds without recourse to the mould-board plough. Minimal cultivation was well established by the mid 1960s, and later implements which sowed seeds into soil obviated the need for any form of prior cultivation. Direct drilled winter cereals increased from about 1000 ha in 1969 to about 130 000 in 1980 (ICI data), by then about one third of the country's cereal crop (winter plus spring) was being established without the use of a mould-board plough (Davies 1984)

On farms where straw was not baled and carted from the field both minimal cultivation and especially direct drilling were generally associated with the widespread practice of straw burning. In the 1970s straw was burnt on about 1.2 million ha - about half the total (Vickerman 1974 - cited by Edwards 1984).

It was feared that abandoning the mould-board plough would increase the risks of foot rots (take-all had been increased by 'no-till' husbandry in parts of the United States). In practice foot rots were seldom made worse by 'no-plough' methods of seed bed preparation and in some cases direct drilling actually reduced the incidence of both take-all and eyespot (Brookes & Dawson 1968, Yarham & Hirst 1975).

Earlier sowing

Straw burning and 'no plough' husbandry also contributed to the steady drift towards even earlier sowing of winter cereals. In 1979 about 12% of winter wheat crops were sown in September; by 1985 that proportion was estimated to have increased to 50% (data cited by Cook & Yarham 1985).

That early sowing favours eyespot has long been recognised and ADAS surveys have also revealed more sharp eyespot in early sown crops. The natural year to year variation in eyespot makes it difficult to pick out long term trends associated with changes in husbandry practice. However, there have been some unusually severe epidemics since September sowing of winter wheat became the rule rather than the exception.

Take-all, too, is favoured by early drilling and in recent years it has become increasingly common to see take-all patches in early-sown crops in the autumn. While crops sometimes grow away from these early attacks disease incidence generally remains higher on early- than on late-sown crops throughout the season. The higher incidence of disease often offsets beneficial effects of early sowing, reducing substantially the yield as compared with an otherwise similar crop sown later - a point illustrated in Table 3.

TABLE 3

Effect of sowing date on take-all at Rosemaund EHF (Simkin et al 1985).

	%plants with take-all.		Yield (t/ha)	
Sowing date 1983	15 Sept	15 Oct	15 Sept	15 Oct
Sowing date 1984	20 Sept	5 Oct	20 Sept	5 Oct
1983 (2nd wheat)	80	41	2.64	5.05
1984 (3rd wheat)	73	51	8.26	8.94

The yield benefits that can accrue from early sowing in first wheats and fourth wheats (the latter being in TAD situations) are shown in Table 4. In second and third wheats on the other hand very early sowing actually depressed yields to some extent.

TABLE 4

Effect of sowing date on yield of winter wheat in East Anglia (Cousins pers. comm.)

	Yield t/ha (number of fields contributing to mean)			
	1st wheat	2nd wheat	3rd wheat	4th wheat
1-30 Sept	7.64 (108)	7.07 (83)	6.96 (42)	7.33 (94)
1-14 Oct	7.44 (103)	7.32 (115)	7.09 (81)	7.17 (140)
15-31 Oct	7.15 (63)	7.09 (65)	6.86 (46)	6.83 (107)

Changes in varieties

The intensification of winter wheat cropping was made possible by the introduction in the mid 1950s of the eyespot resistant variety Cappelle Desprez, which still occupied 37% of the UK winter wheat acreage in 1971, but declined to a 1% share of the market by 1977. It was replaced by varieties which combined eyespot resistance with higher yield potential. Maris Huntsman, which outyielded Cappelle Desprez by 20%, increased from less than 1% of the winter wheat acreage in 1972 to a peak of 35% in 1977. It was gradually replaced by yet higher yielding varieties and by 1981 it occupied only 16% of the acreage. Reduced susceptibility to the effects of take-all in Maris Huntsman (Scott & Hollins 1984) may have contributed to the smaller than expected take-all losses in the mid 1970s, given the increasing intensity of winter wheat cropping, though it is difficult to untangle such effects from those of the sequence of very dry seasons which occurred at that time.

Increasing nitrogen rates

During the middle and late 1970s there was a trend towards increased nitrogen usage on cereals (Church 1979, Church & Leech 1982, Leech & Chalmers 1985 - table 5) and more crops received part of that nitrogen as an early 'split' in late February or early March. Experiments at Rothamsted Experimental Station have shown that these early applications reduce the severity of take-all (Prew pers. comm.)

TABLE 5

Nitrogen usage on winter cereals (kg/ha) 1974-1981

	1974	1978	1981	1984
W wheat	90	125	162	187
W barley	91	106	143	150

The introduction of fungicides.

Stiff strawed and eyespot resistant varieties such as Cappelle Desprez paved the way for the increased nitrogen rates on wheats. Cappelle, however, was not immune to eyespot and increased nitrogen increased the risks of lodging. It was discovered in the early 1970s that MBC fungicides gave good control of eyespot and so these fungicides found a ready market on our winter cereal crops. It was soon observed that they increased the incidence of sharp eyespot, but this was outweighed, by the consistent yield increases (even in the absence of eyespot) from the control of early-season attacks of Septoria tritici and other pathogens. By 1981 about 40% of winter wheat crops in England and Wales were receiving an MBC spray (King 1982), mostly applied in the spring with eyespot as the primary target.

The only fungicide for which commercial claims for activity against take-all have been made is the seed treatment based on triadimenol + fuberidazole. First noted in the USA (Bockus 1983) the effect has also been detected in both ADAS trials and at Rothamsted where the fungicide was found to be beneficial where winter wheat was sown in early September and severe take-all developed (Bateman et al. 1986).

1981 - 1985 THE CHAGRIN OF SURPLUSES

The economic background to the cereal boom of the 1970s was the increase in cereal prices that followed Britain's entry into the EEC in 1973 (Table 6). Profitability of wheat growing was maintained until well into the 1980s and the area grown rose to a record 1 939 000 ha in 1984 (MAFF June returns). By 1983, however we were producing a cereal surplus of about 4.5 million tonnes and the EEC was also in surplus. The record harvest of 1984 boosted profits in the short term, but also reminded the voting public that their taxes were supporting production of grain which, it seemed, nobody wanted. Although profits were being maintained by higher yields, the net margin per tonne of wheat was already falling, (Murphy 1984). Farmers knew that, in the long term, either cereal prices would fall or production would have to be limited by some form of control.

The return to closer rotations

The response of many cereal farmers to the problems they saw ahead was to seek alternative crops. The area of oilseed rape, which had grown from 0.05 million ha in 1975 to 0.12 million ha in 1981, increased still further to about 0.3 million ha by 1985 (Anon. 1986). Some farmers used this profitable crop to break runs of continuous cereals, others increased the numbers of rape crops taken in existing cereal/rape rotations, others reverted to more 'traditional' rotations using rape and pulse crops as breaks. Of the pulses winter beans provide the 'safer' crop as peas share with rape a susceptibility to Sclerotinia sclerotiorum. The effects of break crops on the yields of subsequent cereals are well illustrated by the data presented in Table 7 (Cousins pers. comm.) Yield declined in the 2nd and 3rd wheats after a break crop - the third wheats sometimes (but not always) being worse than the second, but recovered in the fourth year of the sequence. The disadvantages of the "4 years wheat, 1 year rape" rotation favoured on many East Anglian farms are that the field constantly passes through the take-all peak and the extra yield

TABLE 6

The economics of wheat production in the eastern counties 1971-1984 (Murphy 1986)

Financial year	Yield t/ha	Current price	Variable costs	Gross margin current*	Real**
70/71	4.11	30.9	22.3	104.7	546.2
71/72	4.66	31.2	24.9	120.6	569.9
72/73	4.50	35.4	29.6	129.8	583.9
73/74	4.31	59.2	35.8	219.2	706.9
74/75	5.26	58.8	47.2	262.2	681.0
75/76	4.46	66.0	64.0	230.3	551.1
79/80	5.46	98.8	142.0	397.7	583.0
80/81	6.42	106.0	158.8	521.5	682.7
81/82	6.29	112.4	183.5	523.2	622.6
82/83	6.45	119.6	199.2	572.2	635.8
83/84	6.87	126.8	209.7	661.4	687.1
84/85	8.36	113.5	222.9	726.0	726.0

* - all items valued at market prices 1970/84
** - all items valued at 1984 input prices.

following the break crop is achieved in only one wheat crop in four. Falling wheat prices maximise these disadvantages, especially in the peak take-all years when both yield and quality are reduced, because as supply increases, increasingly high quality standards are being demanded by merchants and by the Intervention Board. There is therefore much to be said for the "rape-wheat-wheat-beans-wheat-wheat" sequence which is more profitable than continuous cereal growing even on the well-structured chalky boulder clays of Boxworth EHF (Jarvis pers. comm.) On more take-all prone soils second wheats could still suffer severe losses and while barley still provides a possible alternative to a second wheat, there is increasing interest in the new higher-yielding varieties of triticale in this situation.

Fungicide resistance

Mass mycelial isolates of P. herpotrichoides from two wheat crops which had suffered severe attacks of eyespot in 1981, despite application of MBC sprays, proved to be highly resistant to MBC fungicides (Griffin & Yarham 1983). Subsequent surveys (King & Griffin 1985) showed that: i) MBC resistance in P herpotrichoides was widespread in the main cereal growing areas of England; ii) resistance was highest in fields with a history of MBC use; iii) resistant isolates were obtained more frequently from 'BWR' pathotypes than from 'BW' pathotypes; and iv)

TABLE 7

Yields of wheat crops (t/ha) in East Anglia in the years following breaks. (bracketed figures = numbers of crops contributing to the means)

Wheat crop	1982	1983	1984	1985
1st after sugar beet	--	--	8.95 (162)	6.89 (62)
" " peas	--	--	9.60 (107)	7.22 (35)
" " beans	--	--	9.43 (64)	7.32 (22)
" " rape	--	--	9.79 (266)	7.67 (168)
1st (average)	7.64 (385)	8.02 (417)	9.20 (389)	7.36 (317)
2nd "	7.24 (229)	7.64 (306)	9.03 (335)	7.19 (271)
3rd "	7.26 (115)	7.74 (136)	8.54 (243)	6.94 (179)
4th "	7.43 (103)	7.73 (146)	8.83 (397)	7.08 (366)

both 'BWR' pathotypes and resistant isolates were more common in winter barley than in winter wheat. Occasionally populations of the eyespot pathogen with very little resistance, despite a past history of fairly intensive MBC usage, occurred where there had been no recent barley cropping and where the pathogen population was still dominated by 'BW' pathotypes. The 1970's expansion of winter barley probably facilitated the development of resistance in the fungus by increasing the proportion of 'BWR' pathotypes.

When the pathogen population is dominated by resistant strains MBC fungicides do not control eyespot and may even increase its incidence. Populations in most fields in the intensive cereal-growing areas of Eastern England are now so dominated and, since resistance is now also widespread in both Septoria tritici and Fusarium nivale, it is becoming increasingly difficult to justify the early season use of MBCs. Increasing cost consciousness, however, makes many farmers reluctant to abandon their 'traditional' MBC spray for the more expensive alternative product, prochloraz.

The Advance of the 'Greens'

Coincident with public concern over surpluses is a growing opposition to what are perceived as the excessively-high and potentially-dangerous, inputs of pesticides and fertilisers which produce our current high yields. Few fungicides are regarded as being harmful to the environment, but MBCs can be toxic to soil-inhabiting arthropods and to earthworms (Edwards 1984). To maintain soil structure, some practitioners of direct drilling limited the use of MBCs even before the problem of resistance arose, but a single application of the recommended rate is unlikely to affect earthworm populations significantly.

Public concern has fostered an expanding market for 'originally grown' or 'conservation grade' crops which have received no synthetic pesticides, and there have been demands for restrictions for nitrogen usage which should they be introduced would almost certainly increase the risks of severe take-all losses in second and third cereals.

Other agricultural pollutants - the flames, smoke and smuts from burning straw - have given annoyance at sometime or other to most who live, work or take their recreation in the countryside. Since 1983, public pressure, more stringent byelaws and a more rigorous NFU Code of Practice have led more farmers to incorporate rather than burn their straw. Straw incorporation, like direct drilling, can increase the incidence of cephalosporium leaf stripe (Jordan et al, 1984), but seldom aggravates,(and sometimes reduces) eyespot (Yarham, 1979). Field observations have suggested that straw incorporation without ploughing can sometimes aggravate take-all, but patchy distribution of the disease has made it difficult to demonstrate this conclusively in replicated trials. Table 8 illustrates some data from two 1986 trials from the ADAS Eastern Region, where take-all levels in the spring were highest in the straw-incorporated, cultivated treatments but these differences tended to lessen as the season progressed. Although the move away from straw burning has been accompanied by a move back to ploughing, modern equipment makes it possible to plough and yet still to drill early.

TABLE 8

Effect of straw disposal method on take-all,

	% infection of plants and roots						
	Straw burnt		Straw incorporated				
	Plough	Cultivated 15cm	Plough	Plough 85/6 Cultivated in 1984/5	Cult then plough	Cult 15cm	SED
Bovingdon: March							
% plants	26.0	20.7	17.2	20.6	20.3	41.9	8.06
% roots	7.3	5.0	5.0	4.4	5.2	11.2	2.31
Bovingdon: June							
% plants	63.9	60.5	74.4	64.3	68.7	82.3	13.64
% roots	9.3	8.4	8.9	7.5	6.6	14.6	4.24
Ellington: March							
% plants	47.4	64.4	54.8	69.1	50.0	89.0	5.97
% roots	12.8	16.6	14.5	19.7	13.3	26.4	2.82
Ellington: June							
% plants	86.8	89.1	87.3	79.4	85.6	88.4	4.83
% roots	15.4	20.6	15.7	12.3	12.7	20.5	2.27

Alternatives to fungicides

Both economic and environmental pressures, as well as the problems of fungicide resistance, argue strongly for the development and exploitation of non-chemical methods of disease control. The most successful of such methods is still the breeding of resistant varieties and the introduction of the wheat variety Rendezvous, with its VPM resistance to eyespot, has brought the possibility of effective genetical control of this disease. In winter barley, varietal resistance to BaYMV has been greatly improved in cvs Palomino, Sonate and Torrent. In the area of biological control work is currently underway on the use of suppressive micro-organisms for the control of take-all (Capper & Cambell 1986). These projects are, however, still at the "drawing board" stage with little chance of commercial exploitation in the immediate future.

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THE EFFECT OF CHANGING AGRICULTURE ON SLUGS AS PESTS OF CEREALS

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ABSTRACT

A combination of factors has led to increased slug risk to winter wheat during the last decade. The weather was especially favourable, six years providing damp springs and summers and five of these featured mild autumns as well. The winter wheat acreage increased, so did the area of oilseed rape, which encourages slugs. In 1985 15% of wheat was preceded by oilseed rape but 50% followed wheat and even fourth wheats in rotation are still perceived to suffer slug damage. Traditional ploughing and seedbed preparation reduce the risk. On medium and heavy land the interest in reduced cultivations in the 1970s and 1980s, no doubt, brought greater slug damage. Straw burning reduces slug populations so the need for alternative disposal techniques will contribute to more slug problems. If financial constraints force the farmer to retain reduced cultivation and if there is a tendency to conservation management of headlands, slugs are likely to remain a problem. Control measures will rest with methiocarb or metaldehyde pellets in the medium term. New chemicals, improved baits and seed coatings may offer new opportunities.

INTRODUCTION

Agricultural practices involving cereal production have changed and continue to change rapidly. During the last decade, there has been an increase in total area of small grains grown in the United Kingdom, and since 1978 there has been a substantial rise in the area devoted to wheat, which is virtually all autumn-drilled (Table 1). Concomitantly there has been a significant increase in the growing of rape for oilseed, and currently a renewed interest in peas for harvesting dry. These trends may be important when considering real or perceived changes in the status of slugs as cereal pests.

TABLE 1

Crop area in thousand hectares, UK

Crop	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985
Cereals	3737	3632	3684	3705	3811	3873	3938	3979	4030	3961	4036	4015
Wheat	1233	1034	1231	1076	1257	1371	1441	1491	1663	1695	1939	1902
Oilseed rape	-	39	-	56	-	74	92	125	174	220	269	296
Peas (dry harvested)	-	-	-	-	-	-	-	-	-	29	42	78

Sources: MAFF Agricultural Statistics - United Kingdom, and Bunting, 1984.

Concurrently modifications in cultivation and husbandry methods, partly due to economic pressures and attention to production costs, have occurred. Traditional cultivation techniques, such as ploughing, are costly in energy and on some heavier soils, the working down of ploughed land to produce a seedbed can be extremely difficult to achieve. Direct drilling requires an energy input of only 35-80 MJ/ha, reduced cultivations based on tines and discs, 130-230 MJ/ha; whilst traditional cultivation based on mouldboard ploughing consumes 200-360 MJ/ha (Anon, 1982). Reduced cultivation techniques have caused visible increases in grass weeds but in the case of soil fauna such as slugs, the effect of changing agronomy on populations is less obvious but may be just as real.

Depending on the taxonomic authority, there are between 25 and 35 species of slugs in Britain (Quick, 1960; Cameron *et al*, 1983), but the most important of these in agriculture, *Deroceras reticulatum* (Muller), the grey field slug, is the species most frequently damaging wheat crops (Brown, 1955; Gould, 1961, 1962). Others including *Arion hortensis* (Ferrusac) the garden slug, the large black slug, *Arion ater* (L.), *Arion fasciatus* (Nielsson) and *Milax gagates* (Drap.) have all been recorded feeding on wheat grain though damage by the grey field slug is usually more distinctively confined to the germ end.

Leaf grazing can continue during the whole of the growing season and may be serious to small seedlings, in retarded or thin crop stands. In wet summers, such as 1985 and 1986, slugs are frequently observed feeding on the upper leaves of winter cereals (MAFF Pest and Disease Intelligence reports) causing loss of photosynthetic tissue at a crucial period. Such grazing damage has so far been disregarded though in a commercial wheat crop (cv. Corinthian) during 1985 (N.J. Kemp, pers. comm.) some tillers lost 30% of the flag leaf area and grain weight from these was significantly less.

Slug populations are difficult to assess, but very high numbers have been estimated, especially of *D. reticulatum*. Gould & Webley (1972) calculated that an average of about 250 000 slugs per hectare were killed by an application of 5.6 kg/ha of methiocarb pellets and up to 600 000/ha on some parts of the treated field. In a mixed clover and grasses habitat, South (1965) estimated populations from soil samples and found the equivalent of >6 million *D. reticulatum* per hectare. Hunter (1966a) estimated slug populations in arable land in Northumberland from soil samples and found the equivalent of >12 million *D. reticulatum* per hectare. More recently Glen *et al*, (1984) calculated a population of 760 000 slugs per hectare.

Multiplying sample results to estimate population size has statistical dangers because slugs have an aggregated distribution. Estimating numbers of corpses after molluscicide treatment is also inaccurate because baits have little effect on juvenile slugs. However, it is clear that large numbers of slugs may be present in arable fields.

THE EFFECT OF WEATHER

Weather greatly influences slug activity, affecting population size and potential damage to cereals. High populations may be prevented, by the weather, from causing economic damage, conversely, favourable conditions may encourage damage by relatively few slugs. Rainfall, humidity, light and temperature all influence slug movement and feeding. Slugs also have daily and seasonal activity rhythms though there is disagreement as to the

relative importance of light and temperature as factors governing these rhythms (Dainton, 1954; Dainton & Wright, 1985; Wareing & Bailey, 1985).

Slugs lose body water if the humidity at the skin surface falls below 99.5% r.h. at 20°C (Machin, 1975). This loss occurs even if slugs are quiescent but accelerates as activity increases due to mucus production. Therefore a suitable slug environment is one which remains moist for much of the time (Dainton, 1954) and a film of moisture on soil or plants is essential for movement and feeding (Barnes & Weil, 1945). Hence rainfall and soil moisture influence numbers, activity and resulting crop damage; regular precipitation being more important then infrequent, heavy rain which may actually discourage activity (White, 1959). Though slugs have little control of water loss they tolerate considerable fluctuations of soil moisture and *D. reticulatum*, *A. ater* and *L. maximus* are able to survive a 50% loss in weight due to dehydration (Dainton, 1954).

Frost and drought can directly reduce populations but adverse weather may indirectly do the same by lengthening the life cycle (Runham & Hunter, 1970). When soils dry out and adults retreat to greater depths reproduction ceases, and eggs take longer to hatch and may die because they require a soil moisture content of 60-85% (Carrick, 1942; Godan, 1983).

Though slugs are largely nocturnal, *D. reticulatum* is often active in daylight especially during dull, cool and showery weather. In fact daytime activity occurs all year round provided temperatures are above 0°C and conditions are sufficiently moist (Wareing & Bailey, 1985). A peak of activity just after dawn is probably due to the return to daytime shelter (Cook, 1979). A pre-dusk activity peak in *D. reticulatum* is governed by fall in temperature when over 94% of daily activity occurs in the following three hours. If temperature remains stable, or actually rises, activity is reduced but prolonged into the night (Wareing & Bailey, 1985). *D. reticulatum* can move and feed down to 0°C (Mellanby, 1961) but the optimum feeding temperature is 14°C in winter and summer. The optimum temperature for movement, however, varies between seasons and is 17°C in summer but falls to 13°C in winter. These findings emphasise the versatility of the grey field slug as a pest of winter cereals regardless of changes in agronomic practice.

THE EFFECT OF SOIL TYPES, STRUCTURE AND MOISTURE STATUS

It is generally agreed that heavy, wet, little-cultivated soils with an open structure of large aggregates encourage the highest slug populations. In a field survey of wheat in East Anglia 79% of crops grown on clay loams were damaged by slugs and 64% on silty clay loams; only 27% were damaged on silty loams and none at all on peaty loams (Gould, 1961).

Hunter (1969) asked ADAS district advisers to define the soil texture of fields where slug damage had occurred. Clay and silt soils provided 58% of the cases whilst loams, organic soils including peats and sands provided 39%, 2.5% and 0.5% respectively. *D. reticulatum* prefers heavy soils which allow it to move easily and to rest between moist soil aggregates maintaining maximum body surface contact with the soil water film. Cloddy soils, and those with dense vegetation, also provide more shelter and daytime resting sites. *D. reticulatum* prefers a loose, albeit finer tilth for egg-laying, though both juveniles and adults choose medium to coarse soil textures in which to settle (Stephenson, 1975b).

