SESSION 3A

INTEGRATION OF CEREAL CROP MANAGEMENT WITH WEED CONTROL: PRACTICAL ASPECTS

CHAIRMAN	MR A. J. BIDE
SESSION ORGANISER	MR M. N. TALBOT
INVITED PAPER	3A-1
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THE INTEGRATION OF PEST AND DISEASE CONTROL WITH WEED CONTROL IN WINTER CEREALS IN GREAT BRITAIN

J. H. ORSON

Agricultural Development and Advisory Service (ADAS), Block B, Government Buildings, Brooklands Avenue, Cambridge, CB2 2DR.

ABSTRACT

The intensive growing of cereals, particularly winter cereals, has been made possible by herbicides and chemical fertilisers. Such systems now depend on relatively high inputs of pesticides which often need to be applied at specific times. The number of spray occasions available to the farmer is frequently severely limited by the weather. This limitation has led increasingly to the use of tank-mixes for the control of weeds and/or diseases and/or pests. It has been argued that such tank-mixes are often applied when the conditions or the growth stage of the crop are not correct for one or more of the constituents. Spray applications for pest and disease control and for growth regulation are usually time specific. However, modern herbicides can be applied at a range of timings to obtain reliable weed control. In addition there is a long period of time over which weeds can be controlled before they reduce yield. Hence, the integration of economic chemical weed, pest and disease control and growth regulation in the form of tank-mixes of pesticides is often possible without conflict or compromise. The notable exceptions to this conclusion are highlighted. The integration of cultural control measures for pest, disease and weed control are also discussed and again there are some notable conflicts between objectives. Also briefly discussed is the impact of weeds on the pest and disease status of cereal crops and the direct influence of herbicides on cereal diseases.

INTRODUCTION

Cereal production has been transformed by the introduction of chemicals that "feed and weed" crops and thereby largely replace the role of the traditional rotation. "Artificial" fertilisers, based on inorganic chemistry, were introduced in the last century and selective herbicides, based on organic chemistry, have been introduced over the last 40 years. Together they have made it possible for farmers to intensify cereals on land suited to their production. Not only has there been an intensification of cereal production but also a trend to the higher yielding winter rather than spring crops. Virtually all the wheat and two thirds of the barley is now autumn sown. This has led to a further increase in weed pressure which in turn has led to further herbicide use. Effective herbicides have also allowed changes to some of the major husbandry practices of winter cereals, particularly primary cultivation methods and date of drilling. This in turn has led to even further encouragement of weeds, particularly annual grasses. Intensification of cereals has also resulted in greater disease and pest pressure. The

introduction of more effective insecticides in the 1960s and more effective fungicides in the 1970s now contributes towards the reliable production of cereals in intensive cropping sequences including continuous winter cereal production on heavy land. As a result of artificial fertilisers and pesticides, plus the advances in plant breeding, mechanisation and drainage, the yield of cereals has increased dramatically over the last twenty years (Silvey, 1986).

Therefore, modern cereal production, particularly intensive winter cereal production, can involve large usage of herbicides, fungicides and insecticides. In addition, growth regulators are commonly used. This has resulted in the farmer adopting tank-mixes to save on application costs and to ensure that with the often limited number of spray occasions, the relevant crop protection agents are applied at the correct time (Table 1). This paper examines the impact of weeds on the pest or disease status of the cereal crop, the effect of herbicides on cereal diseases and the integration of weed control with other crop protection measures and discusses whether there is any conflict or compromise between the differing objectives. Both cultural and chemical weed control measures are discussed. The impact of husbandry practices on weed control in cereals has been discussed in detail elsewhere (Orson, 1987). Limited space does not allow for a review of the impact of all husbandry factors on the integration of weed, pest and disease control.

TABLE 1. Number of spray occasions (Spackman, 1983) of five hours at Waddington (Lincolnshire) available for a low-ground pressure vehicle between 1970 and 1988 (Spackman, pers. comm.).

	Month								
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Soil-applied herbicides									
Average	23	17	9	6	5	9	13	20	23
Worst year in 10	17	10	4	3	1	0	6	10	13
2-(Aryloxy)alkanoic acids									
Average	19	10	1	0	0	1	3	11	17
Worst year in 10	10	4	0	0	0	0	0	2	8

IMPACT OF WEEDS ON THE PEST AND DISEASE STATUS OF WINTER CEREALS

In general, there is little significant interaction between weed infestations and pests and diseases but there are important exceptions (Heitefuss, 1986).

Emerged volunteer cereals act as a "green bridge" and if not controlled can be a source of obligate parasites, such as Puccinia striiformis (the yellow rust fungus) and barley yellow dwarf virus, to emerging crops in the same or neighbouring fields. Emerged volunteer cereals and some annual grass weeds, if not controlled at drilling,

can act as primary sources for aphid borne barley yellow dwarf virus and cause problems with Oscinella frit (frit fly) in late drilled crops of cereals which otherwise would not suffer from the problem. There has been an association between high populations of Alopecurus myosuroides (black-grass) and Claviceps purpurea (ergot) in wheat in years when there has been heavy rain during the flowering period of the weed. The presence of Elymus repens (common couch) or its rotting rhizomes has encouraged Gaeumannomyces graminis (the take-all fungus). Weed control in cereals suffering from take-all is particularly important because of the need for adequate nutrition of the limited root system. Avena spp. (wild-oat) and certain other weeds can act as hosts to nematodes which occasionally attack cereals on light soils. Finally, high levels of vegetation, such as a weedy cereal crop, may encourage slugs.

CULTURAL CONTROL OF WEEDS, PESTS AND DISEASES IN WINTER CEREALS

Between successive winter cereal crops there is limited time available for cultural measures to control weeds, pests and diseases. It is partly for this reason that intensive cereal systems rely heavily on pesticides. The cultural control measures which are attempted aim to kill weeds which are difficult if not impossible to control in the following cereal crop, such as Bromus sterilis (barren brome) and volunteer cereals, or which increase the risk of the transfer of barley yellow dwarf virus or fungal pathogens such as P. striiformis to the following crop.

The objectives of cultural control of weeds and obligate parasitic diseases may conflict. The objective for weed control is either to bury the seeds to a depth from which they will not emerge or to encourage viable seeds to grow so that they can be destroyed by cultivation or non-selective herbicides. This latter objective can be different from that of trying to prevent a green bridge for cereal diseases or pests.

Straw burning

Straw burning reduces weed, pest and disease problems in the succeeding winter cereal crop. It kills some weed seeds, including volunteer cereals, on or near the soil surface but this does not mean that the need for weed control in the following crop is avoided or indeed significantly reduced. The exceptions are B. sterilis, other Bromus spp. and volunteer cereals which can be controlled effectively by straw burning but not reliably with selective herbicides. Obviously, the control of weed seeds is dependent on the heat generated and the proportion of the field effectively burnt. The straw burning operation can also reduce the number of slugs and may destroy green material which will act as a green bridge for cereal diseases or as a host for the aphid vectors of barley yellow dwarf virus. The inoculum of trash borne pathogens such as Pyrenophora teres, the net blotch fungus of barley, can also be reduced. Environmental pressures have decreased the amount of straw burning in recent years.

A possible conflict between straw burning for weed control and disease control is that surviving weed seeds are often less dormant. Provided that the soil is moist, there is often a "bloom" of annual grass weed growth after straw burning. This "bloom" presents an ideal

opportunity to destroy the plants from weed seeds surviving straw burning. However, it can act as a green bridge for the survival and transfer of cereal diseases and pests to the following or neighbouring crops if it is not destroyed before these crops emerge.

There are also drawbacks associated with straw burning. Burnt straw adsorbs many soil-applied herbicides. If straw burning is an annual event and the burnt residues are not dispersed by relatively deep cultivation or by ploughing, the efficacy of many of the soil-applied herbicides can be reduced.

Stubble cultivation

The use of non-selective herbicides rather than stubble cultivation offers more effective control of some weed species and diseases.

In the absence of straw burning, many farmers carry out shallow cultivations immediately after harvest for various reasons. Where straw is to be incorporated without ploughing there is a distinct advantage to an early start in terms of yield of the subsequent cereal crop. Cultivation prior to ploughing encourages a more rapid break down of the straw. However, this does not result in a higher yield from the succeeding crop. On the other hand, incorporation prior to ploughing can have practical advantages. With better mixing of the straw and soil there is likely to be more nitrogen immobilised during the winter months and hence less nitrate leaching. Also, on heavier soils there is the prospect that cultivation prior to ploughing may ease the production of a seedbed after ploughing. Finally, cultivation straight after harvest may stimulate the germination of some weed species. However, whilst early cultivation may stimulate the germination of B. sterilis and in some years the germination of volunteer cereals, it prevents the loss of Avena fatua seed on the soil surface and in some years can increase the dormancy of volunteer cereals. Therefore, with certain weeds the common objective of preventing a green bridge and reducing viable weed seed is best served with the use of non-selective herbicides rather than cultivation. There is the added advantage that the use of a non-selective herbicide, such as paraquat, can reduce the green bridge transfer of the aphid vectors of barley yellow dwarf virus. Non-selective herbicides can also suppress the sporulation of pathogens such as P. teres and Septoria nodorum though not the sporulation of Rhynchosporium secalis on plant debris on the soil surface (Harris & Grossbard, 1978; Jordan & Allen, 1984; Stedman, 1980). The practical significance of this effect may be small since the reduction of sporulation of P. teres did not lead to a significant reduction of net blotch in the succeeding winter barley crop (Jordan & Allen, 1984).

Where straw burning is carried out, farmers are legally obliged to cultivate within 36 hours. This may encourage a fuller germination of Bromus spp. and volunteer cereals. However, it is of little or no advantage for encouraging the germination of A. myosuroides and may prevent loss of A. fatua from the soil surface.

Stubble cultivation used to be one of the most effective measures for the control of perennial grass weeds. However, it was necessary to repeat the cultivations in order to reduce significantly the number of viable buds on the storage organs and/or to exhaust them. This inevitably resulted in a delay in drilling. The introduction of glyphosate has given

the farmer access to more effective control without a delay in drilling. Therefore, it is now unusual for farmers to try to control perennial grasses by cultural means.

Primary cultivation for winter cereals

Historically, ploughing has been used to reduce weed problems, to minimise the risk of infection with some fungal diseases and to reduce the risk of certain pests in successive cereal crops by burying all residues of the previous crop.

Ploughing is now commonly carried out to control B. sterilis and volunteer cereals. It also reduces the herbicide adsorption of the soil surface layers, due to organic matter and burnt straw residues which build up with repeated shallow cultivation techniques. It is also used to bury large numbers of viable annual grass weed seeds, which may also be a result of previous shallow cultivation techniques associated with intensive winter cereal production. However, ploughing often produces a more cloddy seedbed than shallow cultivation on heavy soils. In this situation, slugs are usually more mobile in the surface horizons of the soil and hence can cause more crop damage. Similarly, if a cloddy and loose seedbed results, there can be a more rapid build up of root diseases such as take-all. Finally, ploughing down aphid infested plants of volunteer cereals and some of the annual grass weed species without allowing a sufficient interval before drilling creates, when the seedbed is warm and dry, a reservoir of these pests which may invade the following cereal at or below the soil surface. A severe attack of barley yellow dwarf virus can result if these aphids are carrying the virus. In this situation, the weed plants should be sprayed with a dessicant non-selective herbicide prior to ploughing. This application of herbicide may not be necessary for weed control alone.

Ploughing can also have other drawbacks. It can produce a cloddy and loose seedbed unsuitable for the activity of soil-applied herbicides and where weeds may establish their secondary root systems deeper than normal, which thus makes them more difficult to control.

Non-ploughing techniques may encourage the emergence of infected volunteer cereals which, if not controlled, will act as a source for the spread of barley yellow dwarf virus and some other cereal diseases. In the absence of straw burning, when large amounts of straw debris are allowed to build up on or near the soil surface, there is evidence that slug populations will be encouraged.

Delayed drilling of winter cereals

Delayed drilling of winter cereals can be an advantage for both weed and disease control particularly in reducing the risk from barley yellow dwarf virus. Although risky on heavy soils where conditions may become too wet for drilling, some farmers have used this technique where there are extremely heavy populations of B. sterilis and A. myosuroides. Later drilling of winter cereals is likely to result in lower weed populations with weed plants emerged at the time of drilling being killed by the final cultivations or by a non-selective herbicide. However, delayed drilling can result in a slower germinating crop growing under poor conditions and therefore, on heavy soils, more likely to suffer from slug damage.

Delayed drilling of winter cereals reduces tillering of individual crop plants and this may reduce the crop's ability to withstand an attack of Delia coarctata (wheat bulb fly) which in its later instars moves from the tiller initially attacked. D. coarctata only rarely occurs in intensive rotations of solely autumn-sown crops and then usually after a crop of oilseed rape.

EFFECT OF SELECTIVE HERBICIDES ON CEREAL DISEASES

There is some very limited evidence that herbicides can increase disease levels. Mecoprop salt, dicamba and ioxynil have all been associated with an increase in take-all (Tottman & Thomson, 1978). In ADAS trials, apart from the application during the winter to barley grown on sandy soils, there has been little concern regarding the yields obtained from the recommended use of these herbicides. However, there have been instances of disappointing yields when ioxynil with mecoprop has been applied to winter wheat in ideal conditions for weed control. The reason for these lower yields has not been investigated (Orson, 1988).

Herbicide usage out of recommendation may decrease the plants' ability to withstand disease infection. For instance, application of mecoprop salt to winter barley later than recommended can increase the level of powdery mildew (Orson, 1983). On the other hand, the use of the herbicide difenzoquat as recommended reduces the levels of powdery mildew (Erysiphe graminis).

CHEMICAL CONTROL OF PESTS, DISEASES AND WEEDS IN WINTER CEREALS

Pesticide usage in winter cereals

Pesticide use in winter cereals is dependent on the intensity of cropping on the farm and in the surrounding area, cultivations used, cereal cultivar, soil type and location within Great Britain. Not all these factors affect the level of pests, diseases or weeds. Weeds are mainly influenced by the rotation, soil type and cultivation; pests by rotation and location; diseases by cultivar, location and intensity of cropping on the farm and within the surrounding area. Additionally, diseases, weeds and some pests are encouraged by early drilling.

With the increased intensity of winter cereals and the earlier drilling that has been carried out in the 1980s, the use of pesticides, notably herbicides and fungicides, has increased dramatically over the last 20 years. It is difficult to find reliable data to demonstrate in detail this increase in usage but the MAFF surveys in 1977 and 1982 give an indication of the trend (Table 2). Market research and surveys by ADAS indicate that the usage of herbicides and fungicides tended to plateau from 1984 onwards. Insecticide and molluscicide use varies considerably between seasons depending on the mildness of the winter and summer weather for aphids and the wetness of the summer and autumn for slugs. These are the two main pest problems in winter cereals. Unlike herbicides and fungicides, the use of growth regulators in winter cereals has apparently increased since 1984. This reflects recently introduced cultivars being more susceptible to lodging than their predecessors and to the increased importance of grain quality and the rate of harvesting, both of which can

be impaired by crop lodging. Evidence that winter barley may respond in yield to the use of chlormequat, even in the absence of lodging, has particularly increased usage.

The increased usage of herbicides is due to the application of soil-applied products primarily for the control of annual grass weeds which are a result of the more intensive growing of winter cereals. Virtually all herbicides are applied as sprays. Most fungicides are foliage-applied sprays. In addition, seed treatments of mercury based compounds are usually used but there has been a significant recent usage of triazoles with or without morpholine based fungicides as seed treatments. Cereal seed is also treated with an insecticide to control soil pests where necessary. Spray applications are used to control aphids and some other pests. Slugs are treated with pellets containing molluscicides and bait and hence their usage is not included in Table 2. Growth regulators are used as foliage-applied sprays. Chlormequat is commonly used at the pseudo-stem erect stage of winter cereals (Zadoks et al, 1974). Plant growth regulators based on 2-chloroethylphosphonic acid are less commonly used and are applied later at the second node detectable to flag leaf ligule visible stage of the crop. Their sequential use is advised where there is a very high risk from crop lodging.

TABLE 2. Usage of pesticide products/ha applied either as sprays or granules to winter wheat and winter barley in England, Wales and Scotland in 1977 and England and Wales in 1982 (Sly, 1981; Sly, 1986).

	Selective Herbicides		Fungicides		Insecticides		Growth regulators	
	Winter Wheat	Winter Barley	Winter Wheat	Winter Barley	Winter Wheat	Winter Barley	Winter Wheat	Winter Barley
1977	1.54	1.21	0.37	0.45	0.48	0.01	0.17	0.00
1982	2.32	1.94	2.02	1.56	0.25	0.12	0.37	0.14

Time of application of pesticides to prevent yield loss in winter cereals

Weeds

The general conclusion from a bibliography of papers on weed competition in all crops compiled by Zimdahl (1980) was that the critical time to control annual weeds in order to prevent yield loss was prior to the period of exponential growth of the crop. This period of growth usually starts in winter cereals in Great Britain at the first node detectable stage. Experimental evidence with annual weeds in this country tends to back this view unless there are exceptional levels of weeds when earlier application may be necessary (Baldwin, 1979; Moss, 1987; Orson and Marshall, 1985). However, in practice, it is unwise to delay weed control until the last moment, particularly if earlier applications of herbicides give economic and effective season long control of weeds. In addition, weeds may be too large to be controlled effectively by herbicides at the time of the pseudo-stem erect stage of the crop or the weather may be unsuitable for optimum activity of herbicides.

Farmers try to apply annual grass-weed herbicides in the autumn and winter. By the late spring, these weeds, with the exception of Avena spp. are too large to control reliably with the current commercially available herbicides. Again with the exception of Avena spp., these weeds are mainly controlled by soil-applied herbicides, which require a period of moisture in the surface layers of the soil. These conditions are less likely in the spring, particularly after mid-February. Avena spp. can be effectively controlled by foliage-applied herbicides in the spring and yield loss prevented, provided that conditions are suitable for the activity of the presently available herbicides before the critical growth stage. These conditions are by no means guaranteed, particularly in the early sown crops, which has again resulted in a significant usage of herbicides to control these weeds in the autumn (Baldwin, 1979). Annual broad-leaved weed control is possible either by autumn or spring application. However, season long control of some species may not be possible from autumn/winter usage of soil or foliage-applied herbicides and of other species from spring usage of foliage-applied herbicides.

It is not possible to control perennial grass weeds selectively in cereals although pre-harvest glyphosate application is often carried out. Perennial broad-leaved weeds do not emerge until advanced stages of the crop and some selective herbicides can reduce populations if they can be applied before the growth stages of the crop when they may cause phytotoxicity. Pre-harvest glyphosate is often used against these weeds.

Diseases

The time of application to prevent yield loss is now reflected in field practice. There is little spraying of foliage-applied fungicides in the autumn and winter unless the weather is exceptionally mild and there is a build up of powdery mildew in barley or yellow rust in wheat. Most of the spraying commences in the spring. Winter barley is usually sprayed with a broad-spectrum fungicide or fungicide mixture at the beginning of spring growth and often a further spray is applied at flag leaf ligule emerged stage. Winter wheat commonly receives two or three sprays. With the recent development of widespread benzimidazol (MBC) resistant Pseudocercosporella herpotrichoides (the eyespot fungus), the 'spray window' has been lengthened as prochloraz (now the most effective fungicide against the disease) can give good control when applied as late as the second to third node detectable crop growth stage. Earlier sprays are not usually necessary except where there is an active yellow rust infestation. Spraying at the flag-leaf ligule emerged stage is the single most important timing for the control of foliage diseases in winter wheat. A further spray may be required at ear fully emerged stage, especially in wet seasons.

Pests

Yield loss from pests is prevented by insecticide application usually when infestations reach action thresholds in the field. Slugs are treated with pellets during the autumn and winter. Insecticide sprays are applied in high risk areas every year and in other areas when the need arises for the control of the aphid vectors of barley yellow dwarf virus. The timing is after the winged invasion but before the population builds up and colonises other plants. This is usually from mid-October to mid-November. D. coarctata is commonly treated by sprays applied at egg hatch from the

middle of January or when damage symptoms are seen from late February. Aphids causing direct damage are sprayed when the need arises which is usually in June or July.

Management of chemical pest, disease and weed control

The wide period of time during which herbicides can be applied before yield loss from weeds occurs makes it possible to tank-mix with insecticides, fungicides or growth regulators which have to be applied at more specific times. For instance, in the autumn, a post-emergence spray for weeds can be applied with an insecticide for the control of barley yellow dwarf virus or in the spring, a spray for the control of broad-leaved weeds can be applied with a growth regulator and also a fungicide in barley.

There are some notable exceptions where tank-mixes of pesticides may not provide the ideal means for the optimum or economic control of pests, diseases and weeds. For instance, in Scotland, Wales and the west of England the use of relatively cheap pre-emergence herbicides is often preferred for the control of Poa spp. (meadow-grasses). The post-emergence option which can be tank-mixed with an insecticide offers a more expensive alternative. The mixing of an insecticide and grass weed herbicide in the autumn can cause problems where application of the insecticide is deemed to be necessary in mid-October. This may be too early for the optimum control of grass weeds with herbicides such as isoproturon, when the following weather is mild and moist leading to rapid degradation in the soil. Galium aparine (cleavers), one of the most common and certainly the most competitive annual broad-leaved weed, usually has to be sprayed in the spring whether or not it has been treated in the autumn. Weather conditions may be too cool for some herbicides at the pseudo-stem erect stage and later spraying may be necessary to achieve optimum control, particularly in early-drilled crops. Fortunately, G. aparine can be controlled a little later than other annual broad-leaved weeds before yield loss occurs but such a timing may be too late for the safe and effective use of chlormequat (Orson, 1988). Perhaps one of the greatest conflicts in the timing of herbicides, growth regulators and fungicides is where the fungicide is being applied for the control of eyespot in winter wheat. In terms of the rational use of fungicides, the optimum time for the control of MBC resistant strains of P. herpotrichoides and for the first application against some leaf diseases is often at a later time than for the application of herbicides to prevent yield loss from annual broad-leaved weeds, with the possible exception of G. aparine, and is too late for the safe and effective use of chlormequat.

There are sometimes other problems with the use of tank-mixes. The addition of some insecticides to isoproturon has occasionally predisposed the crop to frost scorch. The addition of mecoprop further increases this risk. One of the most common tank-mixes used in the spring is mecoprop salt for the control of broad-leaved weeds and chlormequat. On occasions, the addition of chlormequat reduces the efficacy of mecoprop on G. aparine (Sansome, 1989).

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THE INCIDENCE OF WEEDS IN WINTER CEREALS IN GREAT BRITAIN

R. WHITEHEAD, H. C. WRIGHT

Schering Agriculture, Stapleford, Nottingham NG9 8AJ

ABSTRACT

A survey of 1476 fields of winter wheat and 883 fields of winter barley was carried out throughout Great Britain in autumn 1988, to assess the occurrence (but not the level of infestation) of grass and broad-leaved weeds in crops which had not received herbicide treatment at the time of inspection. Care was taken to ensure that the sample was as representative of the total area of each crop as possible. The survey covered 4.3% of all farms growing winter wheat and 4.1% of those growing winter barley. The most common broad-leaved weeds were Stellaria media, Veronica persica, Matricaria spp and Galium aparine. Volunteer rape was more common in wheat. Poa annua was by far the most frequently recorded grass weed in wheat (74%) and barley (85%). Avena spp and Alopecurus myosuroides were the next most frequent in both crops. The findings are presented and discussed, including reference to earlier similar surveys.

INTRODUCTION

The growth in number and spectrum of selective herbicides since 1945 has created a continuous need to assess the spread and abundance of weed species. Choice of herbicide is governed, at least in part, by the weed species present, while development of a herbicide must be directed towards control of those weeds likely to be present in future. Both situations require knowledge of weed distribution. Numerous weed surveys have been carried out in UK which vary so widely in objective and scope that strict comparisons are virtually impossible. Some were directed at individual weed species or groups of species (Thurston, 1954; Elliott et al., 1979), while others covered a very limited geographical area (Elliott et al., 1968; Froud-Williams & Chancellor, 1982; Chancellor & Froud- Williams, 1984). Most surveys have used "a field" (usually with some measure of randomisation of choice) as the unit of measurement and have recorded presence or absence of weed species, usually associated with a simple measure of relative abundance (e.g Ingram, 1975).

One survey (Chancellor, 1977) used the tetrad (2 km x 2 km) positioned according to the Ordnance Survey National Grid lines as the unit of measurement, but unfortunately confined itself to recording presence or absence of a pre-selected list of 40 species, very few of which appear in the lists of most abundant species shown by other surveys.

Most surveys were carried out in the spring (typically March-May for broad-leaved weeds and July-August for grass weeds) which has the advantage of ensuring that all weed germination will have occurred, but the disadvantage that the record will be of species which have survived herbicide treatment rather than those which may have succumbed.