A survey of slug problems in winter wheat fields over the three harvest years 1983-1985, involving some 3 000 to 4 000 fields in each year, provided practical evidence of the importance of slugs on medium and heavy soils (Table 2) and calcareous soils of varying texture.

The use of excessive nitrogenous fertiliser can also increase the population density in many soils. Neumann in Godan (1983) reported that an excess of nitrates in the soil produces particularly attractive and nutritious plant tissues, which increase the fecundity of slugs. Numbers of slugs and variety of species is greater on alkaline soils, Carrick (1942) reporting a proliferation of *D. reticulatum*, *A. hortensis*, *A. subfuscus* and *A. circumscriptus* resulting in extensive crop damage in potatoes.

The water holding capacity of soils was first shown to be an important element in affecting slug populations by Carrick (1942). Oviposition of *D. agreste* (= *D. reticulatum*) was greatest in soils with a 75% moisture content. In soils with both 50% and 25% moisture, oviposition was delayed until the slugs were able to penetrate to a depth of 3 in. to lay the major proportion of eggs. Farm practices which affect soil moisture status influence the damage risk to winter cereals, for example, irrigation of the previous crop increases the risk whilst good drainage may reduce it.

TABLE 2

Soil type and slug problems in wheat

Soil textural group	% Soils with slugs 1982/83	1983/84	1984/85	All years (mean)
Sands - light soils	7	9	5	7
Calcareous soils	12	17	10	13
Medium soils	11	20	12	14
Heavy soils	11	26	19	18
Organic and peaty soils	4	6	6	5

Source: ICI Farm Advisory Service

THE EFFECT OF CULTIVATION

It is generally accepted that cultivations reduce slug populations, and slug damage to winter wheat is often less severe on more compacted headlands. Gould (1961) reported that on 30 fields where slug damage was recorded, headland wheat was normal or had only slight damage. Several fields were seen where extra cultivation or consolidation after drilling had apparently reduced slug damage. On one field the wheat crop was very thin except within tractor wheelings.

Grant *et al* (1982) compared a direct drilling regime for alfalfa with mouldboard ploughing, followed by disc and drag harrowing. Both foliage damage and the population of *D. reticulatum* were greater in the direct drilled plots. Hunter (1967a) compared the effect of three cultivation regimes on subsequent numbers of *D. reticulatum*, *A. hortensis* and *M. budapestensis*. In the first, the ground was ploughed; in the second a

fine tilth was produced firmly compacted and sown with grass which was kept mown; in the third, a fine tilth was produced, but not subsequently compacted or sown with grass. All regimes reduced slug numbers but the second was most effective. Edwards (1975) compared soil fauna populations from direct drilled with those from ploughed plots, and found that slugs were much more plentiful in the former. Glen *et al* (1984) found least slugs on plots following traditional burning compared with various methods of straw incorporation. Shallow incorporation did not reduce slug numbers, but resulted in significantly less damage to wheat compared with direct drilled plots. Burning clearly reduces the potential slug problem whilst straw incorporation has the opposite effect (Table 3).

How cultivations create their impact on slugs is uncertain. However, the exposure of large numbers of slugs to predation from birds and other vertebrates when the land is ploughed must have a marked effect. Birds following the plough are searching for soil fauna and slugs are a major constituent of the diet of birds such as starlings and gulls (Runham & Hunter, 1970). The breaking of soil clods into smaller aggregates must also lead to direct injury and destruction of slugs coupled with further predation. Edwards (1975) noted that some species of carabid beetles which prey on slugs were more plentiful in crops grown on ploughed land compared with direct drilled crops.

TABLE 3

The effect of post-harvest straw and stubble treatment on perceived slug problems

		Good burn	Poor burn	Baled	Chopped and incorporated	Incorporated untreated	Other
1983/84	No. of fields	1470	221	958	201	200	140
	% with slugs	18	29	17	48	29	19
1984/85	No. of fields	1198	304	711	451	207	165
	% with slugs	10	15	10	28	17	14

Source: ICI Farm Advisory Service

Advanced embryos of *D. reticulatum* tolerate considerable dehydration which could occur in warm autumn weather after cultivation, as Bayne (1969) found that they could survive a 60-80% weight loss (by dehydration) from the eggs. However, autumn days can be very sunny and maximum temperatures at the soil surface may be some 10°C higher than those measured in screen (J.L. Monteith, pers. comm.). It is not unusual to record maximum air temperatures of between 20°C and 30°C in October and therefore, eggs and juveniles exposed by cultivations could experience high radiant temperatures. Carrick (1942) showed that adults of *D. reticulatum* died within 1 hour when exposed to a temperature of 35°C whilst at 30°C, 80% died within 4 hours. Eggs died in 12 hours if kept at a constant 25°C. Adults and juveniles will seek cooler positions but injured or very small slugs might be incapacitated

before reaching a safe refuge and eggs exposed to these high temperatures and resulting dehydration, would probably die.

Soil consolidation reduces crop damage probably by killing some slugs and preventing others reaching the grain and subterranean shoots. Stephenson (1975a) showed that when small soil aggregates were broken down and firmed over wheat seed by moderate or heavy pressure, slug damage was reduced but larger aggregates did not break down so well and many seeds remained exposed and were damaged. So rolling a rough, cloddy seedbed after drilling is unlikely to be successful. Slugs move and find wheat seeds easily in cloddy soils which explains why many of the worst cases of slug damage have occurred on heavier land with poor seedbeds.

The heavy land farmer is in a dilemma; if he ploughs and works down he must try to ensure a fine seedbed and if he uses minimal cultivation techniques, he starts with a potentially greater slug problem. If he direct-drills, not only will more slugs be present to damage the crop, but they may inflict greater losses by using the slots as 'motorways' with feeding points at regular intervals (Allen, 1981).

THE EFFECT OF CROP ROTATION

The influence of rotation is largely indirect by improving the slug environment under dense crop or weed cover and post-harvest debris, thus increasing population.

The increase in the area of winter as opposed to spring cereals provides a favourable slug habitat for a greater part of the year, particularly during autumn, winter and early spring, aiding population survival and extending the traditional breeding season of slugs. In 1975, the proportion of winter cereals to spring cereals in Great Britain was approximately 51%, whereas by 1985, winter cereals represented 75% of the total cereal area, with an increase of 54% in the United Kingdom wheat area during the same period.

TABLE 4

Frequency of perceived slug problems in wheat by position in rotation

	Number of perceived slug problems			
Position of wheat crop	1982/3	1983/4	1984/5	Total
1st Wheat	441	338	242	1021
2nd Wheat	239	150	91	480
3rd Wheat	101	63	32	196
4th Wheat	182	170	69	421

Source: ICI Farm Advisory Service

Recent survey results from the harvest years 1983-85 show that slug problems are most frequent in first wheats (Table 4) which follow crops such as oilseed rape and peas (Table 7). When wheat follows wheat (half the current wheat area) slug problems continue to be experienced, primarily in

second wheats but even in the fourth wheat in rotation (Table 4). In a 1958 survey dry harvested peas were found to provide the most serious slug risk to following wheat crops; beans were next, followed by preceding cereal crops, brassicas for seed and grass/clover leys (Gould, 1961). Little damage to wheat was experienced after potatoes and none was observed after sugar beet or fallow. The most severe damage, as high as 90% loss in plant stand, occurred following combining peas or brassicas for seed (Table 5).

Stephenson & Bardner (1976) considered the increase in oilseed rape growing a major reason for the greater frequency of slug damage to wheat crops due to its dense foliage and post-harvest debris. This crop has increased in recent years from 3.8% of the total area of wheat in 1975 to 15.6% in 1985 (Table 1) and in the 1985/86 growing season, 15% of winter wheat surveyed (Table 7) was preceded by oilseed rape. Thus virtually all oilseed rape crops are followed by winter wheat. Since 1975, therefore, the larger proportion of wheat grown after oilseed rape, represents an increase of 413% in wheat crops at risk and certainly helps to explain the perception of worsening slug damage. Rape introduces a substantially greater slug threat to wheat than any other preceding crop in the rotation (Table 6) and once again legumes, cereals and grass enhance the risk, whilst sugar beet and potatoes are relatively innocuous in this respect, which bears out the 1958 results in Table 5.

TABLE 5

Slug damage to wheat in relation to previous cropping (after Gould 1961)

	Dry harvest peas	Clover/ grass leys	Beans	Cereals	Brassicas for seed	Po- ta- toes	Sugar beet
No. of fields	23	23	3	5	15	16	5
No. of fields with slug damage	22	14	3	5	11	2	0
% loss of stand on damaged fields (range)	20-90	5-70	5-35	15-70	5-90	5	0
Mean % loss of stand	30	20	Not given	53	55	-	0

In autumn 1985, a survey was made involving almost 2 000 ha of winter wheat throughout the UK, the majority of the crops exceeded 20 ha and were evenly distributed between 50-100 ha, 100-200 ha and over 200 ha. The crops associated with the greatest damage to wheat (Table 6) are amongst those most frequently preceding it in the rotation (Table 7), again emphasising the validity of the present perception amongst farmers and field advisers of the widespread threat of severe damage by slugs to winter wheat crops in Britain.

TABLE 6

The effect of previous crop on perceived slug problems in winter wheat

Previous crop	Number of fields with perceived slug problems 1982/3	1983/4	1984/5	Total
Oilseed rape	248	202	175	625
Grass	122	64	40	226
Peas/beans	35	42	11	88
Potatoes	12	12	4	28
Sugar beet (tops ploughed in)	10	9	8	27

Source: ICI Farm Advisory Service

TABLE 7

Crops preceding winter wheat in Britain

	W. wheat	OS rape	Peas	Grass	S. barley	Pota- toes	S. beet	W. barley	W. oats	Others	N.A.
Wheat area (%)	50	15	6	5	4	4	4	4	4	5	2

Source: Farmstat 1985/86. Total wheat area surveyed: 1 968 ha (G.B.)

THE EFFECT OF CEREAL SOWING DATE

The effects of drilling time on slug damage to cereals are linked to other factors such as previous crop, soil type and crop type. Winter barley traditionally is sown earlier, on lighter, more friable and easily managed soils whilst winter wheat is generally sown later. Port & Port (1986) consider that because winter barley is usually sown earlier than winter wheat, germination is more rapid, tillering capacity is greater in most barley cultivars and compensatory growth following seedling damage and leaf grazing renders the crop less susceptible. However, the sowing of winter wheat has become progressively earlier in recent years. In 1974 only 22% of the crop was sown by mid-October whereas this had risen to 47% by 1977 (Port & Port, 1986) and over two thirds of the crop was drilled by mid-October in 1985 (Table 8).

It is doubtful whether the risk to earlier sown wheat is greatly reduced, because many early sowings follow oilseed rape, peas and grass leys, all of which encourage high slug populations. The lower moisture content of many earlier prepared seedbeds may not be a limiting factor, especially after oilseed rape, and the warmer soils might be more favourable to slug foraging and feeding, offsetting the possible benefit of more rapid germination and early seedling vigour.

Experience in the autumn of 1985 suggested that drier seedbeds, may be more detrimental to wheat germination and rapid establishment than to slug activity. Many wheat crops on medium to heavy soils were sown into dry, extremely coarse and cloddy seedbeds due to soil compaction during a wet

TABLE 8

Proportion of wheat area sown at various dates

	Time of sowing										
	Aug/E. Sep.	M. Sep.	L. Sep.	E. Oct.	M. Oct.	L. Oct.	E. Nov.	M. Nov.	L. Nov.	Dec./ Jan.	N.K.
Wheat area (%)	0.3	1	10	25	31	17	6	7	1	0.5	1.2

Source: Farmstat 1985/86 G.B. E = early, M = mid, L = late. N.K. - not known

harvest followed by a long dry period. The moist summer conditions in preceding crops had also created high slug populations (as evidenced by bait traps) which thrived in the ideal, open cloddy seedbeds. The coarse tilth inhibited germination but not slug feeding, so damage was exacerbated by the prolonged period of crop establishment.

MAGNITUDE OF THE SLUG PROBLEM IN CEREALS

There have been a number of attempts to estimate the scale of the slug problem (Strickland, 1965; Hunter, 1969; Stephenson & Bardner, 1976). Using the figure of 0.22% of the value of the wheat crop lost to slugs (Stephenson & Bardner, 1976), Port & Port (1986) calculated that losses in 1967 were equivalent to £191 700, in 1974 they were £600 000 and in 1985, £2.69 million. So far these figures are not compatible with our best estimates of molluscicide usage on cereals. A synthesis of a number of market research reports plus internal Bayer market statistics indicate that in Great Britain 367 000 ha of cereals received a molluscicide in 1984 and 400 000 ha in 1985. Port & Port (1986) estimated an even higher figure for 1982 of 550 313 ha in England and Wales. What is the reason for the discrepancy? The evidence seems overwhelming that recent changes in cropping (Table 1) should be held responsible for the increase in slug damage during the last decade. We think that there is another equally significant factor. Surveys or estimates of actual damage are often retrospective. They are also prone to consider only cases where depredations due to slugs are the major contributory factor in loss of plants, loss in yield or the decision to re-drill. Wheat growers have to anticipate and slugs represent one of the factors which may upset plant stand. They have been educated to pay greater attention to the parameters of cereal yield. Numbers of seeds sown, plants, tillers, ears and hence grains per square metre are all part of modern cereal growing parlance. Slugs and other biotic factors including seed-borne diseases, foliar diseases, soil insects and even seed vigour can combine to thwart the farmer's aim to produce an adequate plant stand; together they represent a considerable risk and the perception of each as a potential problem has been heightened in recent years. Some farmers therefore, use a molluscicide prophylactically and obviously consider slugs very important pests closely

rivalled only by aphids (Tables 9, 10).

TABLE 9

Frequency of pests, diseases and weeds as perceived problems in wheat (%)

Pests		Disease		Weed	
Slugs	46	Septoria	42	Cleavers	40
Aphids	34	Eyespot	28	Blackgrass	22
BYDV	5	Take-all	11	Pansy	9
Frit fly	5	Fusarium	8	Wild oats	8

Source: Farmers Weekly, May 23, 1986. Results of LAMA survey.

CONTROL MEASURES

The farmer can manipulate his cultivation and rotational strategy in such a way as to reduce the scale of potential slug problems. However, it is unrealistic to expect that farmers can easily modify a chosen cropping system because there are often more powerful economic and cultural arguments for the policy adopted. Potato cultivars show marked differences in susceptibility to tuber damage from slugs so some are more suitable for fields known to be at risk. Unfortunately, this information is lacking for cereals though nitrogen content is known to be important (Godan, 1983).

TABLE 10

Frequency (%) of occurrence of perceived pest problems in wheat fields

	Aphids	Slugs	Leather-jackets	Wheat bulb fly	Frit fly	Wire-worm	*Opomyza*	Others	Fields recorded
1982/83	6	23	1	1	2	1	<1	2	4210
1983/84	17	20	1	1	1	<1	<1	3	3612
1984/85	22	13	1	1	4	1	<1	1	3345

Source: ICI Farm Advisory Service

So far no mention has been made of the role of natural invertebrate parasites and predators. They can be found among the protozoans, brachylaemid flatworms, lungworms, lampyrid beetles, staphylinid beetles, carabid beetles, drilid beetles, and sciomyzid flies (Port & Port, 1986). Recent work by Jones & Selman (1984) showed that the microsporidian parasite, *Microsporidium novocastriensis* could reduce the fecundity of the grey field slug by 40%, growth rate by 30% and longevity by 23%. Unfortunately, the use of natural enemies has not yet been refined to allow exploitation in agriculture.

In the past chemical methods of control included soot, lime, tar oils,

copper sulphate and copper aceto-arsenite and Godan (1983) has summarised current knowledge. In Great Britain metaldehyde, mainly in the form of bait pellets has been recommended since the 1930's (Gimingham & Newton, 1937) Bayer developed methiocarb as a pelletted bait in the 1960s (Martin & Forrest, 1969). It was shown to be more reliable than metaldehyde especially in moist conditions when metaldehyde baited slugs often recover (Crawford-Sidebotham, 1970) and so methiocarb bait (4%) has become the material of choice in recent years.

It has been suggested (Frain & Newell, 1983; Kemp & Newell, 1985) that counting dead slugs in field trials favours methiocarb because metaldehyde poisoned slugs crawl away and die elsewhere; and though methiocarb kills significantly more slugs after a single ingestion those recovering from metaldehyde are more likely to feed on bait again. However, there is conflicting evidence from video recordings of slug behaviour. Wedgewood & Bailey (1986) found that metaldehyde poisoned slugs were usually immobilised after a bait meal, seldom re-encountered baits, seldom fed again and only 13% found a refuge. In other experiments not only did 4% methiocarb pellets kill more slugs than other baits when weathered for thirty days (D.R. Wareing, pers. comm.) but mortality of baited slugs significantly increased after weathering (Hogan, 1985). In only two of 44 comparisons between metaldehyde and methiocarb (Port & Port, 1986) was metaldehyde considered better than methiocarb though differences were not always significant. It seems that good slug kill may be obtained from both materials but methiocarb is more reliable in cool moist conditions when slugs are usually most troublesome.

Though there is renewed interest in sprays of copper, aluminium and boron salts in mixture, Glen *et al* (1986) found that treatment of soil with this combination did not reduce the number of slugs or the damage to potatoes grown in the treated soil. Bait pellets are therefore still the most effective chemical weapons available to the farmer, but their optimal use is difficult.

Slug populations may recover if pellets are applied too early and treatment of standing crops prior to drilling winter wheat is problematical because there is alternative food. However, there is great interest in the treatment of oilseed rape crops prior to drilling winter wheat and more research is needed. Gould & Webley (1972) found no differences between treatments applied to the stubble of the previous crop, after ploughing or after drilling. In other work on winter wheat following oilseed rape, pellets applied seven weeks pre-drilling failed to reduce slug damage. A treatment 4 weeks before drilling was effective but most tillers survived slug attack when pellets were broadcast on 6 December (Rogers - Lewis, 1977). Sometimes simultaneous drilling of wheat seed with pellets admixed is effective and especially when crops are direct drilled. In studies with wheat seed planted in rows on the surface Hogan (1985) found slugs tended to follow the drill lines, contacted seeds and fed several times in one night. When methiocarb pellets were included in the drill lines, slugs were attracted to the bait almost immediately and died within 48 hours.

THE FUTURE

Undoubtedly slugs are now a major pest of winter wheat crops in Britain. Agriculture is dynamic and a number of factors seem to have combined to increase the slug threat over the last decade. The effect of weather is commonly underestimated but the British climate is particularly suitable and

in the last decade there have been six years with damp springs and summers (Hough, pers. comm.). Mild autumns have also featured prominently with seven occurring in the same period, five of which coincided with damp summers. The influence of weather is profound but is not under the farmers' control.

Though financial margins are again shrinking it is unlikely that the area of winter wheat will decline substantially. The oilseed rape area is still increasing though it is probably reaching its maximum; the future of the crop will, no doubt, be greatly affected by the role of the new 'double-low' cultivars but this crop will continue to present a risk to following winter wheat. There is already an indication (Table 3) that straw burning is decreasing and the trend seems to have continued in 1986. The incorporation of chopped straw will probably increase and whilst providing advantages on some soils the evidence suggests that the slug risk will worsen. The authors believe that straw burning may slowly be phased out almost entirely. Increased fertiliser application, especially nitrogen, so characteristic of modern wheat growing, is likely to continue particularly for the breadmaking cultivars whose protein levels are improved with nitrogen usage.

Currently mouldboard ploughing seems to be more prevalent and in 1985 minimal cultivation stood at only 19% of the wheat area (Farmstat data). The continued use of the plough may depend upon the financial constraints on farmers and despite other problems the need to use cheaper, low energy consuming equipment might prove paramount. If this is also accompanied by conservation management of headlands slug problems will not decrease. Predicting a potential problem is usually based upon known fields at risk, soil type and conditions, preceding crop, and weather conditions. Ideally, this should be accompanied by an accurate measurement of slug populations. Current methods of estimating numbers involve direct nocturnal counts (Barnes & Weil, 1945), soil sampling and washing (Hunter, 1966b), or flooding (South, 1964), but are difficult and expensive in time and labour, and remain primarily research tools. The most practical and easy method available to farmers and field advisers for the correct timing of a molluscicide involves an assessment of slug activity by poison bait trapping.

Control measures, in the medium term, will rest upon pelleted baits containing methiocarb or metaldehyde but farmers will need to be more aware of the cultural techniques essential for fast crop establishment, so that chemical methods are part of a slug control strategy and not regarded as a panacea. There may be improvements in the attractiveness, palatability and durability of bait pellets as a result of considerable research effort. Whilst the use of conventional seed treatments have shown promise, phytotoxicity and problems of safety to seed eating birds have precluded commercial development so far, but the interest remains and the advent of seed coatings might offer new opportunities.

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RECENT APPROACHES TO YIELD-LIMITING FACTORS IN DIFFERENT CEREAL ROTATIONS

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ABSTRACT

Long term trials showed that, compared to winter wheat in rotation, continuous winter wheat crop had fewer tillers after February and lower yields. These effects may have been caused in part by identified factors such as nematodes and/or take all, but other factors which remain unidentified seemed also to be involved. Except for soil disinfection and the introduction of break crops, attempt to eliminate limiting factors, identified or otherwise, were unsuccessfull in our trials. Further diagnosis suggested that limiting factors have to be sought in the soil. Our work, therefore, is now directed not only towards the aerial parts of wheat, but also towards a study of root growth, development and function, which seem to be the key to understanding large, unexplained variations in yield. Examples of the quantification of root health using a root necrosis index are presented and discussed.