The work reported here is believed to be the largest scale survey of weeds in winter cereals ever conducted in UK, and the only one carried out before any herbicide treatment of assessed crops. It was designed to examine the relative importance of different weed species nationally and regionally and to allow comparison with two earlier surveys conducted in 1967 (Fisons, 1968) and 1972 (Fisons 1973).

METHOD

A total of 2359 winter cereal fields were assessed of which 1476 were winter wheat from 1129 farms and 883 were winter barley from 742 farms. The survey covered 4.3% of farms growing winter wheat and 4.1% of those growing winter barley (Table 1). These farms represented 6.1% and 5.4% respectively of the national areas of these crops.

TABLE 1 The achieved sample for each crop as a proportion of the total farms available for inspection nationally

	WINTER WHEAT Farms	WINTER BARLEY Farms
Sample achieved	1129	742
% Availability nationally	4.3	4.1

The assessments were made by 120 technical sales representatives in 14 territories, comprising 4 regions, covering the arable areas of England, Scotland and Wales. Each assessor was asked to provide a sample of farms which was as representative as possible of his area in terms of the range of soil types, cropping patterns and topography of that area. Each assessor surveyed 10 farms growing a minimum of 20 hectares of winter cereals in 1988/89. The sample results were grossed up to provide national projections of weed occurrence based on the area of each winter cereal crop by farm as a proportion of the total area of that crop within the sales territory.

In order to maximise the crop area covered by the survey, assessors were asked to inspect a minimum of 2 fields per farm, taking the largest appropriate fields on the farm. As a result a disproportionately high area of crop was surveyed in relation to the number of farms included in the sample. The data were therefore weighted back according to the 1987 MAFF Census statistics on 3 distinct crop area bands (20-49 hectares, 50-99 hectares, 100+ hectares) for each of the 14 territories to provide data which reflected the national distribution of each crop. On farms where only one field of winter cereals met the above requirements, that field was assessed.

Only crops which had received no herbicide at the time of inspection were included in the survey, to minimise the influence of herbicide treatments on weed incidence. For each field the weeds present were recorded on a pre-printed form together with details of the previous crop and the soil type using a classification based on the Soil Texture (85) System (MAFF, 1985). The survey was conducted between October and December 1988.

No attempt was made to record levels of weed infestation as the purpose of the survey was to show the variations in weed occurrence by soil type and winter cereal crop. The assessors surveyed fields as they would to determine herbicide treatments. The method of assessment could not prove the absence of a weed from a surveyed field but only provide a reasonable likelihood of its being recorded if present. The results therefore probably underestimate weed presence.

RESULTS

Fields of winter wheat (1476) and winter barley (883) were assessed and reported separately. However, apart from a few exceptions discussed below, differences between the crops were small. Results are therefore presented for all winter cereals (2359 fields) but the separate data are available from the authors on request. Tables 2 and 3 show the findings for broad-leaved weeds and grass weeds respectively.

TABLE 2 Main broad-leaved weeds in winter cereals (% fields infested)

	Total	Scotland/ N East	Anglia	Southern	Western
Stellaria media	94	98	92	90	96
Veronica persica	72	79	76	69	59
Matricaria spp	67	72	68	63	63
Galium aparine	58	59	60	55	58
Lamium purpureum	47	59	36	47	39
Viola arvensis	45	37	45	49	54
Sinapis arvensis	36	27	41	38	42
Veronica hederifolia	30	28	33	33	26
Capsella bursa-pastoris	23	26	21	20	24
Volunteer rape	23	36	22	10	16
Papaver rhoeas	18	13	27	20	11
Fumaria officinalis	17	21	7	17	20
Chenopodium album	13	17	11	10	13
Aphanes arvensis	12	6	13	17	14
Geranium spp	11	9	11	11	14

TABLE 3 Main grass weeds in winter cereals (% fields infested)

	Total	Scotland/ N East	Anglia	Southern	Western
Poa annua	79	82	66	78	88
Avena spp	42	35	51	45	40
Alopecurus myosuroides	38	25	70	35	26
Elymus repens	21	23	21	19	20
Lolium spp	14	15	7	15	19
Bromus sterilis	13	15	12	12	10
Poa trivialis	7	8	3	12	2
Volunteer cereals	7	7	7	9	5

DISCUSSION

Arable weeds are, by definition, invaders of cultivated land. Changes in agricultural practice, whether chemical, cultural or in cropping pattern, are likely to influence the relative abundance of individual species. Indeed, there are those who claim that excessive use of herbicides has resulted in the elimination of some plant species. The results from this survey suggest that this is far from the truth and that use of herbicides has done little to alter the spectrum of commonly found weeds.

Table 4 indicates the overall abundance and ranking order of the main broad-leaved weeds in this survey compared with data on winter cereals from the two Fisons surveys of 1967 and 1972. The 22 years spanned by these surveys have seen greater changes in herbicide technology, and agricultural practice (including a major swing from spring to autumn sown cropping) than in any similar period before, or likely to be seen again. Yet the top four weeds are the same in each survey and are, if anything, increasing in abundance. Sinapis arvensis, one of the easiest species to control chemically in cereals, is as important and as abundant today as in 1967. Furthermore, our survey (conducted in the autumn) is likely to have underestimated spring germinating species such as Bilderdykia convolvulus, Polygonum aviculare and Polygonum persicaria, all of which featured strongly in the spring surveys of 1967 and 1972.

The rise to prominence of Lamium purpureum (not considered to be sufficiently important for inclusion in the earlier surveys) and Viola arvensis is almost certainly attributable to the switch to winter cropping rather than weaknesses in the available herbicides, as is sometimes suggested, because the advent of hydroxy benzonitriles and, more recently, sulfonyl ureas, has failed to prevent their increase.

The appearance of volunteer rape in the top ten is significant not only because it reflects the enormous increase in oilseed rape grown in UK since the 1972 survey, but also it indicates an increasing awareness of the importance of crops as weeds. Oilseed rape commonly precedes a winter wheat crop, and this was one of the few species which displayed a significant difference in abundance between the two winter cereals, being, as expected, more common in winter wheat. Volunteer rape was also one of the few weeds to show significant differences in regional distribution, being more abundant than average in Scotland/north east England, and of apparently less importance in the south.

TABLE 4 Abundance (and ranking) of top ten broad-leaved weeds in winter cereals compared with earlier surveys

	% fields infested		
	1989	1972[1]	1967[2]
Stellaria media	94 (1)	89 (1)	77 (1)
Veronica persica	72 (2)	55 (2)*	52 (2=)*
Matricaria spp	67 (3)	53 (3)	52 (2=)
Galium aparine	58 (4)	52 (4)	49 (4)
Lamium purpureum	47 (5)	-	-
Viola arvensis	45 (6)	14 (12)	7 (17=)
Sinapis arvensis	36 (7)	33 (6)	39 (6)
Veronica hederifolia	30 (8)	*	*
Capsella bursa-pastoris	23 (9=)	11 (6)	-
Volunteer rape	23 (9=)	-	-

1 Fisons (1973)
2 Fisons (1968)
* Veronica spp not differentiated in 1967 and 1972 surveys
- Not included in survey

Most weeds showed a remarkably consistent distribution across the four regions. This was most marked among the abundant species which again indicates that their success as weeds is little influenced by agricultural practice and they can be expected to remain the commonest and most troublesome broad-leaved weeds in future.

Comparison of grass weed incidence in this survey with earlier surveys is more difficult because attention in the past has been confined to Avena spp, Alopecurus myosuroides, and Elymus repens (eg Fisons, 1973; Elliott et al., 1979). More recently the importance of other grass species, notably Bromus sterilis and Poa trivialis as competitive weeds, has been recognised. These species were included in the survey by Froud-Williams and Chancellor (1982) in central southern England but the strong regional differences in distribution exhibited by several grass weeds in our survey (table 3) make comparisons meaningless. Furthermore, Froud-Williams and Chancellor conducted their survey between 23 June and 26 August 1981 by scanning from suitable vantage points and assessing grass weeds by visible panicles above the crop. As a consequence Poa annua, by far the most frequently observed grass weed in our survey, was omitted from the 1981 survey altogether.

The problem of alien cereals as potential contaminants which can, for instance, reduce Hagberg Falling Number of wheat, has commanded attention only very recently. Indeed the survey reported here is probably the first to provide a quantitative assessment of the problem. Seven percent of winter cereal fields in our survey carried alien cereals as weeds (Table 3). This was marginally greater than the incidence of Poa trivialis, already a familiar weed and one regarded as being a significant competitor in some situations (Sharrott, 1988).

It would be easy to suggest that the results of this survey add little to our knowledge. We suggest the opposite. That the most abundant weeds have remained unchanged for over 20 years during which virtually everything else in agriculture has changed dramatically is a finding of great importance. This survey contains no information on levels of infestation, but the evidence here gives strong support to the view that the main effects of herbicide usage in arable land have been to reduce rather than eliminate weed populations and to change the relative frequency of some species (Chancellor, 1977). Of course, we need to know more about the economic significance of different weeds, but those carrying out such work can concentrate their efforts on the major species. Those involved in the development of new products can be assured that the targets are not moving much, however good and selective our present range of herbicides. Finally, to those who believe that use of herbicides is changing our countryside, the answer is that the evidence of our survey does not support them. If anything, the weeds are winning.

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CUTTING THE COST OF CULTIVATIONS, THE LAST SAVING FOR CEREAL GROWERS?

M. GIBBARD, J. COUTTS

ICI Agrochemicals, Woolmead House West, Bear Lane, Farnham, Surrey, GU9 7UB.

D. B. DAVIES

Agricultural Development and Advisory Service, Government Buildings, Brooklands Avenue, Cambridge, CB2 2DR.

J. BAILEY

Agricultural Development and Advisory Service, Government Buildings, Beeches Road, Chelmsford, Essex, CM1 2RU.

ABSTRACT

The need for a reappraisal of the use of reduced cultivations on medium to heavy soil farms in the U.K. is discussed. With machinery and cultivations accounting for 40% of fixed costs, less dependance on the plough is required. Both previous and current work together with advisory experience indicate a range of situations were there are opportunities for shallower cultivations even where straw is not burnt. In taking note of past experience, a flexible but planned approach is required in assessing machinery and labour needs; avoiding soil damage and grassweed build up and using fast shallow cultivations with as necessary a total herbicide in seed bed preparation.

INTRODUCTION

While the prospects for the future profitability of cereal growing remain uncertain, growers need to carefully review all the items of expenditure within the enterprise. Farmers have already looked closely at variable costs and further savings are likely to be small and have a detrimental effect on yield or quality.

To remain competitive farmers must pay more attention to their fixed costs of power, machinery and labour and in particular understand the cost of individual cultivations. Machinery costs are in the range of £200 - £250/ha for mainly cereal and mixed cropping farms and represents 40-45% (Murphy 1989) of fixed costs. Labour and machinery costs together total approximately 65% of fixed costs. Savings of 10-20% are possible in many situations.

On many farms the cost of ploughing (£30-£35/ha on medium to heavy land) is a significant proportion of the establishment cost. If the plough could feature less prominently in the cultivation system, savings would be made. Shallower and faster seed-bed preparation could not only reduce establishment costs but also improve work rates, and therefore profitability provided yields are not reduced.

Up until the late 1960's all cultivation systems were based on the plough. By the early 1970's the swing to winter cereal production brought about a much increased autumn machinery workload. Reduced cultivations including the art of direct drilling were quickly adopted by many farmers who utilized the speed of establishment to maximise the area in winter cereal production. However continual use of these reduced cultivation systems frequently led to a build up of grassweeds and soil structure problems. So with reduced cultivations seemingly out of favour and money not a limitation, a dramatic swing to larger tractors and ploughs on many medium to heavy soil farms took place in the early 1980's. Since then, the decline in straw burning and increased straw incorporation together with a continuation of grassweed problems has presented advisors with some complex situations. These have been extensively discussed in the past (Cussans et al. 1979 and 1987, Orson 1987 and Davies 1988). This paper reviews relevant past and current work and describes a management strategy for reduced cultivations.

REVIEW OF REDUCED CULTIVATION DEVELOPMENT WORK

Early work at Rothamsted Experimental Station (Russell 1945) clearly demonstrated the potential for zero cultivation provided weeds were controlled. In the 1960's ICI showed the major role that the contact herbicide paraquat could play in the absence of ploughing. During the 1970's and early 1980's the potential for shallow cultivation and zero cultivation was explored in several large experimental programmes (Davies 1988) and reduced cultivation was widely practiced on commercial farms.