INTRODUCTION

Factors limiting cereal yields may be collected into three types : 1) those which are identified and for which crop management techniques and decision-based systems fairly well adapted to diverse soil types and climatic conditions already exist (e.g. losses caused by pests and diseases, soil compaction, mineral deficiences in the soil); 2) those which are identified and against which cereal growers have no efficient defence (e.g. take-all, nematodes); 3) those which are not identified and seem to have no precise relationship to identified factors (this situation is often termed 'soil sickness'). The importance of those factors which farmers are least able to influence is increasing, because the successes in intensified cereal production, especially in controlling pests and foliar diseases, allow them to assume a prominent position and rotation practices have changed due to economic pressure (the acreage of second and subsequent wheats has increased considerably in the last 15 years).

We are entering a period when only growers with the best performance will succeed in maintaining, and perhaps improving, their income. Studies on factors limiting cereal yields assume major importance in the present economic climate because of negative effects on yield potential and the need to optimise inputs and decrease production costs. To characterise these phenomena we will present and discuss some of the most significant results observed in both long-term and short-term trials. Recent collaboration with INRA aims at diagnosing the causes of imperfect root development and activity in a multidisciplinary approach involving agronomists, soil scientists, plant physiologists and plant pathologists.

Development and growth of root systems have been studied in natural or controlled conditions by several workers. The relationship between the development of the main root axis and the aerial organs is well know in a few crops (Klepper et al. 1984, Picard 1985) and in certain cases used in whole plant models (Weir et al. 1984). Root growth in soil has been estimated *in situ* by different methods, among which transparent tubes are promising (Maertens & Clauzel 1982) and may allow observations of root necrosis in soil.

Environmental conditions, especially the physical state of soil (Tardieu 1984), which influences the spread of maize roots in soil, and plant water relations are being studied. At present, however, the importance and effects of root health have been little studied, and before methodological problems will have to be solved.

THE TRIALS

Table 1 lists the main characteristics of the four locations in this study. Three long-term trials first provided knowledge of the risks in continuous wheat compared to those in rotation, and then a study of limiting factors in continuous wheat to identify best techniques for starting and managing continuous wheat. The Chêne Chenu trial sown in 1986 is a consequence of the main observations from the long-term trials, which have shown that knowledge of root development and root health is important. We used a third wheat after peas, because in this location, it suffers a greater yield decrease than wheats in rotation. This is attributed to take-all. In our trials we estimate root health by measuring Root Necrosis Index (RNI), with or without fungicide treatments applied to the seed bed or during growth. The methodology of the root necrosis and mycoflora studies is that of Bouhot at the Soil Microbiology Laboratory of INRA in Dijon.

TABLE 1

Trial sites, rotations and yields.

CROPPING DETAILS	LONG TERM TRIALS			ANNUAL TRIAL
	Rouvroy	Aulnay	Boigneville	Chêne Chenu
	OISE (Plateau Picard)	MARNE (Champagne)	ESSONNE (Gâtinais)	EURE ET LOIR (Thymerais)
ROTATIONS	Wheat after sugar beet Wheat after maize Continuous wheat	Wheat after sugar beet Continuous wheat	Wheat after sugar beet Continuous wheat	3rd wheat after peas
10-Years yields average (t/ha)				
- wheat in rotation	6.17	6.89	6.81	
- continuous wheat	4.83	5.99	6.56	
- difference	+ 1.34	+ 0.90	+ 0.25	

RESULTS

Observations were on identified limiting factors against which the farmer can take action (Type1),or on identified limiting factors against which the farmer is powerless (Type 2), or on unidentified limiting factors (Type 3). The results of a few attempts to remove presumed limiting factors, or unidentified ones, are given.

Observations on identified limiting factors that can be treated

Helminthosporium tritici-repentis at Boigneville and Rouvroy

Leaf spot caused by this fungus was diagnosed for the first time in wheat monoculture at Boigneville in 1974. It probably occured in 1980 (5th consecutive wheat) at Rouvroy where it was first noticed in 1982. It was not controlled by fungicides used at that time, and since 1983 propiconazol or prochloraz have efficiently reduced the disease, which, netherless, tended to start in monocultures where minimum tillage is used.

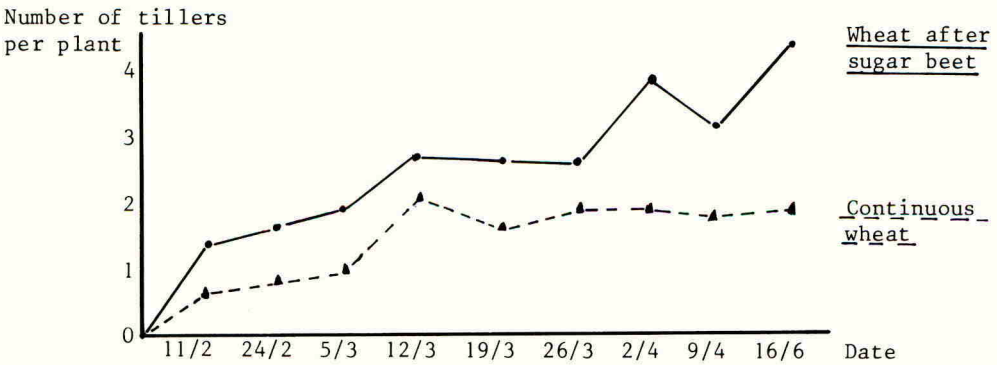
Observations on identified limiting factors that cannot be treated

The effect of monoculture on tillering

This has been tested at Rouvroy and Aulnay. Continuous wheat had lower vigour following emergence, and after February the number of tillers was lower than in wheat in rotation with sugar beet (Fig. 1) and the difference remained until flowering.

Fig. 1

Differences in tiller counts in continuous wheat and wheat in rotation at Rouvroy, 1981.

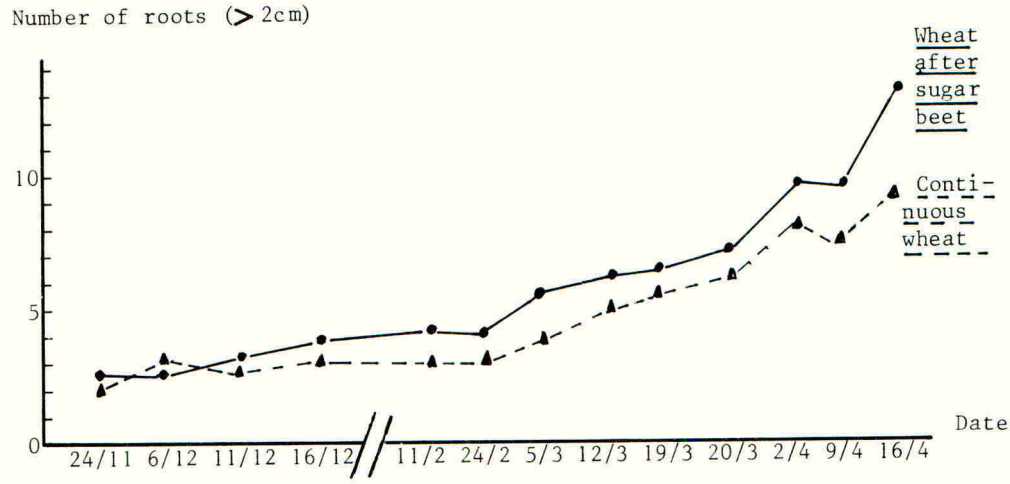


The effect of monoculture on rooting

This has been tested at Rouvroy, where the number of roots (longer than 2 cm) in continuous wheat was lower than in wheat in rotation with sugar beet (Fig. 2). Similar observations were made at Aulnay.

Fig. 2

Differences in roots counts in continuous wheat and wheat in rotation at Rouvroy, 1981.



Nematodes

At both Aulnay and Rouvroy there were smaller populations of Heterodera avenae in monoculture than in wheat rotation. This may because the lower vigour of continuous wheat did not allow multiplication of Heterodera and/or an antagonistic organism progressively parasitised the nematode population in continuous wheat. Research started in 1982 by Cayrol (INRA, Antibes) has shown that eggs of the parasitic fungus Verticillium chlamydosporium were present in July and August 1983.

TABLE 2

Take-all and yields in continuous wheat and wheat after sugar beet at Aulnay.

Year	Take-all (% stems attacked)		Yield difference between wheat in rotation and continuous wheat (t/ha)
	Wheat in rotation	Continuous wheat	
1978	4	71	+ 1.43
1979	26	58	+ 1.58
1980	3	10	+ 0.60
1981	-	-	+ 0.52
1982	4	30	+ 0.35
1983	4	38	+ 0.63
1984	-	-	+ 1.43
1985	0	48	+ 1.34

Take-all

This disease seemed to be the most discriminatory one in the Aulnay trial ; other diseases did not differ between wheat in rotation and continuous wheat. The annual development of take-all in monoculture showed three,

well- differentiated phases (Table 2). Intensity was highest in 2nd (1978) and 3rd (1979) crops and lowest in 4th crop and intermediate in the las year. Changes in incidence of take-all followed changes in the yield of the monoculture closely. At Rouvroy, take-all did not occur in every year, but was always more acute in monoculture than in wheat in rotation (Table 3). It is possible that other limiting factors (Type 3) are also involved, especially at Rouvroy.

TABLE 3

Take-all at Rouvroy.

Year	Tale-all (assessment scale : 0 to 10)				Yield difference between wheat in rotation and Continuous wheat (t/ha)
	Continuous wheat		Wheat after	Wheat after	
	Ploughed	Min. Tillage	maize	sugar beet	
1976	0	0	0	0	+ 0.42
1977	0	0	0	0	+ 1.06
1978	1.3	2.3	0.2	0.3	+ 0.62
1979	1.6	1.4	0.4	0.2	+ 1.31
1980	3.9	3.6	1.7	1.5	+ 0.49
1981	2.6	1.5	0	0.4	+ 1.50
1982	-	-	-	-	+ 2.21
1983	-	0.2	0	-	+ 2.39
1984	-	-	-	-	+ 2.75
1985	-	-	-	-	+ 0.60

Looking for unidentified limiting factors

Analysis and biological tests : an approach to soil sickness

Wheat monocultures and wheat in rotations have been compared in nine trials (including Rouvroy, Boigneville and Aulnay). Soil samples were tested at two levels, applying the multidisciplinary approach to limiting factors related to the soil and the plant developed by Bouhot (1983) and Pierre (1985). Results of field inquiries coupled with more classical analyses (physical, chemical, nematological) are combined to determine with more precision which factors have potential to limit the crop in the locations under study. Continuous wheat can be distinguished from wheat in rotation (Table 4) by a higher total number of potentially-limiting factors. In fact a consequence of continuous wheat is more biological problems in the soil, particularly concerning nematodes, mycorrhizae, and root necroses. Based on tests on wheat in rotation as well as on continuous wheat, a hypothesis of a biological limiting factor has been proposed for Rouvroy. It is that soil-borne pathogens are causing root necrosis (Huet 1985).

Quantification of root health (Rouvroy)

Huet (1984,1985) quantified root health in continuous wheat and wheat in rotation and studied this in relation to growth and accumulating yield at main growth stages (Table 5).

At Rouvroy, as at other locations, the roots of wheat in monoculture were no more necrotic than those of wheat in rotation. At flowering + 350° Cd at

Rouvroy some components of yield (number of ears, and tillering) were related to the amount of root necrosis. Lucas (1985) showed that continuous wheat (10th) soil at Rouvroy had a low receptivity to the take-all fungus, confirming most observations made on the disease decline phenomenon after 3-5 consecutive years of wheat (Table 3). Other organisms may have caused root necrosis, but it is not known which.

TABLE 4

Numbers of limiting factors present in different rotation.

	MEAN OF 9 TRIALS		MEAN OF 3 TRIALS		
	Wheat in rotation	Continuous wheat	Wheat in rotation	Continuous wheat	
				2nd wheat	7th wheat
Number of limiting factors	4.8	6.1	4.3	5.7	4.3
Number of limiting factors differentiating both rotations	0.7	2.3	1.0	2.3	2.3

TABLE 5

Development of root necrosis in different rotations at Rouvroy : Average RNI (Root Necrosis Index) is expressed as % of roots showing severe necrosis. The standard deviation is in parentheses.

Growth stage	10th wheat	Wheat after sugar beet	Wheat after maize
G.S.30	69 % (7)	68 % (5)	61 % (9)
Flowering + 350° Cd	34 % (12)	28 % (6)	34 % (7)

Attempts to eliminate supposed, but unidentified limiting factors

Soil disinfection

Methyl bromide was applied in autum 1980 at Rouvroy. In 1981 the number of ears and yield (6.6 t/ha) were similar in continuous wheat and wheat in rotation in treated plots whereas untreated wheat yielded 4.6 t/ha. We concluded that whatever the limiting factors were, they were eliminated by methyl bromide treatment. In 1983, treated plots were lush and logded completely.

Break crops

Breaking continuous wheat with peas or sugar beet was tested several times at Rouvroy (Fig. 3) and at Aulnay. Wheat following a break crop yielded as much as wheat in rotation. Nevertheless, this effect did not last and yields decreased in the second wheat after a break to the level of continuous wheat yields.

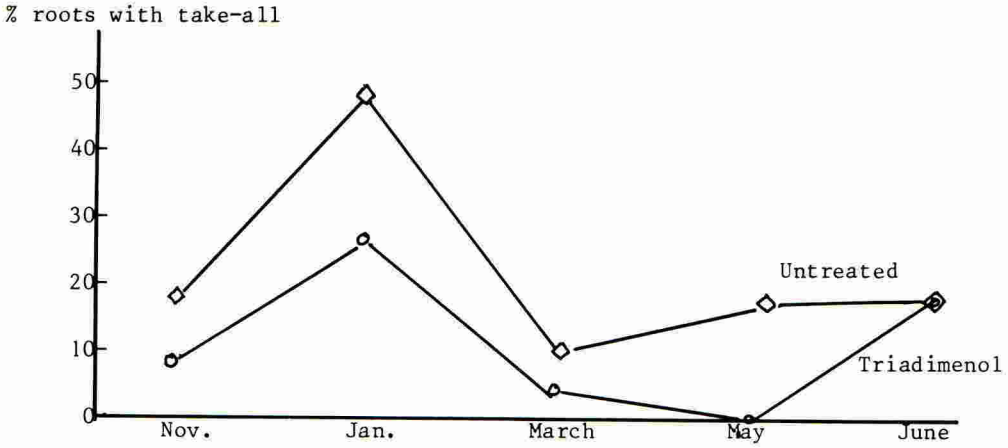
Pesticides in the seed bed (Chêne Chenu, Thymerais)

An experiment at Chêne Chenu, where yield of continuous wheat were less

than those of wheat after peas, tested fungicides in the seedbed and subsequent applications to soil. The chemicals used (triadimenol, metalaxyl, hymexazol, fosethyl-Al, aldicarbe, copper) did not control the development of root necrosis according to assessments of RNI (root necrosis index, where 0 = no necrosis, and 0.5, 1, 2, and 3 = 0.1, 0.5, 0.67 and 0.75 of the root system affected, respectively). Some chemicals partly controlled the quantity of certain pathogic agents associated with necrosis. Triadimenol partially controlled take-all (Fig. 3) without affecting the mycoflora associated with necrosis (e.g. Pythium spp. Fusarium spp.).

Fig. 3

Effect on take-all of spraying triadimenol onto the seed bed.



DISCUSSION AND CONCLUSIONS

The results show that wheat monoculture had a pernicious effect on yield. The component yield most affected was the number of ears and this was affected as early as tillering in normal cultural practice. The introduction of a break crop improved yield but this was shortlived (one year only). Part of the observed decrease in yield may be attributable to known causes : nematodes and espacially take-all appear to be limiting factors that are related to these pernicious phenomena. However sugar beet, a break crop which has little effect on take-all allowed a following wheat to regain the yield level of wheat rotation. Thresholds for nematodes were not reached, yet pernicious effects were obvious. Besides identified factors, factors that remain unidentified seem to be involved. First, therefore, it is necessary to study all limiting factors thoroughly in order to suggest to farmers and technicians those methodes we can trust to give normal yields in the most efficient and cheapest way. The diagnoses suggested so far have been based mainly on examinations of the aerial organs of wheat, but the work described here, clearly shows that limiting factors have also to be looked for in soil. Our research will now be directed towards a study of roots, which seem to be the key to understanding important variations in yields. INRA and ITCF have decided to collaborate on "Growth, development and function of the wheat root system". This work aims at creating physical, chemical and biological conditions that are best for sowing cereals and determine 60 per cent of final yield : maintaining the good start by using appropriate cultural techniques; and following progress by frequent diagnosis. To make a diagnosis it is necessary to have hypothesis about poor root function and then to check this hypothesis to prove that it really provides an explanation. On this matter

we have not advanced beyond stating that limiting factors can be seen to operate as early as tillering. The questions remain very general ones. Is root development disrupted ? (we can at least say that they do not work well). Is there a problem of mineral absorption ? Are there defficiencies or toxicities ? Which factors come into play ? Are pathogens responsible for the disruption and if so are they fungi bacteria, or some other group ? Are we confronted by a complex phenomenon where usefull biological agents are lacking, or harmful agents are present ? Are those agents disrupting the normal cycles of nutritive elements in soil ? From the general biological test made by Bouhot on the trials, we know that biological components in the soil play a very important role at the level of limiting factors. The gap between biologically-essential processes in soil and agricultural practice has to be brigded. A very detailed study of simple, identified factors is necessary, for they play an important part in take-all and nematode infestation. It is also important to develop research on other biological agents, which might prove essential like the genus Pythium (Cook & Haglund, 1982), for instance. Synthetic criteria which may explain differences in yields should be sought to classify hypotheses and to give research the right direction for practical agricultural relevance. It would be interesting to test a few recent hypotheses in long term trials such as those refered to in this paper. Another possible direction would be the use of chemical seed coatings or soil treatments, but that would not preclude the need for a fundamental study of the way these substances affect biological agents. Such information is necessary to tailor advice to each particular field.

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EFFECTS AND CONTROL OF CEREAL ROOT-KNOT NEMATODE IN BARLEY/GRASS ROTATIONS

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ABSTRACT

The effects of cereal root-knot nematode (Meloidogyne naasi) on yield of spring barley have been measured in trials on farms. A one year break-crop of spring oats controlled the nematode without causing differences in those soil factors, such as acidity and overcompaction, which have hitherto complicated interpretation of M. naasi effects on barley. The yields of spring barley following oats were substantially increased compared with those of successive spring barley crops. Observations on the population dynamics of M. naasi in cereal/grass rotations confirmed the value of the non-hosts oats and peas as useful break-crops. Host cereals (barley and wheat) supported dense populations whilst grass leys maintained and sometimes increased M. naasi numbers. Only where presowing population density was very low did grass leys suppress M. naasi to levels below those which would cause damage to spring barley.

INTRODUCTION

The cereal root-knot nematode (Meloidogyne naasi) is common in Wales and south-west England, has been found in several southern and midland English counties and also occurs as far north as Lancashire and Yorkshire (Cook & York, 1980). It has been recorded more frequently in recent years (Empson, 1982), quite probably as a consequence of the decline in the area sown with oats and its replacement with barley in cereal/grass rotations. Damage to spring sown barley has been associated with soil problems, particularly acidity, waterlogging or compaction. This has led to a belief that M.naasi may not be a pest on its own. However, in experiments at the Welsh Plant Breeding Station it caused significant yield loss of barley in field plots and in glasshouse pot tests in the absence of other stress factors (York, 1980). In these tests grain yield was reduced by 3.5% for every 10 juveniles/g soil at sowing. The only present practical means of control is rotation with a non-host crop. Oats, brassicas and potatoes are widely reported as poor hosts and may be expected to give adequate control in a single season (Cook, 1972, Gooris & D'Herde, 1976).

We report observations on Welsh farms on the control of M.naasi by non-host crops, the resulting effects on yield of spring barley and on population changes in cereal/grass rotations and their implications for control.

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MATERIALS AND METHODS

Yield estimates

Assessments of yield responses following control of M. naasi were made on farms in the old county of Pembrokeshire, Dyfed. Fields infested with M. naasi were selected and farmers invited to cooperate by sowing up to half a field with spring oats, and the remainder with spring barley. Nematode populations were monitored and in the following year the yields of spring barley on both areas of the field were recorded. The objective of this approach was to demonstrate and assess M. naasi control in a practicable way, but with minimal effect on husbandry and soil factors.

Soil samples for nematode population estimates were collected in winter or early spring when the eggs had been exposed to low soil temperatures and would hatch readily (Franklin et al., 1971). Subsamples of soil were placed on extraction trays (Whitehead & Hemming, 1965) and juveniles counted after 7 days at 20°C. Estimates of invasion were made on roots of plants dug with a hand trowel and washed free of soil. Galls were counted on randomly selected plants or tillers.

Site 1. Walwyn's Castle

This field had grown spring barley in 1979 and 1980, following a 6-year ryegrass-clover ley. M. naasi damage was observed in 1980 and the post-cropping population was 160 juveniles/g soil. In 1981 half the field was sown with spring oat cv Trafalgar, and half with spring barley cv Varunda. In 1982 cv Varunda was drilled by the farmer across the whole field. Yield trial plots, each 10 drills wide by 22 m long, were sown so that 10 plots followed both barley and oats. M. naasi invasion estimates were made on 13 May and 6 July on the plots, taking 10 and 5 trowel samples per plot and recording galling on 20 plants or tillers. On 6 July 10 samples were taken from the surrounding farm crop. Grain yields were determined on 20 August from 8 x 2 m drill lengths/plot and 2 similar sized bulks cut from the farm crop.

Site 2. Newport

After several crops of spring barley, uneven growth associated with M. naasi galling was recorded in 1981. In 1983 a strip of 2.5 ha of spring oat cv Trafalgar was sown across the field. The remainder of the farm crop (9.5 ha) was spring barley. In March 1984 the farmer's blend of spring barley cvs Patty, Kym and Atem was sown. At harvest (9 August) grain weights were recorded from 350 m long combine cuts (approx. 900 m^2) of barley after oats and barley after barley. M. naasi populations were estimated in spring and winter 1984. Cereal cyst nematode (ccn) (Heterodera avenae) was also present and populations were estimated.