The overall results from these field experiments are given in Table 1 and clearly indicate the great opportunity for reduced cultivation without yield penalty in cereal farming. In these experiments straw was normally burnt, all treatments drilled on the same day wherever feasible and weeds, disease and pests controlled by appropriate crop protection methods.

TABLE 1
The effect of reduced tillage compared with ploughing in U.K. field experiments, 1969-86, cereal yield as percentage of plough (100).

Cultivation System	Clays	Medium Loams	Light Loams
Plough	100	100	100
Shallow Cultivation	100 (81)	101 (88)	101 (42)
Direct Drilling	101 (85)	99 (88)	100 (42)

() Number of Trial Years

ICI have carried out farm studies during 1987 and 1988. In central England this took the form of a recording study on some 55 farms (Butterworth 1988). A wide range of reduced cultivation systems were compared with conventional plough based ones, evaluated in a split field design. In East Anglia more detailed work was carried out on a lower number of sites by ICI, ADAS and independant consultants.

results from the East Anglian work are given in Table 2 (Gibbard 1988). Those from the central England study were very similar.

TABLE 2
Crop establishment costs from 8 winter wheat sites in East Anglia 1987

Cultivation System	Establishment Costs (£/ha)	Work Rate (hr/ha)	Margin over Establishment Costs (£/ha)
Traditional 'Plough-based'	90	5.0	633
'Reduced' Cultivation	66	2.8	673
% Reduction	27%	45%	-
% Increase	-	-	6%

This work identified changes in cultivation practice which could lead to reduced cultivation costs. Significant reductions in establishment costs (27%) and work rates (45%) were achieved. Margin over establishment cost calculated at harvest showed more timely cultivations and drilling slightly improved the yield. These cultivation systems were based on replacing the plough on well structured fields and using the minimum cultivation required to produce a seed bed at each site. On the majority of these a spray of paraquat was used as an integral part of the system to control cereal volunteers and annual grassweeds. The value of effective volunteer control for the reduction of foliar disease and barley yellow dwarf virus has been highlighted in the last few seasons.

STRAW MANAGEMENT AND REDUCED CULTIVATION

The effect of straw incorporation systems are presented in Table 3.

TABLE 3
Straw incorporation systems. Results of ADAS experiments 1983-87.

Soil Type	Very Heavy	Moderately Heavy	Medium	Light
Straw incorporated - cultivation effect				
Yield of disc or tine as % of plough (100)	101	98	98	93
Number of trial years	19	17	15	21
Disc or tine cultivation - effect of straw disposal method				
Yield of straw incorporated as % of burnt (100)	100	99	98	91
Number of trial years	18	18	13	15

In the experiments on reduced tillage already described in Table 1 straw burning was the normal disposal method. Latterly, controls on burning have been strengthened and much more straw is either incorporated or baled. ADAS have been examining the implications of this change on the viability of reduced cultivations in a series of recent experiments the results of which are summarised in Table 3.

These indicate that on clays and medium loams disc or tine incorporation to 10-15 cm is a feasible alternative both to incorporation with the plough and to burning. However this method does increase annual grassweed pressure and rotational ploughing is usually necessary.

MANAGEMENT STRATEGY FOR REDUCED CULTIVATIONS

Time is at least as important as cost in the autumn. On large arable farms, the autumn workload is very high, especially where harvesting of root crops overlaps with cultivations and drilling of winter cereals. The work days available are fewer on heavy soils, particularly in September and October. It is suggested that farmers should plan their labour and machinery complement to meet the demands of the eighth wettest autumn in ten. Table 4 gives the work days available for this eighth year in East Anglia. By using this concept farmers should have seven easier years and two more difficult ones for establishing autumn crops.

TABLE 4
Days available for autumn cultivations in the eighth wettest autumn in ten.

Soil Type	LIGHT	MEDIUM	HEAVY
August	26	26	24
September	24	21	17
October	22	16	7
November	13	9	2

Based on an average annual rainfall of 600 mm [24"].
Source: Agromet Department, ADAS Cambridge.

Faster cultivation helps to ensure that target drilling dates are met even in difficult autumns. No amount of extra machinery can fully cope with the worst weather but it is reasonable to invest in equipment and labour to cultivate and drill all autumn crops during the optimum period in eight years out of ten.

When deciding how to reduce the autumn workload there are several different but interrelated actions which start well before harvest:

1. More farms should consider one large combine rather than two smaller ones. Carefully planned, the one combine should be capable of covering the area to be harvested in the 250-300 hours available, releasing a man to start the primary cultivations.

On many farms the best tractor driver still drives the combine and cultivations tend to be delayed. Releasing one man in this way virtually doubles the time available for main cultivations from 20 to 40 days.

2. Measures taken to prevent compaction reduce the need for remedial cultivation. Compaction and rutting can be minimised by fitting larger tyres running at lower pressures. The main offenders are tractors, combine harvesters and trailers. Both front and rear tyres must be considered. It is now possible for large tractors to carry out draught work on tyre pressures as low as 70 kPa (10 psi) without damage to tyres. Wherever feasible grain trailers should be kept to headlands and tramlines unless soils are dry. If these precautions are taken the need for cultivation below 10 cm may be restricted to headlands in most years.

3. Concentration on essential cultivations only, avoiding those that are just cosmetic, can greatly cut the time and cost of tillage. Table 5 shows the relationship between cultivation depth, relative cost and output for a tined cultivation (Spoor 1989). The deeper the cultivation the greater the cost and lower the output.

Farm studies on heavy land indicate savings of 2-3 hours per hectare from disc or tine cultivation rather than ploughing. For 100 hectares this represents a saving of up to 300 hours, ie one man for all of the five to six weeks normally available for land preparation in the autumn.

TABLE 5
Relationship between cultivation working depth and cost of establishing cereals.

Cultivation depth cm	Relative Cost	Relative Output time/unit area
0*	1	1.0
10	0.9	2.0
15	1.3	3.5
20	1.8	4.0
40	2.8	7.0

*0 = Direct Drilling

4. Savings can not be achieved reliably without detailed knowledge of soil condition in each field. This is best achieved through examination with a spade in March to May when soils are moist. Decisions on depth and time of cultivation can be made at this time and confirmed or modified in the light of any damage to land at harvest and the method of straw disposal.

A record of weed populations should be taken and fields identified where grassweed populations point to the need for ploughing or other measures. In addition ploughing is likely to be the best option where herbicide residues could put the following crop at risk, when crop residues can not readily be incorporated except by inversion and where soil is damaged and too wet for satisfactory tine cultivations.

CONCLUSION

Not since the early 1970s when the area of early sown winter cereals increased rapidly has the need for greater reliance on reduced cultivations been so urgent. However with the knowledge of previous mistakes in mind, it is important that reduced tillage is introduced flexibly on farms with an awareness of where and when it can be safely used. This successful introduction is based on a high level of management expertise applied in a structured but flexible manner. The restrictions and limitations on the use of reduced cultivation, particularly where grassweeds are a problem and straw cannot be burnt, are now well understood. By using this information sensibly on a field by field basis, yield depressions and extra costs can be eliminated.

Where however reduced cultivations are carefully managed by farmers on heavy and medium textured soils, substantial opportunity for greater area capacity and lower costs compared with the routine use of the plough are achieved. On lighter land where the plough and furrow-press system works well there are unlikely to be savings from non-ploughing. Cultivations need to be tuned to the crop and soil requirements in the same critical way as pesticides and fertilizers are tailored to individual crops and fields.

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FACTORS AFFECTING THE CONTROL OF *GALIUM APARINE* (CLEAVERS) IN WINTER WHEAT IN EARLY AND LATE SPRING

G SANSOME

Agricultural Development and Advisory Service (ADAS), Government Buildings, Brooklands Avenue, Cambridge CB2 2DR

ABSTRACT

The results of five trials conducted in 1988 on the control of *Galium aparine* are presented. The addition of chlormequat to mecoprop K salt and mecoprop p isomer reduced *G. aparine* control when applied at GS 30, the largest reduction recorded being 26%. There was no consistent effect of application volume on control and there was no crop yield advantage from removal of *G. aparine* prior to GS 32. The practical implications of these results are discussed.

INTRODUCTION

The performance of mecoprop K salt for *Galium aparine* (cleavers) control has been observed to be reduced by the inclusion of chlormequat based plant growth regulators in field situations (Orson, pers comm). In 1988 53% of winter wheat fields received a tank mix of a chlormequat product with either mecoprop alone or as a component of a herbicide pre-mix or tank mix (ADAS, 1988).

The object of these experiments was to evaluate factors which may cause sub-optimal performance of various herbicides for *G. aparine*, particularly whether chlormequat has an antagonistic effect on herbicide efficacy and to determine the importance of higher volumes of application at later growth stages on herbicide performance.

MATERIALS AND METHODS

Trials were conducted in commercially grown winter wheat crops with naturally occurring infestations of *G. aparine*. There were insignificant populations of other weeds present. The sites were in the ADAS Eastern (Hatfield, Barkway, Arthur Rickwood EHF), Northern (Thirsk, Redcar) and Midlands and West (Evesham) Regions. Trials were of a complete randomised block design with three replicates. Herbicides were applied by knapsack sprayer through the fan nozzles TeeJet 8002, TeeJet 11002 or Lurmark F02-80 at a total volume equivalent to 200 l/ha. TeeJet 8003, TeeJet 11003 or Lurmark F03-80 nozzles were used to give a volume equivalent to 300 l/ha where this was the required treatment. Treatments were applied at GS 30, ear at 1 cm (Tottman, 1987) (timing [a] in Tables) and GS 32, 2nd node detectable, (timing [b] in Tables). *G. aparine* was assessed as number of plants in the spring prior to herbicide application at timing [a] and as green material volume in early July. The minimum population of *G. aparine* was 14/m^2. Weed size at timing [a] was between 25-200 mm across and at timing [b] between 150-300 mm across. Three trials were harvested with plot combines and *G. aparine* seed removed from the sample to provide an accurate estimate of clean grain yield. Percentage control data were

transformed, log (x + 1) for sites E1 and E2, log (x) for other sites to meet the requirements of normal distribution prior to statistical analysis. Log transformation results are only presented for timing [a]. Where comparisons are made in the text these are significant (P = 0.05).

RESULTS

TABLE 1. % reduction in green material volume of G. aparine (Early Spring applications).

Treatment tm = tank mixed with	kg a.i. per ha	Timing	Site E1	E2	E3	N1	N2	MW
fluroxypyr	0.2	[a]	100	100	100	100	100	100
fluroxypyr (tm) chlormequat chloride/ choline chloride	0.2 1.61	[a]	99	100	100	100	100	100
mecoprop K salt	2.39	[a]	76	98	99	95	99	79
mecoprop K salt (tm) chlormequat chloride/ choline chloride	2.39 1.61	[a]	51	99	85	95	88	53
mecoprop p isomer	1.38	[a]	89	100	96	91	99	92
mecoprio p isomer (tm) chlormequat chloride/ choline chloride	1.38 1.61	[a]	78	100	98	97	100	83
fluroxypyr (tm) cyanazine	0.15 0.15	[a]	98	100	100	100	100	100
DPX M6316/ metsulfuron methyl (tm) mecoprop p isomer	0.045 1.14	[a]	97	100	100	98	100	99
Dates of application		[a]	21/4	13/4	26/4	18/4	29/4	31/3
G. aparine m^{-2} in untreated control		[a]	50	29	14	71	40	14

Table 1 lists the results of applications made in early spring at timing [a], when the effect of the addition of chlormequat was investiagted. Levels of G. aparine on the control plots are also presented. Table 2 lists the significance tests conducted for timing [a].

Fluroxypyr was unaffected by the tank mix with chlormequat and performed consistently well across all sites. Mecoprop K salt performance was variable with reduced control at two sites, E1 and MW. The addition of chlormequat to mecoprop K salt reduced control at sites E1, N2 and MW with a similar trend at E3. The largest reductions in control occurred at sites where control from mecoprop K salt alone had been poor. At the time of application sites E1 and MW recorded low night temperatures/frosts.

Mecoprop p isomer efficacy was reduced by the addition of chlormequat at the two sites (E1 and MW) where mecoprop K salt was most affected. At the N1 site, control from mecoprop k salt tank mixed with chlormequat and mecoprop p isomer alone was reduced compared to other treatments. N1 was the only site to show an increase in control from the addition of chlormequat to mecoprop p isomer.

TABLE 2. % reduction in green material volume of G. aparine. Early spring application (log transformation)

Treatment	kg a.i. per ha	Timing	Site					
			E1	E2	E3	N1	N2	MW
fluroxypyr	0.2	[a]	0.055	0.000	2.000	1.999	2.000	2.000
fluroxypyr (tm) chlormequat chloride/ choline chloride	0.2 1.61	[a]	0.174	0.000	2.000	1.999	2.000	2.000
mecoprop K salt	2.39	[a]	1.384	0.167	1.996	1.976	1.997	1.892
mecoprop K salt (tm) chlormequat chloride/ choline chloride	2.39 1.61	[a]	1.653	0.079	1.916	1.976	1.945	1.710
mecoprop p isomer	1.38	[a]	0.842	0.038	1.982	1.958	1.995	1.965
mecoprop p isomer (tm) chlormequat chloride/ choline chloride	1.38 1.61	[a]	1.318	0.005	1.992	1.987	2.000	1.921
fluroxypyr (tm) cyanazine	0.15 0.15	[a]	0.341	0.000	2.000	1.999	1.999	2.000
DPX M6316/ metsulfuron methyl (tm) mecoprop p isomer	0.045 1.14	[a]	0.949	0.000	1.999	1.991	2.000	1.998
SED			0.223	0.061	0.058	0.009	0.006	0.032
d.f			42	42	36	34	36	36
LSD (P = 0.05)			0.450	0.123	0.117	0.019	0.013	0.065

Table 3 lists the results of applications made in late spring at timing [b] and provides comparative data for treatments in Table 1. As before fluroxypyr gave consistently high control comparable to earlier treatments.

Mecoprop K salt in 200 l/ha was more consistent than at timing [a], but was still not comparable with control achieved by fluroxypyr at site E1.

Increasing the water volume to 300 l/ha had no consistent effect. Control from mecoprop K salt was increased at site E1 and control from DPX M6316/metsulfuron methyl tank mixed with mecoprop p isomer was decreased at site E3.

Table 4 lists the yield results from three trials taken to harvest. There was no significant effect from the high volume applications and results are not presented here. At site N1 there was no significant effect between treatments. With timing [a] at site MW there was a reduction in yield due to the poor control from mecoprop K salt and mecoprop p isomer both with or without chlormequat when compared to fluroxypyr. There was an overall trend for the chlormequat tank mix to decrease yield compared to the herbicide alone on the MW site. At site E3 there was a trend for the addition of chlormequat to fluroxypyr and mecoprop K salt to increase yield though not significantly.

There was no yield penalty from delaying control of G. aparine from GS 30 to GS 32 at any of the sites taken to yield. For the three sites taken to yield, the yield penalty averaged 1% yield loss for every G. aparine present at GS 30 and not subsequently controlled.

TABLE 3. % reduction in green material volume of G. aparine. Late spring application

Treatment tm = tank mixed with	kg a.i. per ha	Timing	Volume litres/ha	Site E1	E2	E3	N1	N2	MW
fluroxypyr	0.2	[b]	200	100	100	99	100	100	100
fluroxypyr	0.2	[b]	300	100	100	90	100	100	100
mecoprop K salt	2.39	[b]	200	84	98	83	100	99	100
mecoprop K salt	2.39	[b]	300	94	99	83	100	99	100
mecoprop p isomer	1.38	[b]	200	98	100	95	100	99	100
mecoprop p isomer	1.38	[b]	300	97	100	84	100	99	100
fluroxypyr (tm) cyanazine	0.15 0.15	[b]	200	99	100	96	100	100	100
fluroxypyr (tm) cyanazine	0.15 0.15	[b]	300	97	100	95	100	100	100
DPX M6316/ metsulfuron methyl (tm) mecoprop p isomer	0.045 1.14	[b]	200	-	99	93	100	100	100
DPX M6316/ metsulfuron methyl (tm) mecoprop p isomer	0.045 1.14	[b]	300	-	98	62	100	100	100
Dates of application		[b]		5/5	6/5	17/5	5/5	15/5	21/4

TABLE 4. Yield (t/ha at 85% dry matter) of winter wheat

Treatment (volume 200 litres/ha) tm = tank mixed with	kg a.i. per ha	Timing	Site E3	Site N1	Site MW
fluroxypyr	0.2	[a]	6.47	6.72	8.31
fluroxypyr (tm) chlormequat chloride/ choline chloride	0.2 1.61	[a]	6.71	7.08	7.93
mecoprop K salt	2.39	[a]	6.73	6.66	7.83
mecoprop K salt (tm) chlormequat chloride/ choline chloride	2.39 1.61	[a]	7.14	6.76	7.48
mecoprop p isomer	1.38	[a]	6.91	6.62	7.83
mecoprop p isomer (tm) chlormequat chloride/ choline chloride	1.38 1.61	[a]	6.91	6.22	7.83
fluroxypyr (tm) cyanazine	0.15 0.15	[a]	6.80	6.94	7.90
DPX M6316/ metsulfuron methyl (tm) mecoprop p isomer	0.045 1.14	[a]	6.78	6.99	8.09
fluroxypyr	0.2	[b]	7.03	6.74	8.08
mecoprop K salt	2.39	[b]	6.93	7.14	8.13
mecoprop p isomer	2.39	[b]	6.88	7.24	7.79
fluroxypyr (tm) cyanazine	0.15 0.15	[b]	6.36	7.14	7.60
DPX M6316/ metsulfuron methyl (tm) mecoprop p isomer	0.045 1.14	[b]	6.84	7.34	7.86
Untreated			5.85	3.96	6.40
LSD (P = 0.05) treated vs treated			0.57	NS	0.41

DISCUSSION

The results indicate that the performance of mecoprop K salt can be reduced by the addition of chlormequat as a tank mix, particularly when weather conditions are unfavourable for mecoprop activity at the time of application. Temperature has been reported as the most obvious factor that might influence herbicide activity and this has been well demonstrated (Tottman et al 1988). The largest reduction in control

observed in this trial series was 26% from a chlormequat, mecoprop K salt tank mix compared to mecoprop K salt alone. For the three sites where there was a significant decrease in control from this tank mix, the reduction averaged 21%. Such a result would not be acceptable in commercial practice due to the competitive ability of G. aparine.

Mecoprop p isomer performance was reduced to a lesser extent, by the addition of chlormequat, the reduction averaging 10% at the sites where a reduction occured. Mecoprop p isomer performance from early spring application has been shown to be more reliable than mecoprop K salt in some situations, but this is by no means absolute (Orson, 1988). Fluroxypyr was unaffected by the addition of chlormequat and control was consistently high from both timings. The reasons for the reduced control from the addition of chlormequat with mecoprop cannot be deduced from this experiment. The effect could be chemically antagonistic, or the chlormequat may affect the uptake and metabolism of the herbicide. Similarly the chlormequat could affect the growth and morphology of G. aparine thereby affecting its sensitivity to mecoprop.

The effect of increasing water volume for herbicide applications at GS 32 was inconsistent. Delaying control of G. aparine until GS 32 had no effect on yield as the main competitive effect of G. aparine occurs later. (Wilson et al 1985).

High populations of G. aparine require high levels of herbicide performance. Where conditions are not ideal for mecoprop K salt, or mecoprop p isomer activity it appears that the activity of these herbicides will be further reduced when applied in a tank mix with chlormequat chloride and choline chloride.

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THE EFFECT OF WEATHER CONDITIONS IN THREE SEASONS ON THE CONTROL OF *GALIUM APARINE* (CLEAVERS) IN WINTER WHEAT WITH FLUROXYPYR ESTER AND MECOPROP SALT.

D.R. TOTTMAN and P.M. STEER

AFRC/IACR, Weed Research Unit, Broom's Barn Experimental Station, Higham, Bury St Edmunds, Suffolk IP28 6NP

J.H. ORSON

ADAS/MAFF, Government Buildings, Brooklands Avenue, Cambridge CB2 2DR

M.C.E. GREEN

Dow Agriculture Ltd., Latchmore Court, Brand Street, Hitchin, Herts SG5 1HZ

ABSTRACT

A series of field trials compared the efficacy of fluroxypyr ester and mecoprop salt against *Galium aparine* (cleavers) when applied on a range of dates in the springs of 1987, 1988 and 1989. The results suggest a strong association between efficacy and soil temperatures. Fluroxypyr performed better than mecoprop especially when the 0900 h soil temperatures at 10 cm were less than about 8°C. *G. aparine* grew more vigorously on the heavier soil at Boxworth Experimental Husbandry Farm and were less well controlled by mecoprop at lower temperatures than they were when grown on a light soil at Broom's Barn Experimental Station.

INTRODUCTION

The results of four previous field experiments over two years, exploring the response of *G. aparine* to fluroxypyr ester and mecoprop salt on different dates in early spring have been reported (Tottman, Orson & Green 1987; Tottman, Orson, Green & Martin, 1988). It was concluded that 0900 h soil temperatures at a depth of 10 cm might provide a useful guide to optimum spray timing. Mecoprop required temperatures of 8°C or higher to perform consistently well. Fluroxypyr, given a vigorous and competitive crop, gave high levels of weed control at soil temperatures as low as 4°C.

This paper describes two further field experiments, conducted in 1989, with the same herbicides and concludes with a discussion of all the experimental results obtained over the three years.

MATERIALS AND METHODS

Two field experiments were conducted, one at Broom's Barn Experimental Station on a sandy loam soil and the other at Boxworth Experimental Husbandry Farm on a clay soil. The sites were sown with winter wheat, Mercia at Broom's Barn and Galahad at Boxworth, then over-drilled with the same stock of *G. aparine* seed at about 20 kg/ha in late September. Approximately 20 plants/m^2 at Broom's Barn and 80 plants/m^2 at Boxworth were recorded at the time of the first spray application and very few germinated thereafter. On the first spray date they were small, about 5 cm across, but by the last spray date they had developed into large plants, over 250 mm high (weed growth stages described in accordance with Lutman & Tucker, 1987). Assessments of growth and germination of *G. aparine* were made on each spray date.

Herbicide treatments were fluroxypyr ester (as 'Starane[1] 2') at 200 g a.i./ha and mecoprop K salt (as 'Iso-Cornox[2] 57') at 2.4 kg a.i./ha. They were applied on 9 dates from early March to early May 1989. Whenever possible both experiments were sprayed on the same day; 8, 17, 27/28, 31 March, 9, 15, 26, 28 April & 4 May. Plots were 2 m x 6 m at Broom's Barn and 3 m x 6 m at Boxworth arranged in three replicate blocks with 6 unsprayed control plots per block. The herbicides were applied in 300-330 litres water/ha with pressurised knapsack sprayers and hand-held booms, equipped with Lurmark F03-80 or Spraying Systems 8003 Teejets and operating at 2.1 bar pressure.

Weed control was assessed in late June/early July by estimating the percentage cover of *G. aparine* in 5 x 0.5 m^2 quadrats and multiplying this by the average height of the weeds to give an index of weed bulk in each plot.

Soil temperatures and weather conditions were recorded at permanent meterological sites within a few hundred metres of each experiment. Soil cores were taken to a depth of 60 cm at each site in early March to measure available nitrogen. Subsequent top dressing totalled 200 kg/ha N at Boxworth and 225 kg/ha at Broom's Barn.

RESULTS

Analysis of the soil cores revealed a large difference in the available nitrogen status of the two sites, 99 kg/ha at Boxworth compared to 16 kg/ha at Broom's Barn. The *G. aparine* at Boxworth grew more vigorously than those at Broom's Barn (Fig 1).

The control of *G. aparine* achieved with each of the fluroxypyr and mecoprop treatments is presented in Fig. 2, together with the 0900 h, 10 cm soil temperatures at each site.

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[1]Trade Mark of The Dow Chemical Company

[2]Trademark of Schering Agriculture.

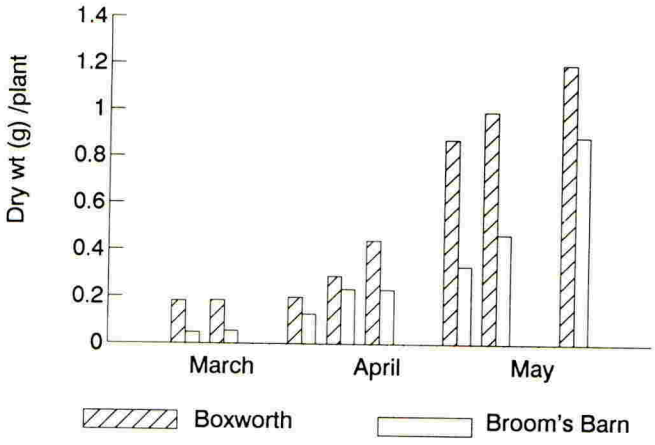

Figure 1. Growth of G. aparine at two sites.