Site 3. Dale

Several crops of spring barley preceded a ryegrass ley undersown in the 1979 crop following M. naasi damage to the 1978 barley. In 1983 about half the field (1.25 ha) was sown with spring oat cv Orlando and half with spring barley cv Atem. In 1984 the whole field was sown in March with Atem. Yield estimates were made from combine cuts of 500 m^2 from both areas on 30 July. M. naasi population estimates were made on samples taken in spring and winter, 1984.

Site 4. Dale

This 5 ha field had grown potatoes on half and barley on half in 1982 and had regularly suffered the symptoms of M. naasi infestation. In 1983 spring oat cv Orlando and barley cv Atem were sown at right angles to

this previous cropping division. In the following year cv Atem was sown on the whole field in late April. Yield estimates were made on 1 August by cutting 8 plots, each 1 m^2, from each of the four areas. Nematode populations were estimated from each area in spring 1983 and spring and winter 1984.

Site 5. Castle Morris

In 1984, spring barley was the sixth consecutive barley on half this field; on the other half the same barley cultivar followed a 1983 swede crop. No initial nematode samples had been taken but galling observations were made in June 1984. Each area was harvested separately and grain and straw yields recorded.

Population dynamics

Observations over several years of cereal/grass cropping have been made on two sites. At the WPBS cereal yield trial site at Llanon, Dyfed, regular cropping with winter and spring barley and wheat in rotation with short term ryegrass leys had led to 4 fields becoming infested by M. naasi. The uniformity of the sites was disturbed and attempts to control the nematode were made by concentrating oat trials into uniform blocks, by growing non-host break-crops of oats or forage peas, and by using spring fallows before sowing ryegrass leys. Nematode populations were monitored in 20 blocks of from 0.7 to 1.5 ha with uniform cropping. Each block was sampled in winter or early spring to estimate post-cropping M. naasi numbers. The means for adjacent areas with identical cropping were calculated.

The second series of observations on M. naasi population changes was made at WPBS, Plas Gogerddan farm. A field infested by M. naasi was sown in 1980, half to spring cereals (barley and wheat) and half to a ryegrass-clover ley. Cropping with spring oats, spring barley and Italian ryegrass (sown annually) was introduced in 1983 with 4 plots of each cereal and 2 of ryegrass on each half of the field. Plots were 40 x 10 m and M. naasi populations were monitored post-cropping for the 3 years of the experiment.

RESULTS

Yield estimates

1982 trials

Yields from the plot trial and farm crop at site 1 are recorded in Table 1, with estimates of nematode galling. In April 1982 the barley after barley was less vigorous than that following oats, but by mid-May there were no visual differences between the crops. However in mid-June barley following oats was earlier with uniform ear emergence, whilst that after barley was later with uneven ear emergence giving the crop a patchy appearance typical of M. naasi infestation. There were clear differences in the extent of galling: the persistence of some M. naasi after oats as indicated by galling (Table 1) was attributed to ryegrass undersown in the 1981 crops. Grain yields were markedly increased following oats in both plots and farm crop. The differences in galling and yield were statistically significant according to a t-test at 0.05 probability.

1984 trials

The pre- and post-cropping nematode densities and grain yields after host and non-host crops in 1983 are presented in Table 2. At site 2

TABLE 1

Yield of spring barley following oats or barley, and infection by Meloidogyne naasi, Site 1, 1982

Crop 1981	1982	M. naasi May 1982 (galls/plant)	M. naasi July 1982 (galls/tiller)	Grain yield (t/ha @ 85% d.m.)
Barley	barley plots	16.9 ± 3.42[a]	4.6 ± 2.11	3.40 ± 0.423
Oats	barley plots	5.8 ± 2.45	1.3 ± 0.80	4.33 ± 0.420
Barley	barley crop	-	7.2	2.70
Oats	barley crop	-	1.7	5.35

(a) Means ± standard deviation

growing the ccn-resistant oat cv Trafalgar reduced populations of this nematode to less than 1 egg/g soil compared with 14 eggs/g after barley. In 1984 these populations increased slightly (to 3 and 25 eggs/g) on the susceptible barley crop. At this site yields of spring barley were 27% greater where both nematodes were controlled. At site 3 where only M. naasi was present, yield of barley was 34% greater after oats. On site 4 a small yield increase (8%) was recorded where barley and oats followed

TABLE 2

Yields of spring barley following barley or a non-host crop and populations of Meloidogyne naasi at 4 sites in 1984

Site	Cropping 1982	1983	1984	M. naasi (juveniles/g soil) pre-cropping	post-cropping	Area harvested	Grain yield t/ha @ 85% d.m.
2	barley	barley	barley	42	185	900 m^2	4.35
	barley	oats	barley	4	34	900 m^2	5.54
3	ley	barley	barley	20	95	500 m^2	3.99
	ley	oats	barley	2	3	500 m^2	5.34
4	barley	barley	barley	228	109	8 x 1 m^2	5.45 ± 1.375
	barley	oats	barley	6	16	8 x 1 m^2	6.89 ± 1.535
	potatoes	barley	barley	19	28	8 x 1 m^2	5.81 ± 0.607
	potatoes	oats	barley	1	2	8 x 1 m^2	6.28 ± 0.501
5	barley	barley	barley	-	612	2.18 ha	3.26
	barley	swedes	barley	-	206	2.55 ha	4.02

TABLE 3

Meloidogyne naasi populations (juveniles/g soil) after cereal, grass and break-crops at Llanon, 1978-1983

Field	A		B		C		D	
	Crop[a]	*M. naasi*[b] (1)	Crop	*M. naasi* (3)	Crop	*M. naasi* (2)	Crop	*M. naasi* (2)
1978	o	0	c	3 ± 4.5	g	75 ± 34.7	g	101 ± 46.0
1979	o	-	c	-	g	-	g	-
1980	o/g	0	c	36 ± 27.8	g/f	11 ± 3.5	g/f	32 ± 11.3
1981	g	1	p	7 ± 4.0	c	40 ± 11.3	c	57 ± 37.5
1982	g	2	g	48 ± 16.3	c	615 ± 120.2	o	11 ± 9.2
1983	g	1	g	21 ± 15.3	f/g	5	f/g	1 ± 0.4

Field	E		F		G		H	
	Crop	*M. naasi* (4)	Crop	*M. naasi* (2)	Crop	*M. naasi* (3)	Crop	*M. naasi* (3)
1978	g	35 ± 40.1	g	14 ± 5.7	c	76 ± 7.4	o	5 ± 2.7
1979	g/f	-	g	-	c	-	o	-
1980	c	44 ± 25.6	g	-	g	49 ± 18.5	g	11 ± 8.5
1981	c	23 ± 33.9	g	11	g	14 ± 3.5	g	8 ± 2.7
1982	c	86 ± 130.8	g	23	g	54 ± 33.3	g	107 ± 55.9
1983	f/g	3 ± 2.9	g/f	1	p&o	2 ± 1.0	p&o	1 ± 0.6

(a) o = oats; c = cereals (barley and wheat); p = peas; g = perennial or Italian ryegrass ley; f = fallow before summer reseeding or after summer ploughing of ley.

(b) mean ± standard deviation, number of areas sampled in parentheses.

potatoes. Where yields of continuous barley were compared with those after a one year oat break the increase associated with M. naasi control was 26%. There were high sampling errors associated with yield determination at site 4 and neither of the differences is statistically significant. This field was sown much later than the others and in the dry 1984 spring it is likely that nematode invasion was reduced. This suggestion is supported by the observation that at sites 2 and 3 marked M. naasi population increases occurred on the susceptible barley whilst at site 4 there were no or only small increases.

At site 5 in mid-June the barley following swedes was taller and more uniform that that following barley. Ear emergence was several days earlier as was noted on site 1 in 1982. Galls were conspicuous on roots of the sixth barley and only a few very small galls were found on the crop after swedes. In addition to the grain yield increase the farmer recorded an improved straw yield on the healthy crop, 137 compared with 124 bales/ha.

Population dynamics

Population changes over six years at Llanon are represented in Table 3. In the 6 years the overall mean annual populations were reduced from 39 juveniles/g after 1978 to 4/g after 1983. Populations were always low after oats and generally high and increasing after barley and wheat, sometimes to very high densities. Continuous oats and other non-host breaks (peas, peas with oats, or a spring fallow), reduced M. naasi numbers. In 1981 the population in field B after peas (7 juveniles/g soil) was less than had been expected due to the crop being infested with meadow grass (Poa annua), a very good host of M. naasi. The 1983 crops of peas with oats in fields G and H reduced nematode numbers by about 95%. In some fields grass leys maintained M. naasi populations or even supported an increase (fields H and G), whereas in others low initial population densities remained low under grass (field A).

TABLE 4

Populations of Meloidogyne naasi after spring-sown barley, oats or Italian ryegrass at Gogerddan, 1983-1985

Cropping to 1982	from 1983	M. naasi juveniles/g soil post cropping (1982)	1983	1984	1985
Grass/clover	barley		19 ± 8.3[a]	77 ± 49.1	70 ± 38.5
Grass/clover	grass	(121 ± 65.4)	27 ± 0.7	17 ± 8.5	42 ± 21.2
Grass/clover	oats		19 ± 8.3	4 ± 3.2	1
Barley/wheat	barley		37 ± 16.1	86 ± 24.3	17 ± 8.1
Barley/wheat	grass	(237 ± 165.3)	37 ± 1.4	257 ± 71.4	74 ± 10.6
Barley/wheat	oats		43 ± 16.1	7 ± 3.0	1

(a) means of 4 plots (cereals), 2 plots (grass) ± standard deviation

The observations at Gogerddan on continuous cropping (Table 4) showed that in 1983 all crops failed to support the initially high populations. Thereafter populations declined only on oats which provided continuing control. The fluctuations in populations under both barley and grass are characteristic of nematodes, influenced by sampling variation, seasonal factors and host-mediated density effects. The annually sown Italian ryegrass plots supported M. naasi at levels which might be expected to cause damage to spring barley. The previous cropping with long term ley or with cereals and annual ploughing had no apparent effect on the suitability of the soil for M. naasi.

DISCUSSION

These farm and plot observations clearly demonstrate the value of oats as a break-crop on soils infested with cereal root-knot nematode, the yields of following spring barley crops having been increased by more than 1 t/ha on most sites. Control of other barley diseases may have played a part in this effect although no differences in root and foot rots were apparent. Other soil factors, such as overcompaction, waterlogging, acidity, often associated with suspected nematode damage, were not affected by cropping with oats. These yield results, together with the nematode population data lend support to the statement, based on controlled field and pot experiments, that M. naasi can be a primary cause of yield loss in spring barley (York, 1980).

The control of M. naasi by oats is long-term and appears to be stable. It has been suggested that because oats vary in their host status (Cook, 1972; Siddiqui & Taylor, 1970) and because some M. naasi populations differ in their host range (Mitchell, et al., 1973) there may be a threat to resistant crops through the selection of virulent M. naasi. This potential problem did not occur in our experiments. Where M. naasi is present the use of grass leys, either short or long term, will not provide reliable control for barley growing. Similar observations can be adduced from French observations (Caubel et al., 1971). It is clear that grasses are very heterogenous in respect of host reaction to M. naasi: fully susceptible and resistant genotypes having been isolated from cultivars (Cook & York, 1985).

Changes in cereal growing have led to the oat area declining from a dominant proportion to only 3% of the cereal area in England and Wales. The use of spring barley in cereal/grass rotations has meant cropping with continuous hosts of M. naasi on many mixed farms in Wales and the west. Our observations have failed to show any decline in M. naasi in this situation, such as has occurred in parts of Britain with ccn in intensive cereal cropping. However a further change in cropping practice may lead to a further change in M. naasi populations in that there is some evidence that winter-sown barley supports fewer M. naasi than the spring crop. We have monitored populations in 3 fields on one farm where after 7 consecutive winter barley crops populations stabilised at around 10 juveniles/g soil. In one field the population under the last spring sown crop was 1000/g soil. On that field the first winter sown crop suffered some damage, estimated by regression analysis of small plot data as 0.18% for every 10 juveniles/g soil (Cook, et al., 1980). The decline of

M. naasi on these winter-sown crops may occur because root development and juvenile emergence occur at different times with relatively few young main axes suitable for invasion in spring.

On those farms where grazing and forage conservation are important and cereal cropping also valued - both for home consumption and to benefit from improved soil fertility after leys - there remains a danger from M. naasi. Both components of grass/cereal rotations may be damaged. However the monitoring of M. naasi populations is straightforward and there are acceptable breakcrops which should allow damange to be avoided.

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SEASONAL CHANGES IN POPULATIONS OF *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* (EYESPOT) IN WHEAT CROPS

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ABSTRACT

Plots in a fourth successive winter wheat crop, previously untreated with carbendazim (MBC) fungicides, were inoculated in October 1984 with mixtures of MBC-sensitive and MBC-resistant isolates of either W or R pathotypes of *Pseudocercosporella herpotrichoides*, or were uninoculated. Plots were either unsprayed or sprayed in November 1984 and April 1985 with carbendazim, prochloraz, or carbendazim plus prochloraz. Sprays were repeated in a fifth wheat in 1985/86. Fifteen per cent of isolates obtained in June 1984, before inoculation, were R-type, 3% were MBC-resistant and none were both. Proportions of R-type isolates and of MBC-resistant isolates in uninoculated, unsprayed plots increased during the 1984/85 season but changed little between July 1985 and April 1986. The main effect of carbendazim was to increase the proportion of MBC-resistant isolates and the main effect of prochloraz was to increase the proportion of R-type isolates.

INTRODUCTION

The proportions of different pathotypes in U.K. populations of *Pseudocercosporella herpotrichoides* (Fron) Deighton, the cause of eyespot in cereal crops, have changed greatly over the last decade (Hollins *et al.* 1985). The predominant pathotype formerly was W-type (wheat-type), which is more pathogenic to wheat than to rye, but is now R-type (rye-type), which is equally pathogenic to wheat and rye. W-type isolates produce colonies with smooth margins on potato dextrose agar (PDA); R-type isolates produce colonies with feathery margins and their radial growth rate is generally about half that of W-type isolates (Scott *et al.* 1975). Two varieties of *P. herpotrichoides* have been described by Nirenburg (1981) and it has been suggested that W-type and R-type isolates are *P. herpotrichoides* var. *herpotrichoides* and var. *acuformis* respectively (King & Griffin 1985).

The incidence of carbendazim(MBC)-resistance in U.K. populations of *P. herpotrichoides* has increased greatly, as a consequence of the regular use of MBC fungicides (Bateman *et al.* 1985, Hollins *et al.* 1985, King & Griffin 1985). MBC-resistant isolates can grow on media containing 1 µg/ml of carbendazim whereas MBC-sensitive isolates cannot. Thus, U.K. populations of *P. herpotrichoides*, which were mainly composed of W-type MBC-sensitive strains, are now predominantly composed of R-type MBC-resistant strains. The increase in the incidence of MBC-resistance, and resultant control failures, has led to greater use of prochloraz, which is active against both MBC-sensitive and MBC-resistant isolates, and of mixtures of prochloraz and MBC fungicides. In field surveys on commercial farms the proportion of R-type isolates has been shown to increase between spring and

summer (King & Griffin 1985, Coskun *et al.* 1986). In a field experiment at Long Ashton the proportion of MBC-resistant isolates recovered had increased from 0 to 90% after 3 years of carbendazim use (Hoare *et al.* 1986). However, changes in the proportions of the different strains in response to different fungicide regimes have not been followed in detail. This paper reports interim results from a long-term field experiment at Rothamsted to investigate the changes in populations of *P. herpotrichoides* in winter wheat crops under different fungicide regimes.

MATERIALS AND METHODS

Field experiment

The experimental site, which had not been previously treated with MBC fungicides, had grown three consecutive winter wheat crops. Plants were sampled from the third crop in June 1984 and the eyespot pathogen population was characterized. Seed of winter wheat (cv Avalon) was sown on 4 October 1984 at 172 kg/ha. Plots were left uninoculated, or were inoculated on 18 October with one of four mixtures of isolates, grown separately on sterilised oat grains: 95% MBC-sensitive plus 5% MBC-resistant W-type isolates; 5% MBC-sensitive plus 95% MBC-resistant W-type isolates; 95% MBC-sensitive plus 5% MBC-resistant R-type isolates; 5% MBC-sensitive plus 95% MBC-resistant R-type isolates. Plots were sprayed in November 1984 and April 1985 with carbendazim, prochloraz, or carbendazim plus prochloraz at the recommended rates, or were left unsprayed. There were two blocks of four plots, one for each fungicide treatment, split into six sub-plots (6.0 x 4.5 m) for inoculum treatments (two sub-plots per plot uninoculated). Sub-plots were separated by a minimum width of 3 m of the same crop. The plots were again sown to winter wheat in October 1985 and spray treatments were repeated in November 1985 and April 1986.

In April 1985 and 1986 two plants per row were sampled from alternate rows along three diagonal transects across each plot (*c.* 100 plants). In July 1985 plants from ten 20 cm row lengths were sampled from each plot (*c.* 60 plants). The presence of visible eyespot lesions on each plant (April and July) or stem (July) was recorded and infected shoot bases from each plot were stored at -15°C until the fungus could be isolated.

Characterization of isolates from eyespot lesions

A modified version of the procedure described by Bateman *et al.* (1985) was used. A single lesion was cut from each infected plant sampled. Lesions from each plot were surface sterilized in sodium hypochlorite (1% available chlorine) for 5 min and rinsed in sterile distilled water before placing them on moist filter paper in Petri plates. After 2 weeks at 10°C under near-ultra-violet light to induce sporulation of *P. herpotrichoides*, each lesion was placed in a small autoclaved bottle containing 3 ml of 0.01% Tween-80. After shaking, 1 ml of spore suspension was spread over the surface of each of two PDA plates, one containing 1 µg/ml carbendazim and one with none. After 2 weeks of incubation at 20°C the presence or absence of *P. herpotrichoides* colonies on each plate was noted. *P. herpotrichoides* was subcultured onto PDA from plates with colonies, and after a further 2 weeks at 20°C isolates were characterized as W-type or R-type on the basis of colony morphology. In 1985, isolates were also tested for growth on PDA containing 1 µg/ml prochloraz. Frequencies of recovery of isolate types are presented as percentages of all plants sampled and as percentages of the total number of isolates from each plot. Because some plots yielded only small numbers of isolates the statistical analysis is not straightforward and it has not yet been completed.

RESULTS

Effects of fungicides on the incidence of eyespot

Eyespot infection was recorded on more plants in July than in April 1985 (Table 1). The incidence in April 1986 was slightly less than that in July 1985. Eyespot was decreased by treatments including prochloraz, but not by treatment with carbendazim alone. *P. herpotrichoides* was recovered from 30% of the lesions in April 1985 and 1986, and from 70% of the lesions in July 1985.

TABLE 1

Percentage of plants with visible eyespot lesions in plots, which were untreated, or treated with carbendazim (C), prochloraz (P), or carbendazim plus prochloraz (C + P). Mean of all inoculum treatments.

Date	Percentage plants infected				
	Treatment				Overall mean
	Nil	C	P	C + P	
April 1985	42	44	14	7	26.7
July 1985	75	73	38	31	54.4
April 1986	68	67	30	31	49.4

Changes in pathotype frequencies

In June 1984, 85% of the *P. herpotrichoides* isolates recovered from the wheat crop before the start of the experiment were W-type and 15% were R-type. In uninoculated plots untreated with fungicides the proportion of R-type isolates recovered had increased to 32% by April 1985 (Table 2).

TABLE 2

Frequency of W-type and R-type isolates from plants with lesions, expressed as percentages of all plants sampled

Treatment		Nil			Carbendazim			Prochloraz		
Sampling date		Apr 1985	Jul 1985	Apr 1986	Apr 1985	Jul 1985	Apr 1986	Apr 1985	Jul 1985	Apr 1986
Inoculum	Pathotype recovered									
None	W	3.4	31.0	16.9	0.9	24.4	11.6	0.4	4.5	3.6
	R	1.6	26.7	9.1	0.5	13.8	15.4	0	7.8	11.4
		(32)*	(46)	(35)	(36)	(36)	(57)	(0)	(63)	(76)
W-type	W	39.2	48.1	17.2	11.8	62.0	20.1	5.5	21.1	2.6
	R	0	7.6	5.3	0.2	3.8	3.5	0.2	3.5	4.1
		(0)	(14)	(24)	(2)	(6)	(15)	(4)	(14)	(61)
R-type	W	2.1	1.1	6.4	0	3.8	4.0	0	0.5	1.3
	R	14.2	41.7	18.8	16.8	43.1	9.0	3.1	43.0	11.9
		(87)	(97)	(75)	(100)	(92)	(69)	(100)	(99)	(90)

* Figures in brackets are the percentages of isolates which were R-type.

There was no further increase between April 1985 and April 1986. Where the untreated plots were inoculated, the predominant pathotype remained so throughout the experiment, although there was some increase in the proportion of R-type isolates recovered from plots inoculated with W-type isolates, and *vice versa*. Treatment with carbendazim produced changes in the proportions of W and R pathotypes which were similar to those in untreated plots. Although the data is based on a few isolates, treatment with prochloraz considerably increased the proportion of R-type isolates recovered from uninoculated plots and plots inoculated with W-type isolates. Results for prochloraz plus carbendazim treatments were similar to those for prochloraz alone, and are not included.

Changes in fungicide sensitivity

In June 1984 3% of the *P. herpotrichoides* recovered from the experimental site were MBC-resistant. The proportion of MBC-resistant isolates increased, both in the uninoculated plots and where the inoculum was predominantly MBC-sensitive, no matter what treatment was applied (Table 3). However, where the inoculum was predominantly MBC-resistant, the proportion of MBC-resistant isolates declined in the absence of fungicide treatment. Treatment with prochloraz increased the proportion of MBC-resistant isolates in some plots, but few isolates were recovered from these plots. No resistance to prochloraz was found in isolates made in 1985.