The data were transformed, log (x + 100), for statistical analysis. Tests for significance cannot easily be applied to the percentage control data but very small differences of 1-2% may be significant at control levels nearing 100%, while bigger differences of around 10% are needed to distinguish between the poorer levels of control. Anything less than about 97% control, left too many *G. aparine* to be commercially acceptable.

Fluroxypyr, at the recommended rate, gave consistently high levels of control whenever it was applied but a few *G. aparine* plants survived the first treatment at Boxworth (Fig. 2). Control with mecoprop was much more variable. Control at Boxworth was poorer than at Broom's Barn and showed more clearly the influence of soil temperature. Performance was worst on the first spray date but had improved by the third date. Thereafter a sharp drop in soil temperature coincided with reduced control levels which rose again with increasing temperature. However, 100% control was not achieved until the 4 May when the soil temperature had risen to 15°C.

DISCUSSION

The discussion is based on the results obtained over the three years. The results of these latest experiments are consistent with those reported previously (Tottman, Orson & Green 1987; Tottman, Orson, Green & Martin, 1989). Control levels were generally high, as might be expected in a season when many of the treatments were applied in warm moist conditions and the weeds later suffered moisture stress, probably reducing their ability to recover from herbicide injury.

Susceptibility to mecoprop salt appears to be influenced more by the environment of the weeds than their size. As a general rule the higher the soil temperature the more effective the control (Figs 3 & 4). Soil temperature recorded at 0900 h at a depth of 10 cm appears to be a useful, if not absolute, indicator of herbicide performance. At

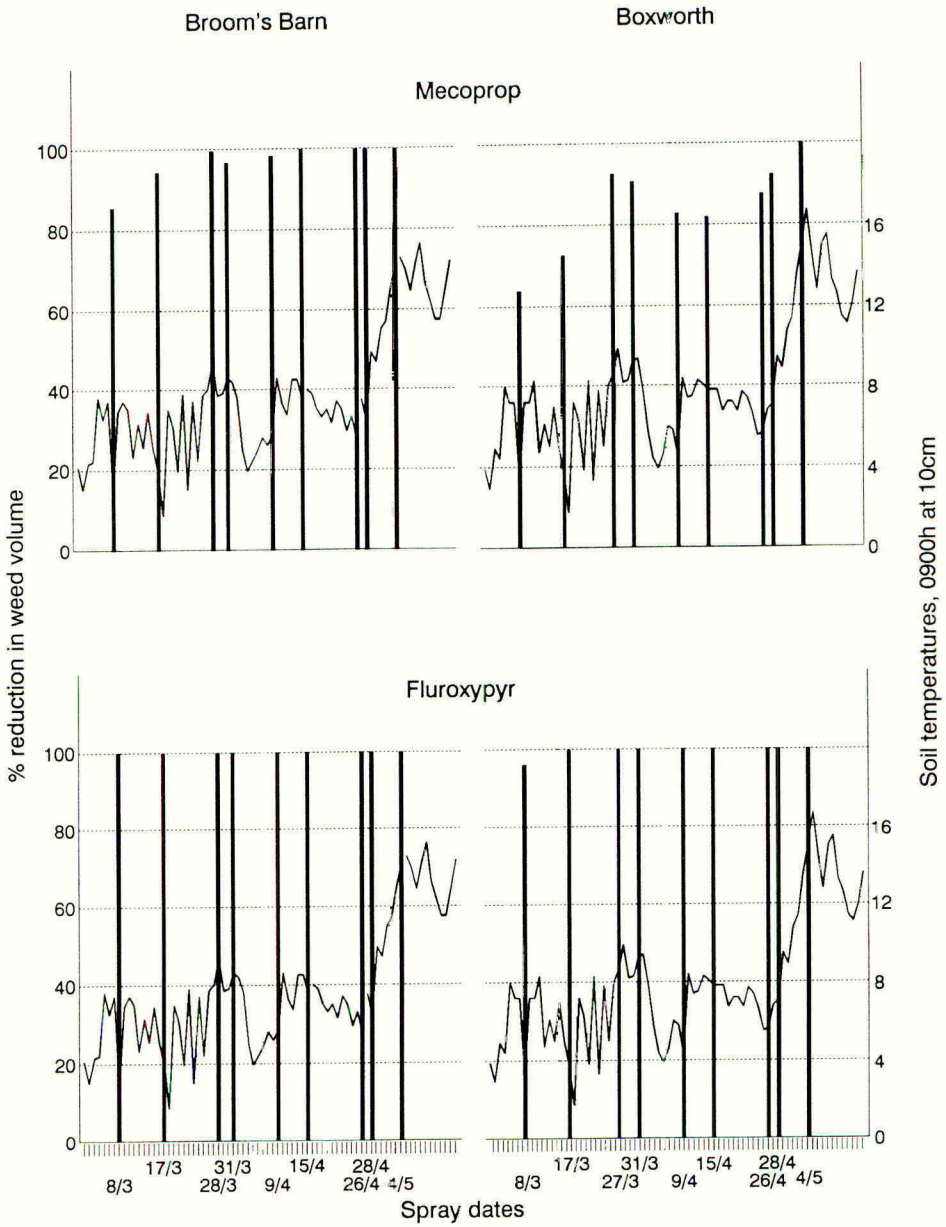

Figure 2. Soil temperatures and percent reduction in weed volume.

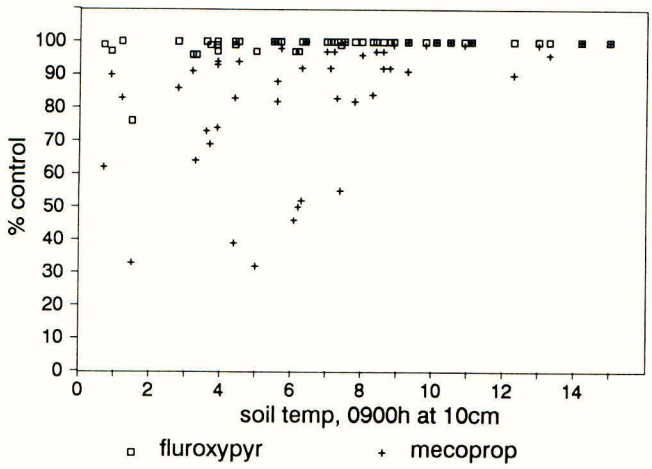

Figure 3. Control of G. aparine over 3 seasons at 2 sites.

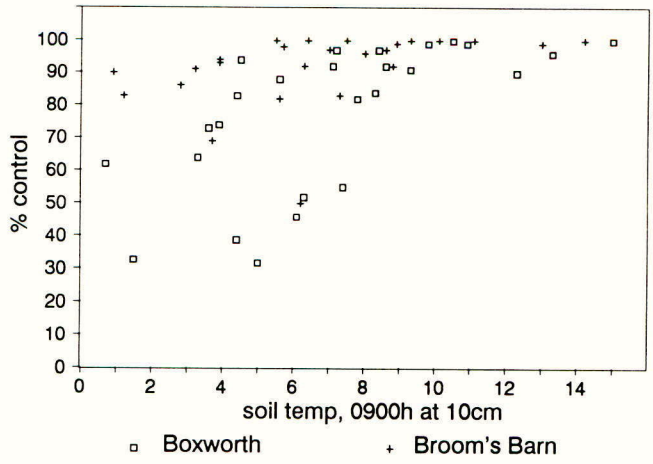

Figure 4. Control of G. aparine by mecoprop at 2 sites.

temperatures less than about 8°C, control with mecoprop was very variable. More effective control is likely to be achieved when soil temperatures rise for two to three days before and after spraying and when the diurnal fluctuation is minimal.

At the recommended rate, fluroxypyr was consistently effective, even at temperatures down to 4°C.

G. aparine grew more vigorously at Boxworth than at Broom's Barn. This may have been due to the better water availability in the heavier soil but is also likely to be a reflection of the higher nitrogen status. Experiments at Long Ashton Research Station have associated higher levels of nitrogen with faster growth rates and greater competitive ability of *G. aparine* (Rooney, J.M. & Wilson, B.J., personal communications).

"Growth regulator" herbicides are usually most effective when the weeds are growing vigorously but the difference in control levels at the two sites suggested the opposite. It seems likely that the more vigorous *G. aparine* at Boxworth were better able to recover from the initial injury inflicted by the herbicide treatment.

Soil temperature provides a useful guide to the timing of herbicidal control of *G. aparine* in the spring. The decision should also take into account the vigour of both weed and crop as well as other factors such as temperature trends and diurnal fluctuations. Over a wide range of conditions fluroxypyr ester gave more effective and consistent control of *G. aparine* than mecoprop salt, at the doses tested. Such advantages are important for the control of such a pernicious weed (Wilson, 1986) in the variable British climate.

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LONG TERM STUDIES OF WEED POPULATIONS IN WINTER WHEAT AS AFFECTED BY STRAW DISPOSAL, TILLAGE AND HERBICIDE USE.

B.J. WILSON, S.R. MOSS, K.J. WRIGHT

Department of Agricultural Sciences, University of Bristol, A.F.R.C., Institute of Arable Crops Research, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF

ABSTRACT

In two experiments in southern England, weed populations were monitored in three successive wheat crops. Alopecurus myosuroides populations built up rapidly, and good control by herbicides was the most significant factor limiting this increase. Fewest plants survived where straw burning supplemented the use of herbicides. In the absence of herbicides, straw burning reduced the initial build up of A. myosuroides. There was an indication that incorporating straw shallowly each year reduced populations of Stellaria media. Rates of build up and decline of A. myosuroides were shown to be density dependent.

INTRODUCTION

In an arable field, the weed species best adapted to the system of husbandry and herbicide use will constitute the dominant weed flora. In winter wheat, weed populations will reflect the balance between the production and losses of weed seeds in past crops. Models have been developed for Alopecurus myosuroides and Avena fatua (Cussans & Moss, 1982; Wilson et al., 1984), to show how populations respond to variations in herbicide efficiency in limiting seed return, and to various cultivation and straw disposal regimes affecting seed survival and seedling establishment. More information is needed to verify these models, especially in relation to changes in husbandry practice, such as in the method of straw disposal.

Two experiments are described in which weed populations were monitored in three successive wheat crops. Data show the effect of reducing the intensity of herbicide use on A. myosuroides and A. fatua, with and without straw burning, and the effect of straw disposal on the build up of A. myosuroides, A. fatua and Stellaria media in the absence of herbicides.

METHODS AND MATERIALS

Experiment 1.

This experiment commenced in autumn 1981 on a clay loam at the former Weed Research Organization, Oxford. Seeds of A. myosuroides (750 m^{-2}) and of A. fatua (250 m^{-2}) were sown prior to drilling winter wheat. The resulting mixed infestation was monitored, and plants were allowed to seed, before treatments commenced after the 1982 harvest. These consisted of two main-plot treatments of straw burning or straw baling and removal, each to be split subsequently for high and low intensity herbicide regimes in sub-plots of 15 m x 8 m. Main-plot treatments were replicated four times, with sub-plot treatments duplicated within each replicate. Two passes with a

rigid tine implement were used to cultivate all plots to 10-15 cm depth, and a seedbed prepared with a spring tine cultivator before drilling winter wheat (cv. Flanders) in September 1982. A. myosuroides seedlings were counted in November. Herbicides were applied (Table 1), and areas of 1 m^2 were covered at spraying, to allow herbicide efficiency to be assessed in March 1983. A. myosuroides heads were counted in June, and A. fatua panicles in July.

TABLE 1. Herbicides and doses (kg a.i./ha) applied in Experiment 1.

	1982/83			1983/84			1984/85
Regime	Nov.	Jan.	May	Oct.	Feb.	May	Oct.
High	IPU (2.5)	IPU (2.1)	DIF (1.0)	IPU (2.5)	IPU (2.1)	DIF (1.0)	IPU (2.5)
Low	DM (0.6)	IPU (2.1)	DIF (0.5)	DM (0.6)	IPU (2.1)	DIF (0.3)	DM (1.1)

IPU = Isoproturon, DM = Diclofop-methyl, DIF = Difenzoquat + 'Agral'

The above treatments and assessments were repeated during 1983 and 1984. In each year the covered areas were cut after assessment to prevent seed shedding. Different areas were covered each year, so that the unsprayed population could be related to the potential population on the sprayed plot. Minimum tillage was followed, and nitrogen fertilizer (130 kg/ha), fungicides and herbicides for broad-leaved weed control applied each spring.