TABLE 3

Frequency of MBC-sensitive and MBC-resistant isolates from plants with lesions, expressed as percentages of all plants sampled

Treatment		Nil			Carbendazim			Prochloraz		
Sampling date		Apr 1985	Jul 1985	Apr 1986	Apr 1985	Jul 1985	Apr 1986	Apr 1985	Jul 1985	Apr 1986
Inoculum	Isolate type recovered									
None	Sensitive	4.8	42.5	19.5	0.8	3.9	1.1	0.2	7.4	6.8
	Resistant	0.2	15.2	6.5	0.6	34.1	25.9	0.2	4.9	8.2
		(4)*	(26)	(25)	(43)	(90)	(96)	(50)	(40)	(55)
95% Sensitive	Sensitive	21.7	26.0	14.4	2.9	4.6	0.6	2.0	21.8	2.4
	Resistant	1.2	18.0	7.6	3.9	46.7	12.8	0	7.8	3.7
		(5)	(41)	(35)	(57)	(91)	(96)	(0)	(26)	(61)
95% Resistant	Sensitive	2.8	2.8	8.5	1.0	0	1.1	1.0	4.6	2.3
	Resistant	29.9	53.5	16.1	21.0	61.4	22.1	4.8	33.8	11.5
		(91)	(95)	(65)	(95)	(100)	(95)	(83)	(88)	(83)

* Figures in brackets are the percentages of isolates which were MBC-resistant.

DISCUSSION

The results presented suggest that, even in the absence of a fungicide treatment which selects for resistant types (e.g. carbendazim), changes in populations of *P. herpotrichoides* within a season (comparing April and July samples) can be greater than those occurring between seasons (comparing

successive April samples). For example, between April and July 1985 there were large changes in the proportions of W-type and R-type isolates, and of MBC-sensitive and MBC-resistant isolates, recovered from unsprayed plots and from plots sprayed with prochloraz. However, in these plots, the predominant pathogen type remained the same. These changes must be interpreted with some caution because *P. herpotrichoides* was not recovered from all the lesions identified as eyespot, and only a few isolates (1-40, average 5) were recovered in April from plots where the more effective treatments containing prochloraz were applied.

The overall increase in R-type strains relative to W-type strains within the 1985 season is consistent with similar increases recorded during surveys on commercial farms (King & Griffin 1985; Schreiber & Prillwitz 1985; Coskun *et al.* 1986). The increased proportion of R-type isolates was largely maintained through to the second April sampling, especially in plots treated with prochloraz. While prochloraz may have some effect in selecting for R-type strains (possibly a significant occurrence in view of the present dominance of R-type strains and widespread use of prochloraz), the changes within a season may be a result of differences in the optimum environmental conditions for infection between the two pathotypes. This possibility is currently being investigated. The two pathotypes may also differ in their interactions with other pathogens and secondary colonizers of eyespot lesions; there is some evidence, for example, that R-type isolates may compete better than W-type isolates with *Fusarium* species (A. Goulds pers. comm.).

The increase in the proportions of MBC-resistant isolates recovered from plots treated with carbendazim was more rapid than might have been expected from experience on commercial farms where MBC fungicides had been used for several years before control failures were noticed in 1981 (King & Griffin 1985). The increases in the proportions of MBC-resistant strains were maintained into the second spring of the experiment. Increases in the proportions of MBC-resistant isolates in untreated predominantly MBC-sensitive plots between spring and summer are less easily explained. Spray drift seems unlikely to have been responsible, as carbendazim-treated plots were separated from other plots by a minimum distance of 12 m.

In some plots there was an apparent association between increases in the proportion of R-type strains and MBC-resistance in the present experiment (Tables 2 and 3). Some survey data suggest a positive correlation between the incidence of R-type strains and MBC-resistance (King & Griffin 1985), although such links have not always been found (Brown *et al.* 1984) and the two characters may be selected independently (Hoare *et al.* 1986).

Detailed monitoring of this experiment will continue, thereby providing opportunities to confirm within-season pathotype changes, to relate these to optimum weather conditions for the two pathotypes and to observe any associations between pathotypes and stem lesion severity. Also, the stability of MBC-resistance in the populations, and its relationship, if any, to pathotype, will be examined.

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SESSION 4B

METABOLISM OF PESTICIDES IN PLANTS, ANIMALS AND THE ENVIRONMENT

CHAIRMAN DR. H. O. ESSER

SESSION ORGANISER DR J. P. LEAHEY

INVITED PAPERS 4B-1 to 4B-5

MODERN STANDARDS IN PESTICIDE METABOLISM STUDIES

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ABSTRACT

Pesticide metabolism studies are designed to assess the fate and the nature and extent of formation of degradation products of a pesticide in plants, in farm animals consuming treated crops and in laboratory animals used for toxicity assessment. Environmental fate studies are needed to determine whether the pesticide and its degradation products pose a potential hazard to man, to non target organisms, to usable land, water and wildlife resources, by direct or indirect exposure. The generated data, together with toxicity and residue results allow safety assessment for the pesticide. The various studies and the important parameters to be observed in these studies are described in detail. Modern metabolism studies can only be conducted using radiolabelled active ingredients. Suitable positions for radiolabelling, the purity and amounts of radiochemical required and the equipment required in metabolism work are discussed. The metabolic transformations to be expected are also summarised. Some ideas concerning registration guidelines and Good Laboratory Practice (GLP) conclude this overview.

INTRODUCTION

Much of the success of modern agriculture is due to pesticide technology. Before a pesticide can be used, numerous studies will have been conducted by the Agricultural Industry in order to establish its safety for the applicator, the consumer and the whole environment. The results of these, often extremely expensive, studies answer questions relating to toxicology, metabolism, residues and environmental behaviour of pesticides.

The metabolism studies will determine the nature of the chemical residue which may result from the use of a pesticide, in crops, animal feed, farm animals and the general environment. The metabolism of the pesticide in laboratory animals, used for toxicity assessment, will also be studied. These metabolism studies can only be carried out using radiolabelled pesticides. This paper will describe the types of study which must be conducted and the results/conclusion which can be obtained.

SCOPE OF METABOLISM STUDIES

Plant Metabolism Studies

Plant metabolism studies are needed to characterize the composition of the "terminal residue" in plants resulting from the use of a pesticide. The objectives of plant metabolism studies are (1) to provide an estimate of "total terminal" or "radioactive residues" (TRR) in the treated crop examined, (2)

to obtain information on the distribution of residues in plants for defining systemic properties of the pesticide, (3) to show the efficiency of extraction procedures for various components of the residue, (4) to identify the major components of the TRR, (5) to characterize minor components of the TRR, (6) to indicate potential concentrations of residues when crops are processed, and (7) to define the components to be looked for in residue quantitation studies.

Plant metabolism studies must be conducted for each type of plant for which use of the pesticide is proposed and in general one metabolism study will be required for each crop group. However, if the results of three metabolism studies on dissimilar crops (e.g., root crop, oilseed and a leafty vegetable) indicate a similar metabolic route in the three crops, then additional plant metabolism studies in other crops, for which use is proposed, will not be required.

Animal Metabolism Studies

Rat Studies

Most toxicity data is generated with the rat as the test species. Data from studies on the absorption, distribution, excretion, and metabolism of a test chemical help in the evaluation of the test results from toxicology studies and in the extrapolation of data from animals to man. The main purpose of animal metabolism studies is to produce data which fortify the understanding of the safety of the chemical in consideration of its intended uses and anticipated human exposure. The rat study is therefore carried out for the following reasons: (1) To determine the amount and rate of absorption of the pesticide at different dose levels - usually a non-effect level and another one which produces slight toxic effects; (2) to determine the pattern of distribution of the test chemical among tissues, organs, and fluid compartments at different dose levels, after single and repeated dosages and oral and intravenous exposure modes; (3) to identify and, to the extent possible, quantify significant metabolites; (4) to characterize the routes and rates of excretion, in urine, faeces and expired air; (5) to determine any possible bioaccumulation and/or bioretention of the pesticide and/or metabolites; (6) to determine absorption, metabolism, excretion, and distribution as a function of single or repeated doses; and (7) to provide adequate radioactivity balances.

The test species are young male and female (non-pregnant) adults, and sufficient numbers are used to allow statistical treatment of results. Additional studies are sometimes required to clarify possible problems. This could include identification of tissue residues; binding by macromolecules in the blood, liver, gonads, and other tissues; biotransformations by specific organs, tissues, and cell fractions; and also absorption by dermal or inhalation routes of exposure. Additional species are also studied to see if species variations occur, if major differences in metabolism are detected more intensive studies of species variations will be required.

Farm animals

Metabolism studies with farm animals are designed to identify the nature of the residue in products entering the human diet, i. e. meat, milk and eggs. With this information residue methods for quantification of relevant residues can be developed. Animal metabolism studies are required whenever a pesticide is applied directly to livestock, animal premises are to be treated, or residues result in crops or crop parts used for feed. Metabolism studies are required for ruminants and poultry. The species of choice are usually goats and chickens. Non-ruminant (swine) metabolism studies may be required if the rat metabolism study is significantly different from the goat and chicken metabolism. Additional animal studies are required if direct dermal or inhalation exposure of livestock is proposed. These additional studies should reflect the proposed use so that it can be determined whether dermal or inhalation exposure results in the same metabolic pattern as oral dosing.

In order to facilitate the identification of residues, a pilot study in a hen, at a high dose rate (1/10 LD50, rat) may be useful. Identification of the metabolites in the excreta may aid in the analysis of the tissue residues. Subsequent to the pilot study a definite study at a more realistic dose rate will be carried out. The livestock should be dosed with the chemical for at least three days and the animal sacrificed within 6 - 24 hrs of the final dose.

The following results should be provided from livestock studies: (1) The concentration of the TRR in each edible tissue, and milk and eggs where applicable; (2) information on the extractability of the TRR with different solvents at varying pH, or following chemical and/or enzymatic hydrolysis; (3) chromatographic profiles of the metabolites extracted from edible tissue(s) and, if applicable, excreta; (4) structural identification of major metabolites and data on the nature of covalently bound residues.

Environmental Fate Studies

Assessment of the environmental fate is now an extremely important part of the evaluation of a new pesticide. The range and type of study which are carried out are discussed below.

Soil Metabolism Studies

Soil metabolism studies are designed to provide the following information. (1) The rate of degradation of the pesticide in soil and thus an assessment of it´s persistence. (2) An identification of the degradation products formed. (3) The rate of formation and decline of the degradation products. (4) An assessment of the rate at which the pesticide is completely mineralised to carbon dioxide. These studies will thus define the pesticide degradation products to which rotational crops and non-target organisms will be exposed, facilitate assessment of potential disposal problems and indicate which metabolites/degradation products might be available for leaching to groundwater. Information gained from these studies is directly applied to designing and conducting field dissipation and accumulation studies. Degradation under both aerobic and anaerobic soil

conditions will be studied and when an aquatic use of the pesticide is envisaged aerobic and anaerobic aquatic degradation will also be investigated.

Consideration must be given to the soil type(s) used. Normally a light and a heavy soil should be studied. Very critical is the bioactivity of the soils. It is more important to use a biologically active soil than a standard soil, which may be biologically dead. Whether a pesticide is metabolized or degraded abiotically can be determined with a sterile control. The laboratory system used to study soil metabolism should allow for a continuous, slow flow of moist air through the apparatus, with traps to collect evolved volatiles, especially carbon dioxide. The experiment should be temperature controlled and light must be excluded. Completely closed systems have been used, but these are not recommended since the soil may turn anaerobic once the oxygen in the system has been used up. The duration of a soil metabolism study is critical, since the whole system may lose much of its biological activity after 6 months. The value of studies which proceed beyond this time is therefore questionable. Several countries request soil metabolism studies at low(er) temperatures. The need for this information is justified, however, it should be possible to provide suitable studies with unlabelled material(s) using cold residue methodology.

Hydrolysis Studies

Since pesticides can be applied directly to aquatic systems or can enter aquatic systems via leaching or runoff after terrestrial application, knowledge of the hydrolytic fate of pesticides is needed to understand the overall fate of pesticides in the environment. A hydrolysis study provides data on rate of loss of hydrolysis products and the identity of these products.

Hydrolysis studies are usually conducted at 3 to 4 different pH values, e.g. at pH 3, 5, 7 and 9. The pH 3 value simulates stomach conditions in the warm blooded animal and pH 5 to 9 covers the range of expected environmental pH's. Special emphasis has to be given to the composition and stability of the buffer solutions, the temperature, sterility and maintenance of darkness. Cosolvents, if required, must only need to be applied in small quantities in order not to measure solvolysis rather than hydrolysis. Hydrolysis studies should only be performed with compounds which are sufficiently water soluble.

Photolysis Studies

Photodegradation by sunlight is a potential route of environmental degradation for a pesticide. Studies in aqueous systems and also on a soil surface are therefore needed. These studies are important since photolytic breakdown may sometimes generate unique products which are not formed by metabolism in biological systems.

Photolysis studies may be carried out using natural sunlight or artificial sunlight sources. For artificial light sources the nature of the light source, its emission wavelength spectrum and intensity and their relationship to that of natural

sunlight needs to be established. A xenon arc source, filtered to remove wavelengths below 290 nm provides an excellent mimic of sunlight. For sunlight sources the interpretation of the results obtained must take into account the latitude, time of the year, atmospheric cover, and other variables affecting incident light. Studies should continue for at least one half-life or 30 days, which ever comes first. Photoproducts representing more than 10 % of the starting concentration should be identified and volatile photoproducts should be trapped, quantified and, if necessary, identified. In order to distinguish from non-photolysis degradations, dark controls for soil and water photolysis investigations are essential.

Leaching studies

The mobility of the test substance and its degradation products in soil may be investigated in soil columns, by soil thin layer chromatography (soil-tlc) or batch equilibrium (adsorption/desorption) testing. Information gained from these leaching tests can be applied to the design and conduct of field dissipation studies. Results obtained will be an estimate of field movement and will alert as to the depth soil samples will need to be taken.

The soil used to investigate the leaching of degradates must be the same as that used in the aerobic soil metabolism study. The amount of test substance added to the soil should be equivalent to the highest recommended label rate for one application of the active ingredient. Prior to leaching the soil residue of the pesticide should be aged for one half-life or 30 days, whichever is shorter. Another point to consider is the untreated soil column length, which should not be less than 30 cm. The amount of "rain" required to be applied to the column will vary according to the country in which a registration is sought, e.g. for the USA an amount equal to the cross sectional area in cm^2 times 50.8 cm of rain is required. The water, or 0.01-0.02 M $CaCl_2$ solution, is applied without interruption, the water must cover the top of the soil column. The untreated soil portion has to be soaked with water prior to the experiment. After leaching the distribution throughout the column should be investigated in intervals of 5 to 6 cms. A radioactive balance must be made in a leaching study to ensure that losses by volatilisation have not ocurred. The degradation products formed during the study should also be identified.

Rotational Crop Studies

These studies deal with pesticide residue uptake from soil and possible accumulation in rotational crops. Rotational crop studies are necessary to establish realistic crop rotation restrictions (time from application to time when follow-up or rotated crops can be planted) and to determine if tolerances may be needed for residues in such crops. The study will initially determine if residues are taken up by rotated crops. If residues greater than 0.01 mg/kg are detected they must be identified. If the residue is shown to result from the incorporation of radioactivity into the natural plant constituents, e.g. sugars, amino acids, then a tolerance will obviously not be required. Small confined outdoor radioactive plots are well suited and realistic for rotational crop studies.

Rotational crop studies are very lengthy and should be initiated early in the study of a new pesticide. The radioactive pesticide is applied to the soil at the recommended application rate and aged for 30, 120 and 365 days prior to sowing of the rotational crops. These time intervals reflect crop failure, fall and annual replantings. The radioactivity content and identity of major soil metabolites/degradation products should be investigated at each planting date and crop harvest. At least three different crops, a root crop, a broad leaf and a grain crop plus the major expected rotational crop are planted at the three time intervals indicated. Where relevant immature samples in addition to the harvest samples are analysed.

Fish Accumulation Studies

These studies provide predictive information on the accumulative nature of pesticides. A bioconcentration study with fish is conducted when certain criteria apply such as an unfavourable octanol water partition coefficient, relatively long water half-life and high fish toxicity. When it can be proven that the pesticide does not reach water, then this study is not necessary. Data from fish accumulation studies are used to establish label restrictions e.g. to prevent pesticide applications to certain sites, so that there will be minimal residues entering edible fish or shellfish, such as catfish or crayfish inhabiting rice fields. The data may also be used to provide information for the setting of tolerances or action levels in these organisms where necessary. Flow-through exposure studies are recommended. Radioactive residues which have been taken up should be identified.

TYPE OF METABOLITES/DEGRADATION PRODUCTS

Metabolic products are often described as either "free" or "conjugated". Free metabolites (or abiotic degradation products) are usually formed by a range of reactions; e.g. hydrolysis, oxidation, epoxidation, hydroxylation, desulphuration, dehalogenation etc. These free metabolites are usually considered as those extracted with organic solvents and remain in this phase when partitioned against water. Free metabolites are usually resolved and ultimately identified, because they are generally apolar and are relatively easily separated from each other and from other interfering materials in the solvent extract. Their structures are often quite similar to the parent molecule and are formed by "phase I" reactions which are usually highly predictable.

Conjugated metabolites are products of secondary (phase II) metabolism which involves the reaction of the pesticide and its metabolites (exocons) with endogenous substrates (endocons), such as sugars, glucuronic acid, sulfate, amino acids, to form chemicals which are not natural components of the cell. These metabolites are usually extractable from the substrate with polar solvents but generally do not partition from water into apolar solvents. The term "water-soluble" metabolites is used interchangeably with conjugated metabolites as a means of classifying radioactive residues in the water phase of a sample extract.

Conjugated metabolites have traditionally been identified by cleavage of the conjugate with enzymic, acid or base hydrolysis and identification of the exocon released. However, modern methods now allow the isolation and purification of unhydrolysed conjugated metabolites and identification of the intact conjugates by mass spectral and nmr measurements. Such identification is now becoming more common, although it is not necessary in evaluating the safety of a pesticide.

Bound radioactive pesticide residues are considered as those resulting from the bonding of the pesticide or its metabolites/degradation products with cellular components to yield nonendogenous products which cannot be removed from the sample matrix by exhaustive extraction with apolar and polar solvents. Often acid and base treatments of the sample matrix at elevated temperatures may render some of the residues extractable into organic solvents. However, the problem with this latter approach is that the treatment may so extensively degrade the pesticide moiety of the complex (exocon) that the subsequently released unbound exocon bears no resemblance to its original structure in the untreated residue. Further suggestions to address this problem see Huber 1983 and Kovacs 1986.

Metabolism of a radiolabelled pesticide to small fragments followed by incorporation of these fragments to yield radioactively labelled natural plant or animal constituents can also occur. However, if the investigator can show that such incorporation has occurred, then these radioactive residues will clearly be of no toxicological concern.

Relevance of metabolites/degradation products

The total terminal residue includes all radioactive components of the residue. The total toxic residue represents that portion of the TRR which is of toxicological relevance (parent pesticide and any metabolites, free, conjugated or bound, which may retain a toxic effect). Analytical methods will need to be developed for the constituents of the total toxic residue. The registration authorities of different countries may sometimes differ in their opinion as to which metabolites are still toxic. Thus it is usually sensible to discuss metabolism results with individual registration authorities.

Laboratory Versus Field Studies

Field studies with the radiolabelled compounds theoretically are always preferred, because the results are more realistic than those from laboratory experiments. However, confined, outdoor field experiments for plant, crop rotation or leaching (lysimeter) studies are extremely expensive and may be ruined by bad weather conditions. Radioactivity licenses for outdoor studies also may be difficult to obtain.

Simulation of Realistic Conditions

The application rates, modes, frequency, timing, collection of interim and harvest samples should be simulated as much as possible as for the intended use. This may be not always be practical because e.g. the final formulation may not be known, nevertheless a "best guess" formulation is better than just applying the a.i. as an acetone solution.

METHODS

Radiochemicals

The use of radiolabelled pesticide compounds usually is the only satisfactory way of providing metabolism data. In choosing the position to be labelled, assurance is required that a labile position is not chosen. Usually ^{14}C labelling is preferred, although ^{32}P, ^{35}S or other elements may be more appropriate if no carbon, or only labile carbon side chains exist in the molecule. Tritium labelling is strongly discouraged. Ring labelling is preferred for most aromatic or cyclic compounds. In aliphatic chains labelling at branching locations is favoured in the order tertiary - secondary - primary C-atoms.

The specific radioactivity of the starting material should be as high as possible (up to 2000 MBq/mMol) to allow low level characterizations and identification of metabolites/degradation products. The radiochemical purity should be better than 96 %, preferably 98 %. High specific radioactivities though may require frequent purification prior to analysis because of degradation by autoradiolysis. For a whole registration project a total of about 4000 MBq may be required per crop.

Reference compounds

Cochromatography of reference compounds (standards) on tlc-plates, in hplc or glc gives initial characterization of metabolites and/or degradation products. Based on experience, several of these compounds can often be predicted and should be made available at the start of the metabolism studies.

Radioactivity detection

Liquid scintillation counting (LSC) is normally used to measure radioactivity in liquid samples/extracts. Solid samples are combusted and the combustion gases containing $^{14}CO_2$ trapped in suitable scintillation cocktails prior to radioassay. Animal tissues frequently are "dissolved" in appropriate solubilizing cocktails. Modern LSC machines differentiate radioactivity from chemiluminiscence and thermoluminiscence by appropriate corrections.

Radio-tlc scanners, radio-hplc and radio-glc flow through detectors are very sensitive nowadays and allow, besides detection of radioactivity, also its immediate computation through the coupling to microcomputers. Modern metabolism studies are impossible without such equipment. The "old autoradiography" is lengthy but still has its advantages with regard to high resolution, documentation and for plant translocation studies.

Purifications of metabolites/degradation products

Tlc, hplc, lc, glc etc. are used to purify/isolate metabolites/degradation products prior to their structural determinations by ms and sometimes nmr. Modern hplc machines with normal and reversed phase columns, and gradient elution capabilities have simplified metabolite separation, isolation, purification and structure determination. Serial combinations of radioactivity detectors with e.g. uv or diode array detectors are frequently used in hplc.

Structure determination

Adequate modern ms equipment like glc-ms, hplc-ms, ms-ms with different ionisation methods - electron impact, chemical ionization, fast atom bombardment, secondary ion-ms, thermospray interface, laser desorption, and ^{252}Cf-ms can be used for structure elucidations. High field nmr can also be used if suffient amounts of a metabolite can be adequately purified. Final confirmation of a structure is obtained when an authentic synthesised sample gives an identical mass spectrum (and nmr spectrum if possible) to the isolated metabolite.

GUIDELINES, GOOD LABORATORY PRACTICE

Guidelines for different countries are not always the same. This can result in very expensive duplication of effort, with virtually no increase in understanding of the fate of a pesticide. All efforts to bring about harmonization of the different requirements of different countries should be of high priority.

Before concluding a metabolism study it is essential to carefully evaluate the requirements of the country in which a registration is sought. The study should then be designed to satisfy that country's guidelines. This situation will continue until harmonization of registration requirements is achieved.

In addition to registration guidelines, draft guidelines for evaluating registration petitions (standard evaluation procedures, SEP's) and for reporting results (standard reporting guidelines, SRG's) have recently appeared. It is possible that this increased formalisation of the application of "guidelines" will restrict the freedom of the metabolism chemist to design the best experiment for the particular pesticide he is working with. Such a situation would be very undesirable for both the registration authorities and the pesticide companies.

Good Laboratory Practice (GLP) will soon be a precondition for the mutual acceptance of test results. GLP is concerned with the organisation and the conditions under which studies are planned, performed, monitored, recorded and reported. GLP is designed to ensure that all studies are carried out to a high standard and that the raw data for a reported study is available for inspection and justifies the results reported. Most pesticide companies believe that GLP is a sensible requirement and has had a beneficial effect on the conduct of metabolism work.

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THE ROLE OF LABORATORY AND FIELD STUDIES, USING RADIOLABELLED MATERIALS, IN THE INVESTIGATION OF THE DEGRADATION AND MOBILITY OF TEFLUTHRIN IN SOIL

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Abstract

The key environmental questions relating to the behaviour of tefluthrin in soil were initially answered by laboratory experiments and later confirmed with a field study. In the laboratory, ^{14}C tefluthrin was degraded readily (half-life 3-20 weeks) in all except sterile soil. The degradative process involved both hydrolytic and oxidative pathways which lead, ultimately, to extensive mineralisation of both the 'acid' and 'alcohol' moieties of the parent ester to $^{14}CO_2$. Laboratory studies also showed that tefluthrin and its degradation products would not leach.

The results of a ^{14}C-field study, carried out in Mississippi, USA, were totally in accord with those from the laboratory experiments. Tefluthrin was degraded with a half-life of about 1 month, thus giving scope for sustained pest control without any risk of residue build-up in the soil. The degradative pathways were the same in the field as in the laboratory. The radioactive residue did not leach below 20 cm and volatilisation of the active ingredient from soil incorporated granules was not an important dissipation mechanism.

Tefluthrin was degraded at similar rates in each of two matched pairs of carbofuran 'problem'/'non-problem' soils (half-lives 7-11 weeks) whereas carbofuran was degraded much faster in the 'problem' soils (half-life about 10 days) than in the 'non-problem' soils (half lives about 14 weeks). Thus, tefluthrin is not susceptible to 'enhanced degradation' in carbofuran 'problem' soils.

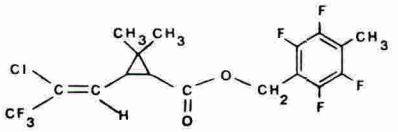
INTRODUCTION

Both the agronomic benefits and the environmental impact created by the application of a pesticide can be profoundly affected by the behaviour of the compound in soil. This is particularly true for those compounds which are applied directly to, and which exert their pesticidal activity via this medium.

In order to have a good understanding of the possible effects of a pesticide in soil, it is necessary to know not only its intrinsic activity but also how quickly the compound degrades, the nature of the degradation products and the mobility of the parent compound and its degradates. The way in which these factors may be studied is illustrated in this paper by reference to laboratory studies and confirmatory field studies with the recently developed soil insecticide tefluthrin, "Force" (Jutsum *et al*.1986). Laboratory studies on the potential for the 'enhanced degradation' of tefluthrin in soil are also described.

STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES

The structure and physico-chemical properties of a pesticide can be used to predict, at least in qualitative terms, the way in which the compound is likely to behave in the environment. Tefluthrin (Figure 1) is structurally related to several pyrethroid insecticides. As with other pyrethroids, hydrolysis of the ester moiety was therefore expected to be an important degradative reaction in soil, resulting in the formation of the corresponding cyclopropane carboxylic acid and the substituted benzyl alcohol moieties. The 'acid' moiety of tefluthrin is, in fact, identical to that of PP321 and is known (Bewick et al 1984) to be rapidly and extensively further degraded in soil. In contrast, the 'alcohol' moiety of tefluthrin represented an unknown entity, the ultimate fate of which in soil could not be predicted with confidence.



2,3,5,6-tetrafluoro-4-methylbenzyl (1R,3R;1S,3S)-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

Fig.1. Tefluthrin: Structure and Nomenclature

The physico-chemical properties of tefluthrin and several other pyrethroids are compared in Table 1. The listed compounds all have, high octanol/water partition coefficients and very low water solubilities. Like cypermethrin and PP321, tefluthrin was therefore expected to interact strongly with soil organic matter and, consequently, to have an extremely low leaching potential. The vapour pressure of tefluthrin is, however, markedly higher than those of the other compounds, making it more susceptible to movement through the soil in the vapour phase. Indeed, it is this property more than any other which differentiates tefluthrin from its pyrethroid predecessors and renders it such a potent soil insecticide.

Table 1 Physico-chemical Properties

Compound	octanol/water partition coeff. (log p)	water solubility (mgl^{-1} @ 20°C)	vapour pressure (KPa @ 20°C)
Cypermethrin (PP383)	6.6	4×10^{-3}	1×10^{-10}
PP321	7.0	5×10^{-3}	5×10^{-10}
(Lamdacyhalothrin)*			
Tefluthrin (PP993)	6.5	2×10^{-2}	8×10^{-6}

* proposed commom name

LABORATORY DEGRADATION STUDIES

The degradation of pesticides in soil is usually studied by incubating the radiolabelled (usually ^{14}C) pesticide with soil under carefully controlled laboratory conditions. This approach was followed with tefluthrin using, initially, material radiolabelled in the phenyl ring.

In addition to our normal practice of studying the unformulated active ingredient, some studies were also carried out using granular formulations of ^{14}C-tefluthrin in order to model more closely the principle proposed use of the compound for corn rootworm control in the USA.

First Laboratory Degradation Study (^{14}C-phenyl tefluthrin)

Methods

Unformulated ^{14}C-phenyl tefluthrin (60 KBq) was applied to the surface of small pots of soil (25 g) at the 'in-row' application rate of approximately 600 g ha^{-1} (equivalent to about 100 g ha^{-1} across the treated field). ^{14}C-tefluthrin granules were buried to a depth of about 1 cm in similar pots of soil. The treated soils (a sandy loam, 4.6% organic matter and a loamy sand, 2.0% OM) were incubated under both aerobic and flooded conditions at 20°C in flow-through systems (Hill and Arnold, 1978). The effluent air from each incubation system was passed sequentially through absorption tubes of 2-methoxyethanol (intended to 'trap' organic volatiles) and ethanolamine (to 'trap' $^{14}CO_2$).

Immediately after treatment, and at several intervals during a 6 month period, complete pots of soil were sequentially extracted with acetonitrile (150 ml; cold shake for 30 min) and acetonitrile/water (70:30; 150 ml; 3 hr reflux). The soil extracts and surface waters of the flooded soil were each analysed by liquid scintillation counting (LSC), concentrated *in vacuo*, and then analysed by thin layer chromatography (TLC). The unextracted radiocarbon was quantified by combustion and LSC.

Results and Discussion

Immediately after treatment, the applied radioactivity was extracted essentially quantitatively as ^{14}C-tefluthrin. There was a 50% decline in the levels of tefluthrin (Figure 2) after about 40 days in the surface treated aerobic soils and after about 150 days in both the aerobic soil treated with granules and the flooded soil.

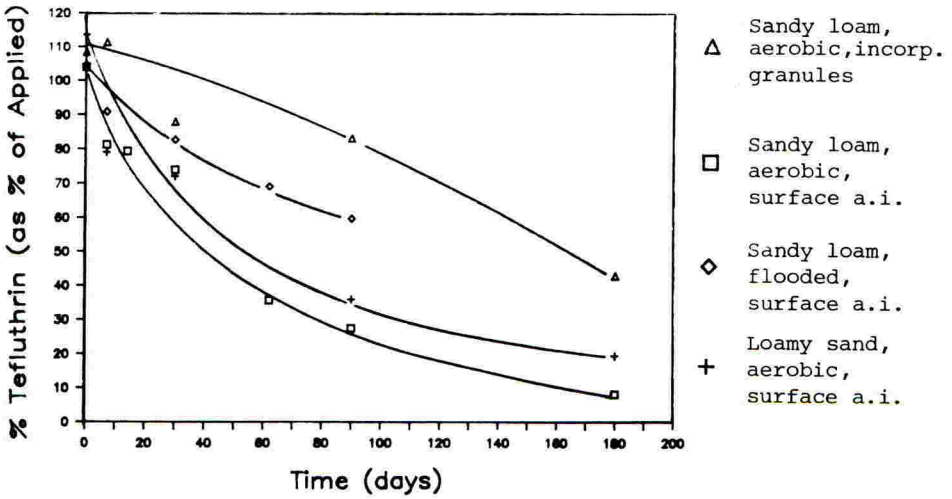


Fig.2. Decline of Tefluthrin in Laboratory Incubated Soils

The soil extracts generally contained three radioactive degradation products in addition to ^{14}C-tefluthrin. These compounds were identified as 2,3,5,6-tetrafluoro-4-methylbenzoic acid (Compound III, up to 2.1% of applied),2,3,5,6-tetrafluoro-1,4-benzenedicarboxylic acid (Compound IV, up to 1.3%) and 4-carboxy-2,3,5,6-tetrafluorobenzyl (1R,3R;1S,3S)-3-(Z-2-chloro-3,3,3- trifluoroprop-1-enyl)-2,2,dimethylcyclopropanecarboxylate (Compound V, up to 1.0%). Compound III was the major product in the flooded soil (12% in the soil extracts, 6.5% in the surface waters). The degradation products were the same for both formulated and unformulated material. Unextractable residues increased gradually, with time, up to 17% after 180 days.

In all the aerobic soils, mineralisation of the applied radiocarbon was an important process with up to 42% evolved as $^{14}CO_2$ during 180 days. Mineralisation was much less extensive in flooded soils (<0.1% in 90 days).

The overall recoveries of the applied radiocarbon from both the flooded soil and the aerobic soil treated with granules were essentially quantitative throughout but those from the surface treated aerobic soils declined to as low as 50% after 180 days. The reason for this 'loss' of radiocarbon was not immediately obvious; volatilisation of tefluthrin or a degradation product seemed the most plausible explanation but no 'volatiles' were found in the 2-methoxyethanol traps.

The distributions of the various radioactive residue components, during the incubation of the formulated (i.e. granular) tefluthrin, are shown in Figure 3.

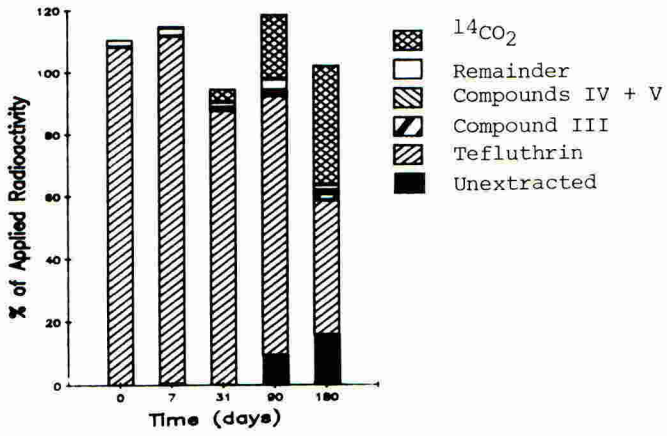


Fig.3. Radioactive Residues in Soil: Sandy loam, aerobic, granular treatment

Second Laboratory Degradation Study (^{14}C-phenyl and ^{14}C-cyclopropane telfuthrin).

The main objectives of the second laboratory degradation study were to (i) test the hypothesis that the 'losses' of radioactivity observed in the surface-treated aerobic soils were due to volatilisation, (ii) to study the fate of ^{14}C-cyclopropane labelled tefluthrin and (iii) to study the effects of temperature and sterility on the behaviour of the compound.

Methods

The methods used in the second experiment were essentially the same as those used previously, the principle differences being that the unformulated ^{14}C-tefluthrin was mixed with the soil and that polyurethane foam bungs were included at the outlet end of each soil incubation system. ^{14}C-phenyl labelled tefluthrin was incubated with the sandy loam soil at 600 g ha^{-1}, at 5,20 and 30°C and also under sterile (autoclaved) conditions. ^{14}C-cyclopropane labelled tefluthrin was also incubated with this soil at 20°C.

Results and Discussion

Significant amounts of volatilized radioactivity were recovered from the polyurethane foam bungs (by soxhlet extraction with acetone). The amounts 'trapped' by the foam were correlated with temperature, being equivalent to 2,7 and 16% of that applied at 5,20 and 30°C, respectively. The foam extracts contained only ^{14}C-tefluthrin.

The times for a 50% decline in tefluthrin levels were about 150, 24 and 17 days at 5, 20 and 30°C, respectively. These rates of decline are faster than those found in the first study, probably due to the fact that the ^{14}C-tefluthrin was mixed with the soil in the second experiment, thus giving a lower and more uniform concentration of the insecticide in the soil pots. No degradation was observed in the sterile soil.

The degradation products of ^{14}C-phenyl labelled tefluthrin were the same as those found during the first study. The degradation products of ^{14}C-cyclopropane labelled tefluthrin were comprised largely of 3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylic acid (Compound Ia; up to 7%) with small amounts of compound V (<1%). No major unidentified degradation products were found from either position of radiolabelling.

Both ^{14}C-phenyl and ^{14}C-cyclopropane labelled tefluthrin were extensively mineralised with up to 45 and 52% $^{14}CO_2$ evolved during 90 days, respectively. Unextracted radiocarbon accounted for up to 19% after 90 days. As a result of the recovery of volatilized ^{14}C-tefluthrin, the overall mass balances in this study (generally >85%) were improved compared to those obtained previously.

Conclusions from Laboratory Degradation Studies

Tefluthrin was readily microbially degraded in both aerobic and flooded soils. Both formulated and unformulated ^{14}C-tefluthrin were degraded via the same hydrolytic and oxidative pathways and, in aerobic soils, the intermediate degradation products of both the 'acid' and the novel 'alcohol' moieties were rapidly and extensively mineralised to $^{14}CO_2$. The proposed degradative pathway is shown in Figure 4.

In addition to degradation, a significant proportion of the unformulated insecticide was volatilised from soil. This route of loss was not important when the formulated (ie granular) material was incorporated into the soil. Volatilisation will not, therefore, be of major importance in the field when tefluthrin is applied as a granular formulation and incorporated into the soil.

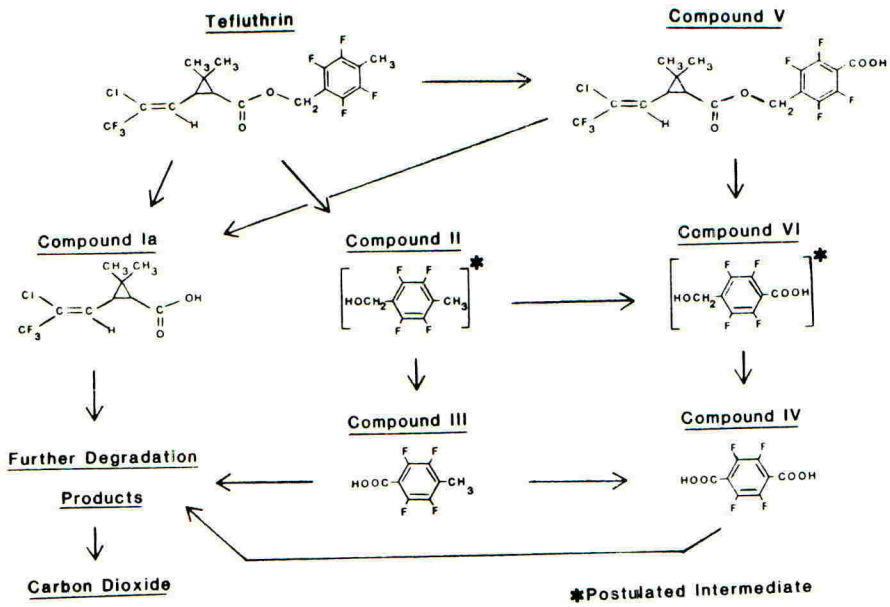


Fig.4. Proposed Degradative Pathway for Tefluthrin in Soil

LABORATORY LEACHING STUDIES

The potential for the leaching of pesticides and their degradation products from soil may be evaluated in a laboratory 'aged-leaching' experiment. This approach was followed with tefluthrin.

Methods

Complete pots of the sandy loam and loamy sand soils which had been treated with ^{14}C-phenyl labelled tefluthrin and incubated aerobically for 30 days, were transferred to the tops of each of two columns (30 cm long x 5 cm ID) containing the respective soils. Artificial 'rain' (30 ml 0.01M $CaCl_2$ solution; 14.7 mm of precipitation) was applied to the top of each column on five days a week for nine weeks. Thus, the columns were eluted with a total of 600 mm 'rain' under non-saturated flow conditions. The leachate from the columns was collected three times a week and analysed by LSC. At the end of the leaching period, the columns were divided into six 5 cm segments and the radioactive residues in each determined by combustion and LSC.

Results and Discussion

Radioactive residues in the leachate from all the columns were in most cases, below the limit of determination ($<0.0002\ mgl^{-1}$) and always less than $0.0003\ mgl^{-1}$ Radioactive residue levels in the column segments were undetectable ($<0.002\ mgkg^{-1}$) below 5 cm.

Conclusions from Laboratory Leaching Studies

On the basis of these data, it was concluded that the agricultural use of tefluthrin will not result in the leaching of either the parent insecticide or its degradation products from soils.

FIELD DEGRADATION, LEACHING AND VOLATILITY STUDIES

The data derived from the experiments outlined above give a very clear indication of how tefluthrin behaves in soil under a range of carefully controlled conditions in the laboratory. Studies of this type have never been found to give a false impression of how a compound will behave in the environment. Nevertheless, it is necessary for regulatory purposes to demonstrate a link between the behaviour of a compound in the laboratory and in the field. Traditionally, this link has been established by conducting 'field dissipation' studies with 'cold' (ie non-radiolabelled) pesticides. It is often entirely feasible to monitor the dissipation of the parent pesticide in this way but rarely possible to account for the formation and subsequent decline of all degradation products. Also, in the unlikely event of degradation products being formed in the field which had not been predicted from laboratory studies, 'cold' residue methods would not normally be expected to detect these compounds. These potential problems were overcome for tefluthrin by the conduct of a field study with ^{14}C-materials.

Methods

The field trial was set up at ICI Americas Research Farm at Vicksburg, Mississippi in June 1985. Plastic cylinders (15 cm ID x 35 cm long) were driven into the soil (Figure 5). Either ^{14}C-phenyl or ^{14}C-cyclopropane labelled tefluthrin granules were then incorporated into the top 1-2 cm of soil in each cylinder at approximately 800 g ai ha^{-1} (2 MBq per cylinder). Immediately after treatment, and at suitable intervals thereafter, complete cylinders of soil were removed for analysis. In order to monitor the volatilisation of tefluthrin, two cylinders were equipped with a polyurethane foam bung 'trap' (Figure 5) during the periods of 0-5 and 30-35 days after treatment. No attempts were made to trap $^{14}CO_2$. Prior to analysis, the soil cores were sectioned into 0-5, 5-10, 10-20 and 20-30 cm horizons. The soils were extracted and analysed using the methods developed previously during laboratory studies.

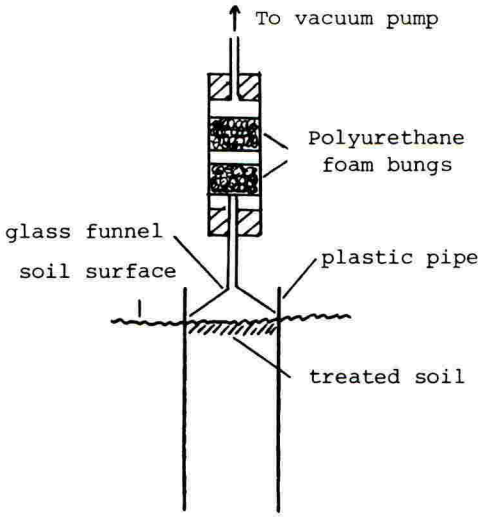


Fig.5. Apparatus for ^{14}C-Field Study

Results and Discussions

Radioactive residues in the soil core sections are shown in Table 2. Despite over 30 inches of rainfall during the trial, the residues always remained close to the soil surface; the highest residues were consistently found in the 0-5 cm sections and, below 20 cm, were always equivalent to <0.2% of that applied (ie <0.001 mgkg^{-1}). The total recoveries of radioactivity were very close to that nominally applied at zerotime, but declined to about 10% after 9 months.

Only small amounts of radioactivity were recovered from the foam bung traps. During the first 5 days after treatment up to 0.31% of the applied radiocarbon was 'trapped' and during the 30-35 day period, only 0.01%. The 'trapped' material was ^{14}C-tefluthrin.

Since the overall decline of radioactive residues was not due to either leaching or volatilisation of ^{14}C-tefluthrin it must have been due to the evolution of $^{14}CO_2$.