Experiment 2.

This experiment, done on a silty clay loam at Long Ashton Research Station, Bristol, commenced in September 1985. Seeds of A. myosuroides (300 m^{-2}) and of A. fatua (600 m^{-2}) were sown in separate plots on to a stubble, and ploughed in. A further 30 seeds m^{-2} of each species were added to the ploughed surface in late September and mixed in with seedbed cultivations. Plots were also included to allow the natural broad-leaved weed flora to be monitored. Wheat (cv. Avalon) was sown, and weeds were allowed to seed during summer 1986, before treatments commenced in the autumn. The experiment was a split plot randomised block, with four replicates. Main plots (12 m x12 m) of four straw disposal and cultivation treatments were split (4 m x 12 m) for A. myosuroides, A. fatua and the natural broad-leaved weed flora. Main treatments, repeated annually, were:-

1. Straw burning followed by tine cultivation to 10 cm;
2. Straw baling and removal followed by tine cultivation to 10 cm;
3. Straw chopping followed by rotovating and tine cultivating to 10 cm;
4. Straw chopping followed by rotovating to 10 cm and ploughing to 20 cm.

After harvest, straw was baled and removed from the experiment. Straw bales (equivalent to 7 t/ha of straw) were returned to treatments 1,3 and 4, and either spread and burnt (1) or chopped and incorporated by rotovating to 10 cm (3 and 4). The weeds under study were allowed to build up unchecked during 1986/87. By the autumn of 1987 A. myosuroides had built up to very high numbers; isoproturon was applied in 1987/88 to control this high population. A. fatua and broad-leaved weed (mainly S. media) plots remained unsprayed. In each year A. myosuroides seedlings were counted in October and heads in June. A. fatua panicles were assessed in July, and S. media seedlings in October.

RESULTS

Experiment 1.

TABLE 2. Numbers of A. myosuroides plants m^{-2} and heads m^{-2}.

Straw disposal	Herbicide regime	1981/82 Plants (Feb.)	1981/82 Heads (July)	1982/83 Plants (Nov.)	1982/83 Heads (June)	1983/84 Plants (Oct.)	1983/84 Heads (June)	1984/85 Plants (Oct.)	1984/85 Heads (June)
		(before treatment)							
BURN	Unsprayed			3388	3428		3517		632
	High				9	1341	25	337	33
	Low				112	1279	41	384	90
		76	845						
BALE	Unsprayed			5343	4561		4125		764
	High				22	1917	22	626	19
	Low				185	2102	57	889	202
S.E.D.	(unsprayed treatments)			344.9	467.0		137.0		154.2
	(sprayed treatments)				21.1	85.4	17.1	66.4	22.2

TABLE 3. Numbers of A. fatua plants m^{-2} and panicles m^{-2}.

Straw disposal	Herbicide regime	1981/82 Panicles (July)	1982/83 Plants (March)	1982/83 Panicles (July)	1983/84 Plants (May)	1983/84 Panicles (July)	1984/85 Panicles (July)
		(before treatment)					
BURN	High		28	2.23	15	2.08	3.69
	Low		19	1.43	11	0.55	2.19
		4.45					
BALE	High		31	3.65	35	2.81	26.25
	Low		23	4.56	47	7.05	31.38
S.E.D.			5.9	0.793	6.1	1.344	9.18

The initial population of A. myosuroides built up rapidly (Table 2), 76 plants m^{-2} in February 1982 producing 845 heads m^{-2} in July, which resulted in averages of 3388 and 5343 plants m^{-2} in the next crop after straw burning and baling respectively. The high regime of herbicides (Table 1) averaged over 99%, and the low regime over 96% control of heads in 1983. Despite this good control, untreated populations remained high during 1984, but declined in 1985. Straw burning significantly reduced A. myosuroides populations compared with baling. In successive years, burning reduced A. myosuroides plants in the following crop by 37%, 35% and 52%; for the same populations, reductions in heads due to burning in the previous autumns were 25%, 15% and 17%.

In contrast, A. fatua numbers remained low throughout the experiment (Table 3). Because of the low density, it was not possible to cover large enough areas to determine unsprayed populations and herbicide efficiency. A pre-treatment average of 4.4 panicles m^{-2} in 1982 was followed by an average of 25 plants m^{-2} before spraying in 1983. The response to burning increased with time, with significantly fewer panicles on the burnt areas in 1985.

Experiment 2.

TABLE 4. Numbers of A. myosuroides plants m^{-2} and heads m^{-2}.

Straw disposal	Cult.	1985/86 Heads July	1986/87 Plants Oct.	1986/87 Heads June	1987/88 Plants Oct.	1987/88 Heads June*	1988 Plants Oct.
BURN	Tine	35	134	468	2488	19	721
BALE	Tine	64	638	1057	5525	3	3320
CHOP	Tine	77	801	1296	5827	8	3371
CHOP	Plough	74	167	614	2861	6	3573
S.E.D.		27.1	232.1	206.0	548.2	2.9	681.8

* Isoproturon applied in 1987/88

TABLE 5. Numbers of A. fatua panicles m^{-2} and S. media plants m^{-2}.

Straw disposal	Cult.	A.fatua 1985/86 Panicles July	A.fatua 1986/87 Panicles July	A.fatua 1987/88 Panicles July	S.media 1985/86 Plants Oct.	S.media 1986/87 Plants Oct.	S.media 1987/88 Plants Oct.
BURN	Tine	21	33	133	11	613	1260
BALE	Tine	13	28	39	122	710	1056
CHOP	Tine	14	16	19	2	115	483
CHOP	Plough	14	14	25	9	325	710
S.E.D.		12.4	7.1	8.1	50.2	215.3	301.9

A. myosuroides (Table 4) built up most rapidly with the bale tine and chop tine treatments (17-fold increase in heads between 1986 and 1987) and least rapidly with ploughing (8-fold increase). Despite good control being achieved with isoproturon in 1987/88, populations in the following crop remained high. Numbers of plants were similar on the three non-burning treatments, but about 80% lower after burning. Numbers of plants with ploughing were higher than in the previous year. In contrast A. fatua populations (Table 5) were slow to build up. The greatest increase occurred on the burnt plots and in 1988 numbers of panicles were significantly higher on these plots compared with the other treatments. S. media populations (Table 5) built up rapidly with all treatments; populations were significantly lower where straw was chopped and incorporated shallowly with tine cultivation, compared with baling or burning followed by tine cultivation.

DISCUSSION

These experiments show how straw disposal, cultivation and herbicides interact to influence long term trends in weed populations. The successful use of herbicides was the most significant factor; this was most obvious with A. myosuroides with a high potential rate of increase; there was a 70-fold increase in plants and a 17-fold increase in heads in the first year of Experiments 1 and 2 respectively. After two years of herbicide use (Experiment 1), fewer plants emerged in the third compared with the previous years, which suggests that the very good control of seed return was effecting a reduction in the seedbank. Fewest plants were recorded where straw burning supplemented herbicides. However more plants survived in 1985 with only a single herbicide application. These results suggest that a consistently very high level of over 95% control of seed production is necessary with continuous minimally tilled winter wheat to reduce or eliminate A. myosuroides. There is little prospect of achieving this where control with herbicides is unreliable.

In the absence of herbicides, S. media and A. myosuroides increased more rapidly than A. fatua, reaching very high densities in two years. Straw burning reduced the build-up of A. myosuroides. Apart from burning, the cultivation associated with the straw disposal had more influence on populations than the straw disposal per se. A. myosuroides increased more rapidly with tine cultivation than with ploughing, the rate of increase with tine cultivation being similar for straw removal or straw incorporation.

Although populations were uneven at the start of the experiment, there is a suggestion that S. media was reduced by shallow straw incorporation. Plant numbers were lower in 1987 and 1988, compared with straw baling and removal, possibly due to toxins from the decomposing straw. Crop seedlings were also stunted and lacking in vigour during the winter months following the shallow incorporation of straw.

The rate of population increase of Galium aparine has been shown to be density dependent (Wilson & Froud-Williams,1988). In the work reported here, both the build-up and reduction of A. myosuroides populations are shown to relate to density, and data are provided which may usefully be incorporated into existing population models (Cussans & Moss, 1982; Doyle, Cousens & Moss, 1986). Table 6 shows that plants at the lower densities in 1986 produced more heads/plant, and the population increased more rapidly, than at the higher densities. Five plants emerged in October 1987 for every head present in the previous crop, independent of density. Thus it is the prolific tillering at low densities which is the main reason for the rapid increase of low populations, and a good example was shown by the A. myosuroides in the early phase of Experiment 1 when each plant produced an average of 11 heads.

TABLE 6. Factors involved in the increase of A. myosuroides in Experiment 2.

Straw disposal	Cult.	Plants m^{-2} Oct.86	Heads/plant 1987	Plants 87 / Plants 86	Plants Oct.87 / Heads June 87
BURN	Tine	134	3.5	18.6	5.3
BALE	Tine	638	1.6	8.7	5.2
CHOP	Tine	801	1.6	7.3	4.5
CHOP	Plough	166	3.7	17.2	4.7

Similarly the effect of any control measure in reducing the population is likely to be buffered by compensatory tillering. For example, in Experiment 1, where unsprayed populations were always decreased by straw burning, reductions in seedlings as a result of burning were always greater than reductions in heads for the same population.

This work helps to explain the intractability of A. myosuroides in continual winter cereals despite intensive herbicide use (Wilson & Scott, 1982), and emphasises the need for an integrated approach to long term control.

ACKNOWLEDGEMENT

We are grateful to the Experimental Husbandry Team at Long Ashton for carrying out the husbandry operations in Experiment 2.

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THE EFFECT OF CROP DENSITY ON HERBICIDE EFFICACY AND YIELD RESPONSE IN CONTINUOUS SPRING BARLEY

D L EASSON

Agricultural Research Institute of Northern Ireland, Hillsborough, Co. Down, BT26 6DR

A D COURTNEY

Department of Agriculture for Northern Ireland, Newforge Lane, Belfast, BT9 5PX.

ABSTRACT

A spring barley experiment has been carried out in which herbicide and crop density treatments have been imposed on two cultivars on the same plots for four years. In the absence of herbicide broad-leaved weed numbers have progressively increased from 106 m^{-2} to 194 m^{-2} over the four years. This contrasts with the Poa annua population which has exhibited a general decline on all treatments. The herbicide treatments have reduced broad-leaved weed numbers and progressively increased yields. Reducing the crop density has influenced weed biomass rather than weed numbers with the highest response to herbicides occurring at the lowest crop density. The herbicide treatments have shown significantly different yield responses which appear to be attributable to factors other than weed control efficacy.

INTRODUCTION

If situations are to be identified where there could be a reduced herbicide input without economic loss then more information is needed on the relationship between crop density, growth habit, weed competition and herbicide response, particularly where the weed population may be allowed to build up over successive years without herbicide use. An experiment was therefore begun in 1986 in which two cultivars of spring barley have been grown at three densities for four seasons in the same plots. The effects of these treatments, and of a range of herbicides with activities against broad-leaved weeds and annual grasses, on weed numbers, weed biomass, crop growth and yield are being monitored. Preliminary results of the weed measurements from the first two years have been reported by Courtney, Easson and Johnston (1988).

MATERIALS AND METHODS

The experiment was conducted at the Agricultural Research Institute of Northern Ireland, Hillsborough, Co. Down. The site had a sandy clay loam soil of glacial origin and had previously grown a range of arable crops. In the season prior to the experiment the crop grown was flax.

Two varieties of spring barley, Atem a tall cultivar and the more prostrate and free tillering variety Triumph were sown each year at seed

rates of aproximately 100, 200 and 400 seeds m^{-2}. The mean plant stands given in the years 1986 to 1988 are given in Table 1. The fertilizer application in each of the years was a standard dressing of 80 to 100 kg/ha. A single broad-spectrum fungicide treatment was applied in each of the years in mid-June (Table 2).

The weed counts (8 x 25 cm square quadrats) in the crop prior to spraying were taken in mid May (Table 2) and the pre-harvest biomass samples (single 0.5 m^2 area) in September.