Table 2

Recovery of Radioactive Residues from Soil Cores: Vicksburg

	Sampling Interval (weeks after treatment)	Recovery of Radioactivity (as % of applied) Core Section (cm) 0-5	5-10	10-20	20-30	Total
^{14}C-cyclopropane tefluthrin	0	99.5	ND	NA	NA	99.5
	2	81.8	0.3	ND	ND	82.1
	4	53.7	0.2	ND	ND	53.9
	8	37.4	0.6	ND	ND	38.0
	12	19.8	0.6	0.2	ND	20.6
	17	14.2	0.7	0.3	ND	15.2
	22	11.1	0.5	ND	ND	11.6
	40	8.3	0.3	ND	ND	8.6
^{14}C-phenyl tefluthrin	0	100.5	ND	NA	NA	100.5
	2	84.3	0.5	ND	ND	84.8
	4	59.9	0.4	ND	ND	60.3
	8	13.9	0.9	ND	ND	14.8
	12	24.3	1.4	ND	ND	25.7
	17	37.3	1.8	ND	ND	39.1
	22	13.1	1.0	ND	ND	14.1
	40	9.9	1.6	0.4	ND	11.9

ND = None detected (<0.2%) NA = Not analysed

There was a 50% decline in extractable tefluthrin residues after approximately 1 month. After 9 months, the tefluthrin residues had fallen to <2% of that applied. The spectrum of extractable degradation products in the 0-5 cm column sections was the same as had been found in the laboratory. The most abundant product was Compound Ia (up to 3.7% of applied) followed by Compound III (up to 1.9%) and Compound V (up to 0.9%). In total, the other minor radioactive components accounted for no more than 3% of the applied ^{14}C-tefluthrin.

Conclusions from Field Study

The half-life of ^{14}C-tefluthrin was about 1 month under warm/moist soil conditions. The degradation products were both qualitatively and quantitatively as predicted on the basis of laboratory studies. No unexpected degradation products were found.

The total radioactive residues derived from both ^{14}C-cyclopropane and ^{14}C-phenyl labelled tefluthrin declined markedly with time but did not leach below 20 cm. Volatilisation of tefluthrin was not an important dissipation mechanism following the incorporation of the granular insecticide into the soil. The overall reduction in residue levels was therefore attributed to mineralisation to $^{14}CO_2$.

LABORATORY STUDIES ON THE POTENTIAL FOR THE ENHANCED DEGRADATION OF TEFLUTHRIN IN CARBOFURAN 'PROBLEM' SOILS

There is evidence that repeated applications of certain pesticides can result in an increase in the rate of degradation of those compounds in soil. This enhanced rate of degradation can result in reduced efficacy, as evidenced by the poor performance of the soil insecticide carbofuran in controlling corn rootworm in certain 'problem' (or 'aggressive') soils (Felsott et al, 1981). Since it is likely that tefluthrin may be applied to carbofuran 'problem' soils, it was felt prudent to study the rate of degradation of tefluthrin in such soils.

Methods

Matched pairs of carbofuran 'problem' and 'non-problem' soils were sampled from each of two sites in Illinois, USA. The soils were incubated, separately, in the laboratory with both ^{14}C-tefluthrin and ^{14}C-carbofuran. The incubation system described earlier was used to monitor the rates of degradation of the two insecticides.

Results and Discussion

Degradation of ^{14}C-carbofuran was very rapid in both 'problem' soils (Table 3) but much slower in the 'non-problem' soils; this behaviour is consistent with the hypothesis that poor performance in 'problem' soils is due to enhanced degradation of the compound. In contrast, ^{14}C-tefluthrin was degraded at much the same rate in each of the two pairs of soils.

Table 3

Rates of Degradation of ^{14}C-tefluthrin and ^{14}C-carbofuran in 'problem'/'non-problem' Soils

Soil	Half-life (days) ^{14}C-carbofuran	^{14}C-tefluthrin
'Peters Farm' - 'problem'	12	77
- 'non-problem'	104	79
'Grotovant Farm' - 'problem'	10	69
'non-problem'	91	43

Conclusions

Tefluthrin will not exhibit enhanced degradation when applied to carbofuran 'problem' soils.

OVERALL CONCLUSIONS

Tefluthrin has a half-life in soil ranging from several weeks to several months, depending on the prevailing conditions. This is close to the optimum persistence for a soil insecticide.

Tefluthrin was degraded initially by ester hydrolysis. Both the 'acid' and 'alcohol' moieties of tefluthrin were extensively further degraded but neither the parent compound nor its degradates were susceptible to leaching.

The behaviour of the insecticide predicted on the basis of laboratory studies was confirmed, in every respect, by a ^{14}C-field study.

Tefluthrin is not susceptible to enhanced degradation in carbofuran 'aggressive' soils.

ACKNOWLEDGEMENTS

We thank all our colleagues within ICI who were involved in setting up, maintaining and sampling the USA field studies. The analysis of the samples from the field study was carried out by D Kirkpatrick, G M Dean and J Riseborough at Huntingdon Research Centre.

Thanks are extended also to Dr A Felsott of the University of Illinois for assisting in the selection and sampling of the carbofuran 'problem'/'non-problem' soils.

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PHASE I AND PHASE II METABOLISM OF PYROQUILON IN ANIMALS AND PLANTS

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ABSTRACT

The phase I and phase II metabolic reactions of pyroquilon, i.e., 1,2,5,6-tetrahydro-4H-pyrrolo[3,2,1-i,j]quinolin-4-one, were investigated in paddy rice and rats using [2-^{14}C] labelled material. Phase I reactions in plants and animals were shown to be dominated by oxidative mechanisms at different sites of the molecule, the combination of which led to a wide array of hydroxylated metabolites. The reactions in rats were essentially the same as those identified in rice plants but proceeded further in the metabolic transformation. Regarding phase II reactions, most of the phase I metabolites in rice were conjugated with neutral carbohydrates. In rats conjugation was with sulfuric acid and β-D-glucuronic acid.

INTRODUCTION

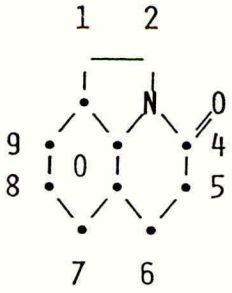

Fig. 1. 1,2,5,6-tetrahydro-4H-pyrrolo[3,2,1-i,j]quinolin-4-one-(pyroquilon).

4B—3

Pyroquilon, 1,2,5,6-tetrahydro-4H-pyrrolo[3,2,1-i,j]quinolin-4-one (Fig. 1) is a systemic fungicide used for blast control in rice. The compound is moderately toxic to mammals, practically non-toxic to birds and only slightly toxic to fish.

During the development of pyroquilon the metabolic fate of this compound was investigated in a laboratory paddy rice system and in rats using [2-^{14}C]-labelled material.

This paper focusses on the structural transformations of pyroquilon elucidated in these biological systems.

MATERIALS AND METHODS

Paddy rice system

Rice seeds (variety Yamabico) were sown in a small seedling box. After 28 days the seedlings (2 to 3 leaf stage) were treated with a granular formulation of ^{14}C-pyroquilon. The amount of pyroquilon applied corresponded to the recommended application rate of 1.5 g a.i./per seedling box (0.18 m^2) containing 800 rice plants.

Twenty four hours after treatment the seedlings were transplanted into glass tanks filled with a clay loam soil to a depth of 15 cm and flooded with water to maintain a level of about 5 cm above the soil surface.

The rice plants were cultivated under controlled conditions (lighting: 10'000 Lux for 14 h/d, r.h.: 70 %, temp: day 30 °C, night 24 °C) and harvested 47 days and 134 days after treatment, i.e., at approximately one half plant maturity and at the fully ripe grain stage, respectively. The stalks of the plants harvested at both time periods were combined and used for the isolation of metabolites, as the metabolite pattern in the grains was essentially the same, but the residues were too low for this purpose.

Rats

Male rats (strain: Tif: RAI f (SPF)) of about 200 g body weight, kept under controlled conditions (light for 12 h/d, r.h.: 50 - 65 %, temp: 21 - 24 °C), received a single oral dose of ^{14}C-pyroquilon (24.5 mg/kg body weight) dissolved in 1.0 ml ethanol/water (1/9 v/v) by stomach intubation.

The urine collected within 24 h after administration of the compound, representing 72.5 % of the dose, was used for the isolation of metabolites.

Isolation of metabolites

Metabolites in rice stalks

Metabolites in rice stalks were successively extracted with methanol/water (80/20 v/v) and water and subsequently partitioned between water and dichloromethane. Metabolites present in the organic phase were separated and purified using multiple hplc steps (silica gel and diol columns). Polar metabolites present in the aqueous phase were prepurified by liquid chromatography on Amberlite XAD-4 followed by separation into neutral and acidic metabolites by ion exchange chromatography on SP-Sephadex-C-25 and QAE-Sephadex-A-25. The neutral fraction was treated with cellulase to cleave the conjugates. The exocons released were separated and purified by multiple step hplc (RP 18 columns). Metabolites in the acidic fraction were separated and ultimately purified by multiple step hplc (RP 18 columns).

Metabolites in rat urine

Lipophilic metabolites in rat urine were extracted with chloroform and separated and purified by multiple hplc steps (RP 18, CN and silica gel columns). The metabolites present in the aqueous phase being mainly in form of conjugates were prepurified by liquid chromatography on Servachrom-XAD-4 and subsequently separated by ion exchange chromatography on DEAE-Sephadex A 25. The metabolites were purified by liquid chromatography on RP 8 and RP 18 and finally by multiple hplc steps (RP 18 and CN columns).

Identification of metabolites

The structures of the metabolites isolated from rice stalks and from rat urine were identified by nmr (Bruker WM 400 or Bruker HX 360) and ms using electron impact (EI), field desorption (FD) and fast atom bombardment (FAB) techniques (Varian Mat CH 5-DF, Finnigan Model 4000).

RESULTS

Identification of the metabolites isolated allows metabolic pathways for pyroquilon in rice (Fig. 2) and the rats (Fig. 3) to be proposed.

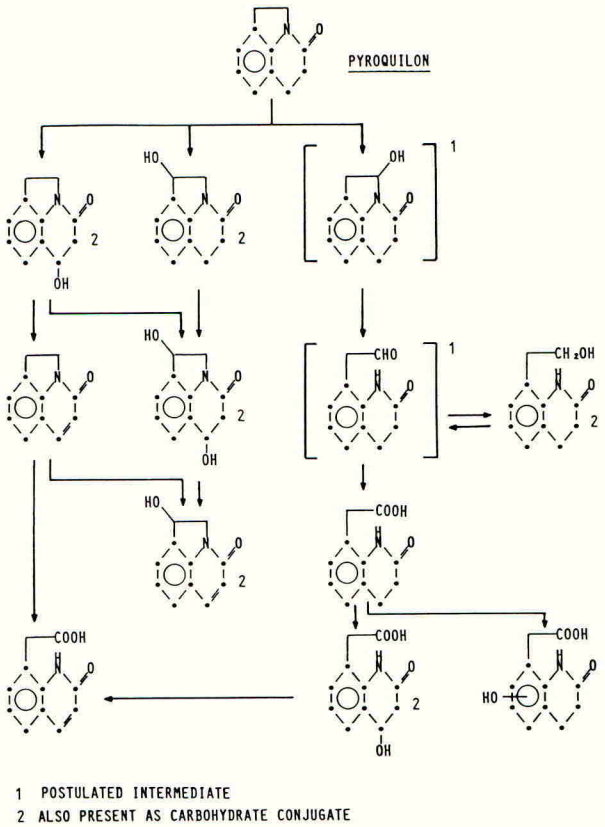

Fig. 2. Metabolic pathways proposed for the degradation of pyroquilon in rice plants.

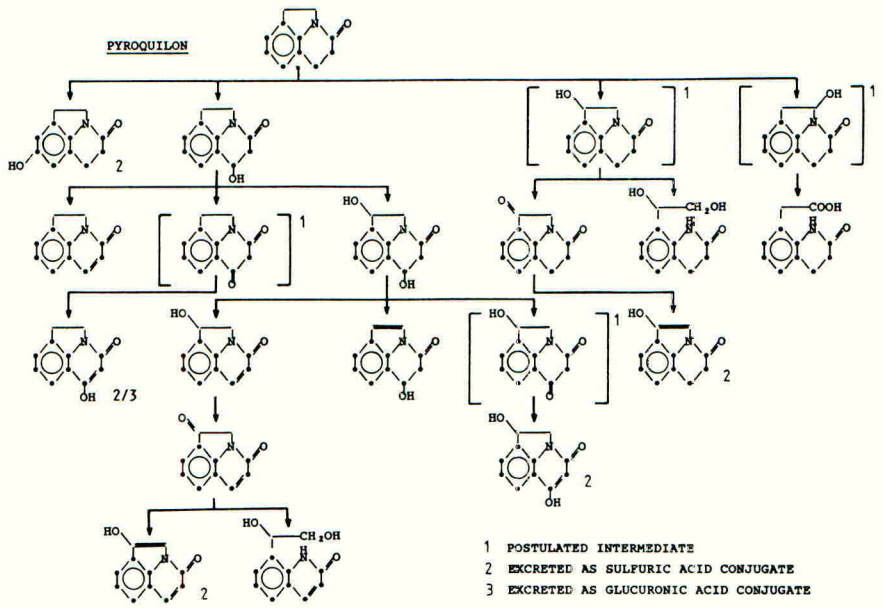

Fig. 3. Metabolic pathways proposed for the degradation of pyroquilon in rats.

DISCUSSION

Phase I reactions

Most of the phase I reactions observed in both experimental systems were oxidative in nature. A condensation reaction caused by a loss of H_2O was found operative in rice and rat. The plant demonstrated also one reductive mechanism leading from a hypothetical aldehyde derivative to the corresponding alcohol.

In rice and rats pyroquilon was attacked at all three ring systems. The combination of the different target sites with the various reactions observed resulted in a complex metabolite pattern.

Analysing the metabolic reactions in rats and rice plants at the different target sites of the pyroquilon molecule it is evident, that all reaction mechanisms operative in the plant also occur in the rat. However, the metabolic processes in the rat generally proceed beyond the stage reached by the rice plant and the rat utilizes additional pathways not found in the rice plant.

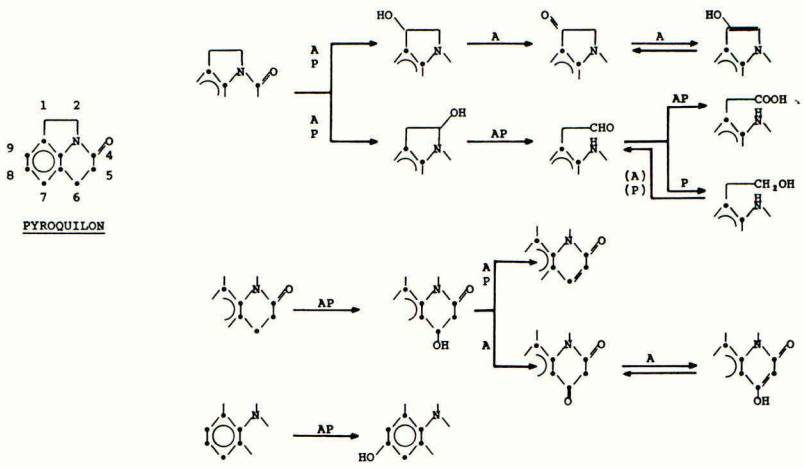

Fig. 4. Comparative scheme of the phase I - reactions of pyroquilon in rats and rice plants (A = Animal P = Plant).

Phase II reactions

Phase II reactions are commonly different in plants and animals, so also in this case. Although the nature of the endocons in the rice plant was defind only by the neutral character of the conjugates and their susceptibility to cellulase, β-D-glucose or its dimer (gentiobiose) may be assumed to be the conjugating moieties. The conjugates in rat urine were isolated and identified in their genuine forms, so that the structures of the endocons were unequivocally determined as β-D-glucuronic acid and sulfuric acid. Some site specific pecularities were observed with these conjugation processes.

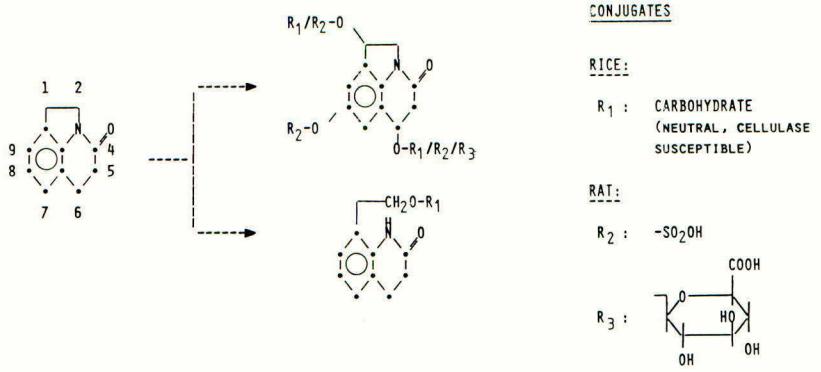

Fig. 5. Comparative scheme of the phase II - reactions of pyroquilon in rats and rice plants.

All aliphatic hydroxy derivatives (positions 1, 6 and at the C atom representing the former position 2) were conjugated in the rice plant. In contrast, a phenolic hydroxy derivative was only found in the free form.

Rats utilized β-D-glucuronic acid only for conjugation at position 6, whereas conjugation with sulfuric acid was found at all aromatic and aliphatic hydroxy groups (positions 1, 6, 8).

Hydroxy derivatives carrying two hydroxy groups at an open chain (at the C atoms representing the former positions 1 and 2) were not conjugated at all.

COMPARATIVE METABOLISM OF THE ENANTIOMERS OF TRIADIMENOL IN BARLEY PLANTS

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ABSTRACT

The systemic fungicide triadimenol possesses four enantiomers which have different biological activities. Uptake and metabolism of these enantiomers were investigated in barley plants after seed application. Uptake and conjugation were greater for the 1S2S enantiomer than for the 1R2S,1S2R and 1R2R enantiomers. The number and types of conjugates, as determined by radio thin layer chromatography, also differed between enantiomers. A hexose conjugate was formed only from the 1R2S enantiomer. An unidentified polar conjugate was formed by the 1S2S enantiomer but not from the other three. No racemisation or epimerisation of the 1S2R, 1R2R and 1S2S enantiomers was detected in barley plants whereas the 1R2S enantiomer was partially converted to its 1R2R epimer.

INTRODUCTION

Triadimenol(1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol) (Fig. 1) is a systemic fungicide used to control cereal diseases (Frohberger 1978). It is used commercially as a mixture of two diastereoisomers (two pairs of enantiomers) in a ratio of 1RS,2SR(80) to 1RS,2RS(20). Only one report has been published (Haque et al. 1983) on the metabolism of applied triadimenol within plants. There have been a number of reports on the metabolism of triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butanone), the keto analogue of triadimenol, which is rapidly converted to triadimenol in plants (Gastonyi & Josepovits 1978, Rouchaud et al. 1981a, Rouchaud et al. 1981b, Rouchaud et al. 1982). For both triadimenol and triadimefon the major metabolites were identified as the primary alcohol of triadimenol, produced by oxidation of one of the tertiary butyl methyl groups, and the conjugate of this alcohol. A conjugate of the secondary hydroxyl group of triadimenol was also found.

The fungicidal activities of the individual enantiomers of triadimenol against different fungi have been reported by Kramer et al. (1983) and Deas et al. (1986). The 1S2R enantiomer was generally the most fungitoxic (up to 1000-fold more active than the other three) but against some pathogens none of the enantiomers was active.

In view of the differences in antifungal activity between the enantiomers it was considered important to investigate their fate individually in a crop plant. In this study we report on uptake, racemisation/epimerisation and metabolism of each of the four enantiomers of triadimenol in barley plants following seed treatment. Knowledge of the stability of the 1S2R enantiomer in plants is an important factor in considering the suitability of this material for commercial development

as a fungicide. This is the first report of a field based study on the stability of the enantiomers of a fungicide. The metabolism of triadimenol enantiomers in soil will be reported later.

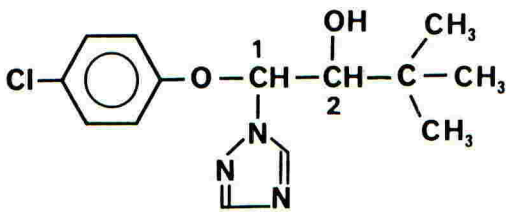

Fig. 1. Structure of triadimenol.

MATERIALS AND METHODS

General

The study was conducted with individual (benzene ring-UL-^{14}C) triadimenol enantiomers (specific activity 67 uCi/mg). All enantiomers had optical (determined by gc and hplc) and radiochemical (determined by tlc) purities > 99%. The formulation corresponded to that of Baytan 25DS (Bayer Agrochemicals), a powder seed treatment used commercially.

Seed coating

Barley seeds (cv. Triumph) were coated by the method of Steffens et al. (1982). The radioactivity per seed was determined by combusting 20 seeds for each enantiomer.

Planting

Seeds were hand sown on 10 April 1985, using standard field depth (2 - 3 cm) and spacing (5 cm) with 7 cm between rows, in well-tilled field soil (fine sandy loam, pH 5.7) at Long Ashton Research Station. Five replicates for each enantiomer were sown, each replicate comprising 2 rows of 12 seeds. Seeds treated with the corresponding non-radioactive enantiomer formulation were sown around each replicate as a single guard row to prevent redistribution between experimental treatments. Normal Baytan 25DS treated seeds were sown between the enantiomer treatments and around the whole plot as further guard rows.

Sampling

From each replicate plot, leaves of 20 plants were harvested 7 weeks after sowing (GS 30 - 31), by cutting 2 - 3 cm above ground level. They were stored at -20°C until analysed. Roots were removed from the soil, air-dried and the radioactivity present measured by combustion (Harvey OX 400) and liquid scintillation counting. Root samples from single plants were combusted separately and total radioactivity determined for each replicate of individual enantiomers.

Extraction procedure

Leaves were macerated (intermittently, 1h) three times (3 x 900 ml) with methanol, water, 0.88 ammonia (65 + 23 + 12 by vol) at room temperature. After each maceration the extract was centrifuged to remove the plant material and the supernatant decanted. The combined extracts were reduced in volume to c. 85 ml (rotary evaporator, 40°C) and made up to 100 ml using methanol/water (10 + 90 by vol). Aliquots of these solutions were then assayed by liquid scintillation counting.

Analysis of extracts

Aliquots were analysed quantitatively by radio-thin layer chromatography (tlc) (Isomess Rita 68000 linear analyser) on Merck silica gel 60 F_{254} (Art. 5715, 0.25 mm thick) pre-coated plates in two different solvent systems: (I) di-isopropyl ether, dichloromethane, acetone, ethanol (100 + 100 + 30 + 11.5 by vol) (II) chloroform, methanol, 0.88 ammonia (75 + 24 + 1 by vol).

Enantiomer analysis

Compounds present in the aqueous extract were partitioned into dichloromethane three times (3 x 100 ml) and the organic phase reduced in volume to c. 30 ml (rotary evaporator, 40°C) and made up to 50 ml with dichloromethane. Aliquots were analysed by radio tlc in solvent system I. The aqueous phase was made up to 100 ml again using methanol/water (10 + 90 by vol) and aliquots analysed by radio tlc in solvent system II. The dichloromethane fraction was taken to dryness (rotary evaporator, 40°C) and the residue was subjected to clean-up on a silica gel column (Brennecke 1984). An aliquot of each replicate after this clean-up was further purified by radio high performance liquid chromatography (hplc). Samples were applied to a reverse phase C-18 column (25 cm x 4.6 mm) and eluted isocratically (1.5 ml min^{-1}) with methanol/water (60 + 40 by vol). The purified triadimenol fractions were analysed by chiral hplc (Gau, pers. comm.) using a combined Hewlett-Packard/Isomess Ramona radio hplc system. Six (S)-N-3,5- dinitrobenzoylleucine chiral columns (Baker No.71150, 25 cm x 4.6 mm) were connected in series and applied samples were eluted isocratically (1 ml min^{-1}) with hexane, dichloromethane, propan-2-ol (94 + 5 + 1 by vol) at 80°C.

RESULTS AND DISCUSSION

The ratio of triadimenol enantiomers present in the normal seed treatment formulation is 40 : 40 : 10 : 10 (1R2S,1S2R,1R2R,1S2S). In this experiment the single enantiomers were each applied at the recommended field rate for triadimenol of 375 ug per gram of seed. Therefore the 1R2S,1S2R enantiomers and the 1R2R,1S2S enantiomers were applied at 2.5 and 10 times their normal field rate respectively. The enantiomers were applied at the same rate so that uptake and metabolism, which may have been influenced by application rate, could be compared directly. At these rates of application no adverse effects on plant growth were noted.

The initial coating of triadimenol enantiomers per seed (Table 1) show that the efficiency of application differ slightly between enantiomers. Total radioactivity in the roots varied from 2% to 4% of the amount initially applied to the seed. Uptake into the leaves, expressed as a percentage of the applied radio-activity, is given in Table 1. There were no significant differences between the 1R2S,1S2R and 1R2R enantiomers but uptake of the 1S2S enantiomer was greater.

Leaf extracts were separated in solvent system I into three fractions: total conjugated components (R_f = 0.00), the 1R2S,1S2R diastereoisomeric pair (R_f = 0.39) and the 1R2R,1S2S diastereoisomeric pair (R_f = 0.49) of triadimenol and from this the ratio of total conjugates to free triadimenol was determined (Table 2). The 1S2S enantiomer was shown to be more highly conjugated than the others, which were comparable. This greater conjugation may have been responsible for the higher uptake of the 1S2S enantiomer, its conjugates being formed

TABLE 1

Initial radioactivity and deposition per seed and uptake into leaves

Enantiomer	Mean seed coating dpm/seed	ug/seed	Mean uptake into leaves (% of initial applied radioactivity)
1R2S	1.414×10^6	9.6	3.25
1S2R	1.756×10^6	11.9	2.84
1R2R	1.976×10^6	13.4	3.43
1S2S	1.497×10^6	10.2	5.10
	SED (75df)0.0914×10^6		SED (12df)=0.473
	LSD (< 0.05) 0.182×10^6		LSD (< 0.05) 1.03

more rapidly. Also tlc solvent system I showed that the 1R2S enantiomer was partially epimerised either to the 1R2R or 1S2S enantiomer. Of the free triadimenol present in the extract of 1R2S treated plants 23% was epimerised. This epimerisation was confirmed by chiral hplc analysis (Fig. 2) which proved the epimer was exclusively the 1R2R form as predicted mechanistically, and confirmed it represented 23% of the free triadimenol. It was probably produced by stereoselective oxidation of the 2S carbon atom followed by reduction of the keto intermediate to the 2R configuration. None of the intermediate ketone was detected which indicated that the reduction was faster than the oxidation step. This reaction in the plant would probably have been catalysed by an epimerase some of which can act by the above mechanism. An example is the mammalian enzyme UDP-galactose-4-epimerase (Stryer 1981). No epimerisation or racemisation was detected with the 1S2R,1R2R and 1S2S enantiomers (Fig. 2).

TABLE 2

Proportions of conjugated components (as % total radioactivity in leaves)

Enantiomer	Total conjugated components (%)	Conj.1(%)	Conj.2(%)	Conj.3(%)	Conj.4(%)
1R2S	52.8	13.1	18.5	21.2	-
1S2R	49.8	24.5	25.3	-	-
1R2R	56.4	23.0	33.4	-	-
1S2S	77.0	20.8	38.8	-	17.4
SED (12df)	4.29	3.09	2.48		
LSD($p<0.05$)	9.4	6.73	5.40		

Individual conjugates were separated by tlc solvent system II (Fig. 3). Four were detected; conjugate 1, which remained at the origin (R_f = 0.00), was unidentified but could possibly be a di-conjugate of the primary alcohol where both hydroxyl groups were bonded to sugar molecules or a malonyl glucosidyl derivative of the primary alcohol. Conjugate 2 is the glucoside of the primary alcohol and conjugate 3 is a hexose

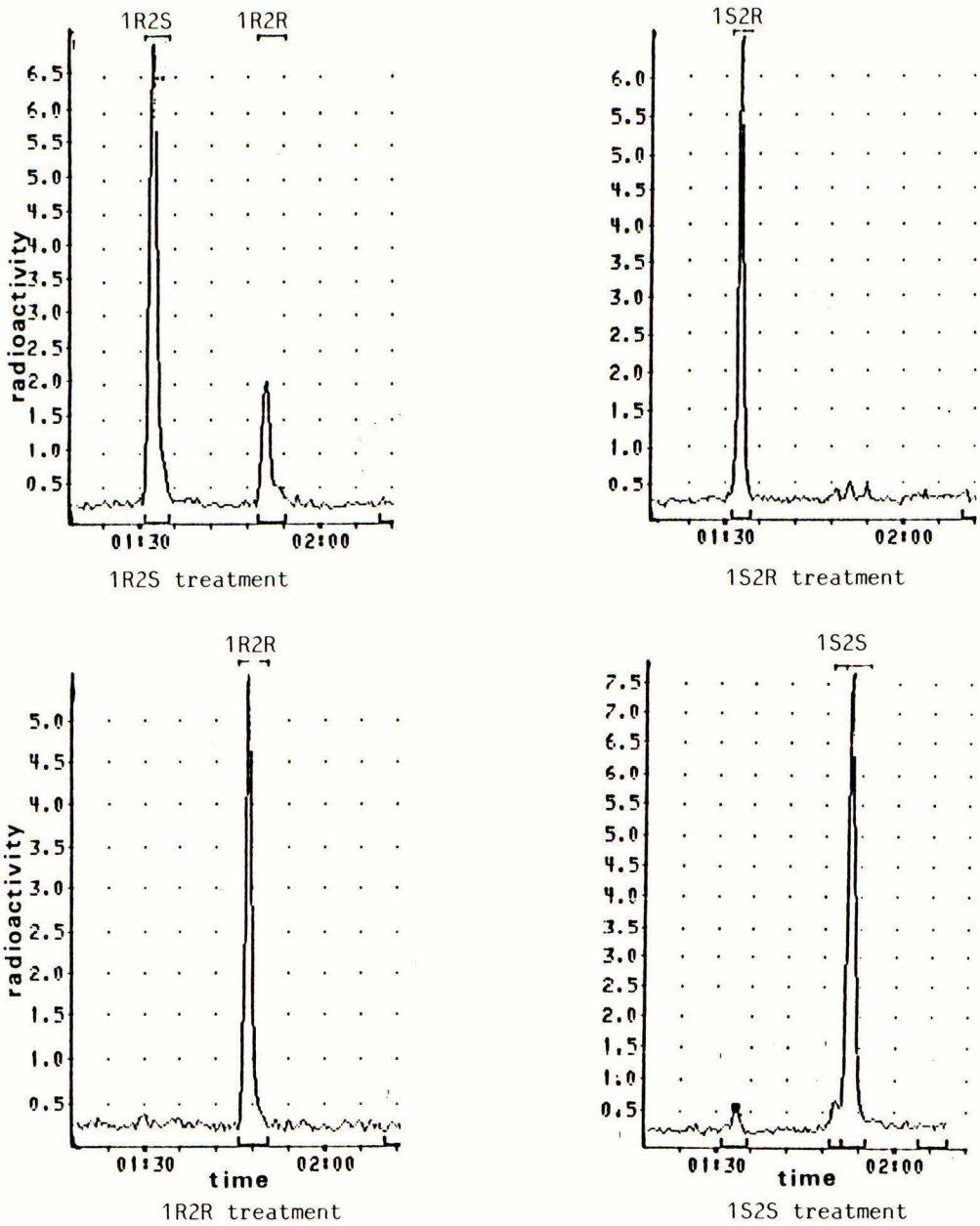

Fig. 2. Chiral hplc chromatograms of purified triadimenol enantiomer fractions isolated from barley leaves.

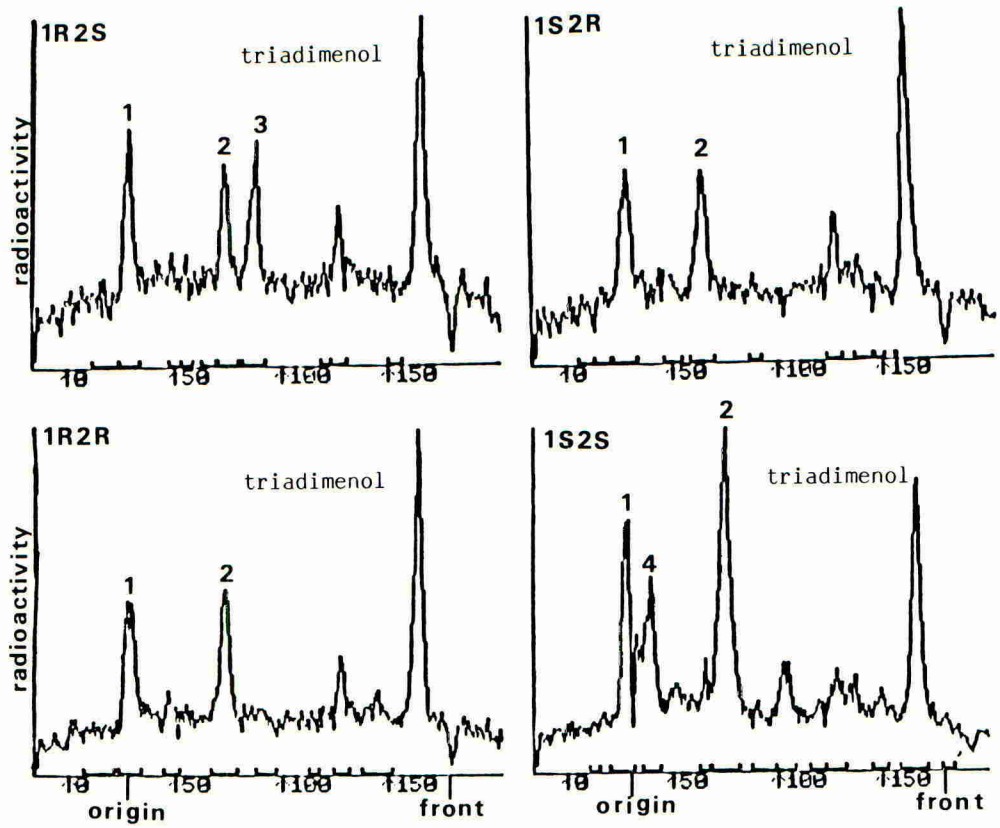

Fig. 3. Conjugate profiles of leaf extracts obtained by tlc in solvent system II.

conjugate of triadimenol, as shown by co-chromatography of the extracts with authentic samples in tlc solvent system II. The structure of conjugate 4 is as yet unknown. Further work is necessary in order to fully characterise conjugates 1 and 4.

Conjugates 1 and 2 were present in extracts of all four enantiomers and the relative proportions of these are given in Table 2. However, there is some evidence that the structure of conjugate 2 of the 1S2S enantiomer differs from that of the other enantiomers. When small aliquots of the 1S2S extract were spiked with aliquots of the other enantiomer extracts and analysed by tlc, developing twice in solvent system II, two peaks appeared in the region of conjugate 2. This was not the case when the other extracts were spiked with one another. Conjugate 3 was only produced by the 1R2S enantiomer, and conjugate 4 only by the 1S2S enantiomer (Table 2). Thus some differences existed between the enantiomers in their conjugation with plant sugars.

The results obtained from the leaf extracts indicated the main metabolic routes to be oxidation and conjugation to sugars, the latter more likely to occur in plants than soil. Thus it seems reasonable to assume that the metabolites were formed by reactions in the plant tissues and not by soil metabolism followed by uptake. However, the epimerisation shown by the 1R2S enantiomer could have occurred either in the soil or in the plant, the latter being more probable. Analysis of the soil samples is currently in progress.

This work has demonstrated that significant differences in metabolism of triadimenol enantiomers occurs in barley plants and therefore a complete understanding of the biological performance of this agrochemical can only be obtained by studying the separate enantiomers. Given the qualitative differences in the metabolism of triadimenol enantiomers it was unexpected that their overall rate of metabolism was similar. The possibility that the rate of conjugation in the plant affected the rate of uptake of the enantiomers needs to be explored more fully. Further studies are also warranted to confirm, where possible, the structures of the unknown conjugates and also to reveal any differences in the structures of the aglycone moieties.

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COMPARATIVE METABOLISM OF THE ACARICIDE CLOFENTEZINE IN ANIMALS

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ABSTRACT

The metabolism of clofentezine has been studied following oral administration to rats, mice, rabbits, dogs, baboons and a calf.

In all species studied, the excretion of clofentezine related material was rapid with most of the administered radiolabelled material being excreted within 24-48 hours. The major route of excretion was via the faeces, with urinary levels ranging from 1-2% in the dog to 25-37% in the rabbit.

The metabolism of clofentezine was qualitatively similar in all species studied with hydroxylation and replacement of an aryl chlorine with a methylthio group being major pathways. Numerous minor metabolites were found in all species.

Quantitative interspecies differences were apparent, most notably the fact that in the calf and baboon, hydroxylation and subsequent conjugation were the most prominent pathways, with methylthiolation being a very minor route of metabolism. This latter pathway was very much more prominent in rodents and the rabbit.

INTRODUCTION

Clofentezine (Figure 1) is a specific mite ovicide of novel structure developed by Schering Agrochemicals Limited.

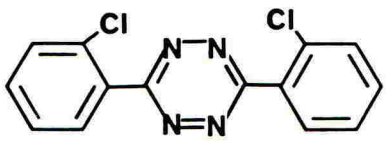

Figure 1 Clofentezine
(3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine)

Clofentezine acts primarily as a mite ovicide with some effect on young motile stages and with long residual activity. It is effective against a range of phytophagous mite species such as Panonychus ulmi and Tetranychus spp. Current recommended usage is on deciduous top fruit, in particular apples and pears, and the compound is also being developed for use on citrus, vines, cotton and other crops including ornamentals.

MATERIALS AND METHODS

Clofentezine (^{14}C)-labelled in the tetrazine ring, was formulated as an aqueous suspension and given orally to the following mammalian species: rats, baboons, mice, rabbits, dogs and a calf. Male and female animals of all species except the calf were dosed at the rate of 10 mg clofentezine/kg bodyweight. The male calf was dosed at 5 mg/kg bodyweight.

After treatment, all animals except the calf were maintained for 96 hours in metabolism cages which enabled the complete collection of urine and faeces at 24 hour intervals.

Urine and faeces were analysed for radioactivity by standard scintillation counting techniques, and levels of excreted radiolabelled material were thus determined.

Metabolites in urine were examined by thin layer chromatography both before and after enzyme hydrolysis (Snail digestive juice) and methylation with silver oxide/methyl iodide. Metabolites and derivatives were thus compared with standard materials synthesised in our laboratories.

RESULTS

Excretion

The excretion of clofentezine related materials over a 96 hour period following dosing is shown in Tables 1 and 2.

The major route of excretion is via the faeces, although significant species differences occur. In the dog, over 90% of the dose is eliminated in the faeces, with only 1-2% in the urine. In the rat and mouse, urinary levels rise to 19-27%, with levels in the mouse tending to be higher than in the rat. Urinary radioactivity levels in the rabbit are highest being in the region of 35%. Levels in the baboon are also high (23% and 35%), but as only one animal of each sex was studied, mean levels in this species are not available.

The rate of excretion appears similar in all species, with most of the administered radioactivity being eliminated within the first 48 hours. With the possible exception of the baboon, there was little evidence of a sex related difference in the route or rate of excretion.

Metabolism

Extensive metabolism studies have led to the proposed metabolic pathway for clofentezine shown in Figure 2. Although qualitatively there are broad similarities in the metabolism of clofentezine in the species studied, there are a number of important quantitative differences. These are discussed under the individual species.

Rat

In the rat, the major urinary metabolites were products of hydroxylation and methylthiolation/hydroxlation in approximately

equal quantities. (The introduction of the thiomethyl group will almost certainly have been initiated by replacement of a chlorine with glutathione (Bakke, 1986)). Thus, free and conjugated 3 and 4-hydroxyclofentezine (and a small amount of 5-hydroxyclofentezine) were found in similar amounts to conjugates of 2-methylthio-3-hydroxyclofentezine. Faeces contained large amounts of unchanged clofentezine, and both urine and faeces contained a large number of minor metabolites.

Mouse, Rabbit

The metabolism of clofentezine in the mouse and rabbit appeared broadly similar and also similar to that of the rat, but with rather less of the 2-methylthio-3-hydroxyclofentezine and rather more highly polar conjugated material.

Dog

Because of low levels of urinary excretion in the dog, quantitative comparison with other species was difficult. However, it was apparent that urine contained small amounts of clofentezine and also hydroxyclofentezine isomers and methylthio-hydroxyclofentezine.

Baboon

The metabolism of clofentezine in the baboon is shown to be very much simpler than in the rat (and most other species studied) in that hydroxylation in the 4- position and subsequent conjugation with glucuronic acid is by far the most dominant route of metabolism. Thus free and conjugated 4-hydroxyclofentezine account for up to 75% of the urinary metabolites. By contrast only 4-5% of the methylthio-hydroxy metabolite is present.

Calf

The metabolism of clofentezine in the calf appears also to favour hydroxylation, with mainly hydroxyclofentezine conjugates present in the urine and little if any methylthio-hydroxyclofentezine present.

DISCUSSION

The results of these studies show that clofentezine is rapidly metabolised and excreted following oral dosing at 10 mg/kg, with the major route of excretion being via the faeces. Urinary levels ranged from only 1-2% in the dog to 35-37% in the rabbit.

The metabolism of clofentezine has been shown to be qualitatively similar in all the species studied, with hydroxylation and replacement of chlorine with a methythio group being major pathways. Numerous minor metabolites were found in all species.

Quantitative interspecies differences were apparent, most notably the fact that in the calf and the baboon, metabolism occurred mainly via hydroxylation and subsequent conjugation. In rodents and the rabbit, methylthiolation became a much more prominent pathway and many more minor metabolites were apparent.

Table 1

Excretion of radioactivity from rats, mice and rabbits dosed with ^{14}C-clofentezine at 10 mg/kg

(Results expressed as percentage of administered dose.)

	RAT		MOUSE		RABBIT	
	5♂*	5♀	3♂	3♀	3♂	3♀
URINE						
0-24 h	17.7	17.8	25.0	22.1	30.0	29.5
24-48 h	1.1	1.9	1.0	2.7	5.8	3.9
48-72 h	0.2	0.5	0.2	0.5	0.9	0.9
72-96 h	0.1	0.3	0.3	0.1	0.9	0.4
TOTAL	19.2	20.4	26.5	25.7	37.2	34.6
FAECES						
0-24 h	71.4	57.9	64.7	60.3	41.7	40.9
24-48 h	5.9	13.5	3.4	5.3	11.8	11.3
48-72 h	0.5	1.0	0.4	0.9	3.1	3.1
72-96 h	0.1	0.3	0.2	0.9	1.3	1.3
TOTAL	77.9	72.8	68.7	67.4	57.9	56.7
TOTAL RECOVERY+	97.1	93.2	95.2	93.1	101.6	97.2

* Number relates to number of animals/group.

\+ Total recovery includes cage debris and washes.

Table 2

Excretion of radioactivity from dogs and baboons dosed with ^{14}C-clofentezine at 10 mg/kg

(Results expressed as percentage of administered dose).

	DOG		BABOON	
	3♂*	3♀	1♂	1♀
URINE				
0-24 h	1.0	0.7	13.2	28.1
24-48 h	0.6	0.4	7.7	6.4
48-72 h	0.1	0.6	1.8	0.4
72-96 h	<0.1	0.5	0.1	<0.1
TOTAL	1.7	2.2	22.8	35.0
FAECES				
0-24 h	38.0	62.2	36.4	44.7
24-48 h	54.1	12.9	24.0	8.7
48-72 h	1.4	10.9	3.1	0.9
72-96 h	0.2	10.8	0.4	0.1
TOTAL	93.8	96.9	63.9	54.4
TOTAL RECOVERY+	96.0	102.8	100.0	100.0

* Number relates to number of animals/group
\+ Total recovery includes cage debris and washes.

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Figure 2 Proposed metabolic pathway for metabolism of clofentezine in animals