Herbicide treatments:

1. Fluroxypyr/ioxynil/bromoxynil ('Advance', Plant Protection Ltd, 9%/10%/10% emulsifiable concentrate at 1.5 l product/ha).
2. Isoproturon/ioxynil/bromoxynil ('Twin-Tak D.F.', May and Baker Ltd, 60%/5%/5% water dispersible grain at 2.4 kg product/ha)
3. Dichlorprop/MCPA ('Seritox 50', May and Baker Ltd, 31.1%/16.2% aqueous solution at 5.5 l product/ha)
4. Metsulfuron-methyl ('Ally', Dupont, 20% water dispersible granule at 30 g product/ha)
5. Unsprayed control.

The herbicides were applied at growth stage 30 in 1986 and 1987, but application was delayed until growth stage 31 in 1988.

The experiment was laid down in three replicate blocks. The herbicide treatments were main plots, with the variety and seed rate treatments fully randomised within these. The individual plots were 2 m x 25 m. Plant, tiller and ear counts were determined from 6 x 0.5 m row lengths, and grains per ear from the ears from these row lengths. Grain and straw yields were taken using a plot combine harvester, and a cleaned grain sample used for thousand grain weight determination. Yields are quoted at 15% moisture content.

RESULTS

Pre-spraying weed populations

In the first year a relatively high number of *P. annua* and broad-leaved weeds were present before spraying (Tables 3 and 4a). The main species present were *Stellaria media* (45 m^{-2}), *Galeopsis tetrahit* (14 m^{-2}), *Polygonum persicaria* (12 m^{-2}), *Chamomilla suaveolens* (17 m^{-2}), *Polygonum aviculare* (9 m^{-2}), *Spergula arvensis* (5 m^{-2}) and *Poa annua* (165 m^{-2}). The number of broad-leaved weeds in the untreated plots increased very little in the first year, but more rapidly in the subsequent years. Where a herbicide had been used in the previous year weed numbers were significantly lower than in the no herbicide plots. In the absence of herbicide broad-leaved weed numbers at spraying were higher at the lowest crop density in 1987 but not in subsequent years (Table 4a). In contrast *P. annua* numbers were generally favoured by reduced crop stand.

In both the control and herbicide treated plots there was a marked decline in the *P. annua* numbers pre-spraying over the three years (Table 3b) with a significantly greater decline at the high rather than at the lower seed rates. The metsulfuron-methyl and fluroxypyr/hydroxybenzonitrile (HBN)

treatments of the previous season tended to increase the P. annua numbers relative to the untreated plots.

Weed populations and biomass at harvest

All the herbicides reduced the broad-leaved weed biomass to low levels at harvest in all years (0 to 9 g/m^2), but only the isoproturon (IPU)/HBN treatment reduced the P. annua biomass. As with the seedbed numbers the other herbicides in fact tended to result in a higher P. annua population due to reduced broad-leaved weed competition.

Where no herbicide was used the number of P. annua present at harvest was very high in 1986 but, as with the numbers at spraying, showed a marked decline in the 2nd and 3rd years to under 2 m^{-2}. At the normal seed rate the numbers were significantly lower than when the seed rate was reduced. The number of broad-leaved weeds present at harvest did not show a consistent pattern other than that lower numbers were recorded in 1988 than in 1986 or 1987. The low numbers, however, were not reflected in a significantly lower biomass, as the total weed DM was broadly similar to that in the other years (Table 4b). Only the P. annua DM showed a significant decline over the years.

Grain yields

In the control plots the yields at the lowest seed rate were significantly below those at the higher rates, the difference widening from 400 kg/ha in 1986 to 900 and 1700 kg/ha in 1987 and 1988 respectively (Table 5). The mean effect of the herbicides was to increase the grain yield at the low seed rate by an increasing amount over the three years, with generally lower positive responses at the higher seed rates (Table 6). Of the individual herbicides, the yield increases with dichlorprop/MCPA and metsulfuron-methyl tended to be greater than those with fluroxypyr/HBN and IPU/HBN treatments (Table 7).

The straw yields were, however, reduced where herbicide was used and this together with the grain yield increases resulted in a harvest index which averaged over 61% with herbicide and 57% without.

The reduced yield at the low seed rate resulted mostly from a lower number of ears per square metre which was not fully compensated for by significant increases in the number of grains per ear and thousand grain weight. The yield increases through the use of herbicide can also be associated with increases in the number of ears in 1986 and 1987, but in 1988 the yield increase was due to increases in the thousand grain weights.

DISCUSSION

At all three seed rates there was a build-up of the broad-leaved weed population over the three seasons as indicated by the weed numbers prior to spraying. However, it was only at the lowest seed rate, which was approximately 25% of the normal seed rate for spring barley, that there was evidence of an increasing competitive effect of weeds on the crop and an increasing yield response to herbicide. At the higher seed rates although there was no increased response over the years, there were

significant yield increases from the use of herbicide but without a consistent trend.

Crop competition significantly reduced weed biomass at harvest as the stand was increased from 25% to 50% of normal with little further reduction at the full seeding rate.

The dramatic decline in P. annua numbers which took place over the three years cannot be attributed to any of the experimental treatments and is likely to have been a reflection of the favourable conditions for P. annua in the wet 1985 season prior to the start of the experiment. At the low seed rate the use of the herbicides which removed the competitive effect of the broad-leaved weeds tended to result in an increase in P. annua compared with that in the control plots.

Although the taller variety Atem had a significantly lower biomass of weeds present at harvest in 1986, with a similar trend in 1988, the yield response to the herbicides was similar for the two varieties. Throughout the course of the experiment the herbicide treatments exhibited signficantly different yield responses, the dichlorprop/MCPA and metsulfuron-methyl treatments tending to give higher yield increases. This does not appear to reflect differential efficacy since the IPU/HBN treatment in each of the years had the lowest total weed biomass at harvest but also one of lower yield responses. It appears probable that some aspect of crop tolerance may also be involved in the differential yield responses recorded. In 1988 due to rapid crop development the crop was at GS31 at spraying. This has partly contributed to the yield response which in 1988 was through greater thousand grain weights, while in the previous years it has been through an increase in the number of ears. However it is also likely the variations in the weather could have influenced this response.

The additional spectrum of activity of the more complex formulations fluroxypyr/ioxynil/bromoxynil and isoproturon/ioxynil/bromoxynil was not reflected in an increased yield response in the three years of this experiment.

TABLE 1. Spring Barley Stand (plants m^{-2})

		Seed Rate (seeds m^{-2})		
	Date	100	200	400
1986	12/5	95	165	300
1987	1/5	109	193	349
1988	23/4	93	255	464

TABLE 2. Dates of main operations

	1986	1987	1988
Date drilled	8/4	17/4	7/4
Fertilizer applied	7/5	28/4	18/4
Fungicide treatment	18/6	12/6	13/6
Harvest	19/9	18/9	13/9
Weed records			
Pre-spraying counts	11/6	28/5	25/5
Pre-harvest biomass	18/9	14/9	13/9
Herbicide spray date	12/6	4/6	9/6
Crop growth stage	15, 30	15, 30	16, 31

TABLE 3. Effect of herbicide treatment on numbers of a) broad-leaved weeds and b) P. annua in the following year, pre-spraying

Number of weeds m^{-2}	1986	1987	1988	1989
a) Broad-leaved weeds				
No herbicide	(106)	112	142	194
Mean of herbicides		76	88	66
		*	*	***
S.E.M.		12.1	17.2	16.1
b) P. annua				
No herbicide	(165)	56	32	8
Mean of herbicides		62	54	18
		ns	*	ns
S.E.M.		6.5	7.4	4.1

TABLE 4. Effect of seed rate on a) pre-spraying broad-leaved weed numbers (weeds m^{-2}) and b) on dry weight of broad-leaved weeds at harvest (g m^{-2}) in control plots

		1986	1987	1988	1989
a) Broad-leaved weed numbers					
Seed rate (m^{-2})	100	-	136	144	178
	200	-	101	144	217
	400	-	99	138	194
			**	ns	ns
	S.E.M.		9.0	12.8	13.8
b) Weight of broad-leaved weeds at harvest					
Seed rate (m^{-2})	100	77.9	104.6	78.8	-
	200	42.6	59.8	89.6	-
	400	43.8	72.4	52.0	-
			***	*	
	S.E.M.	5.60	8.58	8.52	

TABLE 5. Grain yield of control plots (t/ha, mean of varieties)

		1986	1987	1988
Seed rate (m^{-2})	100	4.83	5.05	3.76
	200	5.23	6.15	5.46
	400	5.11	6.29	5.84
		***	***	***
	S.E.M.	0.147	0.126	0.172

TABLE 6. Yield increase due to herbicide (kg/ha, mean of herbicides and varieties)

		1986	1987	1988
Seed rate (m^{-2})	100	418 ns	662 **	1086 ***
	200	287 ns	330 ns	249 ns
	400	657 *	297 ns	580 *
	S.E.M.	147.4	126.2	172.3

TABLE 7. Yield increase from herbicides (kg/ha, mean of seed rates and varieties)

	1986	1987	1988
Fluroxypyr/ioxynil/bromoxynil	389 ns	176 ns	525 *
Isoproturon/ioxynil/bromoxynil	407 ns	375 *	175 ns
Dichlorprop/MCPA	510 ns	549 ***	1013 ***
Metsulfuron-methyl	510 ns	620 ***	840 ***
S.E.M.	186.1	117.5	247.5

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SESSION 3B

THE MODE OF ACTION AND BASIS FOR THE SELECTIVITY OF GRAMINICIDES

CHAIRMAN DR K. WRIGHT

SESSION ORGANISER DR K. E. PALLETT

THE DISCOVERY OF THE SELECTIVE INHIBITION OF ACETYL COENZYME A CARBOXYLASE ACTIVITY BY TWO CLASSES OF GRAMINICIDES

J. SECOR

Dow Chemical Company, P. O. Box 1706, Midland, MI 48641-1706 USA

C. CSÉKE

Dow Chemical Company, P. O. Box 9002, Walnut Creek, CA 94598-9002 USA

W. J. OWEN

Dow Chemical Company Ltd., Letcombe Regis, Wantage, Oxfordshire OX12 9JT, England

ABSTRACT

This review summarizes the work leading up to and confirming the discovery that two classes of graminicides, the aryloxyphenoxypropanoates and cyclohexanediones, inhibit acetyl coenzyme A carboxylase (ACCase) in susceptible species. Initial investigations had showed that both haloxyfop, an aryloxyphenoxypropanoate, and tralkoxydim, a cyclohexanedione, significantly inhibited the incorporation of ^{14}C acetate into lipids in leaf discs from susceptible but not tolerant species. The effect was rapid, within 15 min., and occurred at low (1 μM) concentrations. Further work with leaf discs indicated that these herbicides were reducing the incorporation of ^{14}C acetate into palmitic acid. Thus, based on these and other facts, we tested the herbicides on ACCase extracted from maize and discovered that the activity of ACCase was inhibited by these compounds. The I_{50} values for these herbicides ranged from 0.5 to 1.2 μM for susceptible species and from 10 to 520 μM for resistant species. The herbicidally inactive S(-) enantiomer of haloxyfop does not inhibit maize ACCase.

INTRODUCTION

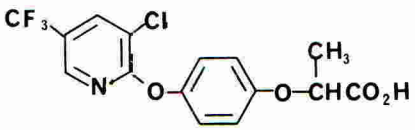
There are many reasons why it is important to understand the mode of action of herbicides. Certainly from the commercial viewpoint, understanding how herbicides kill a plant could aid in the improvement of existing compounds or furnish information leading to the discovery of new chemistries having a similar mode of action. Equally important from an academic perspective, knowing the mode of action of a herbicide can be useful in studies of plant metabolism, understanding gene regulation, and determining the nature of natural and induced resistance to herbicides. Thus, the knowledge derived from this type of research can be beneficial to many researchers for different reasons.

This paper reviews the work leading to our discovery that the aryloxyphenoxypropanoate and cyclohexanedione herbicides inhibit the activity of ACCase (acetyl-coenzyme A: bicarbonate ligase [ATP], E.C. 6.4.1.2) in

susceptible species (Secor and Cséke, 1988; Secor *et al.*, 1989). Similar findings have also been reported by other groups (Burton *et al.*, 1987; Rendina *et al.*, 1988).

BACKGROUND

At the onset of this research, we believed it was plausible to assume that the cyclohexanedione and aryloxyphenoxypropanoate herbicides had a similar mode of action because their symptomology and selectivity were quite similar. This is in spite of the fact that these compounds are quite different structurally (Figure 1). Earlier mode of action studies had shown that these compounds did not interfere with photosynthesis, respiration, protein synthesis or nucleic acid synthesis (Hoppe, 1980, 1981; Hosaka and Takagi, 1987). It was known, however, that these compounds did interfere with other processes such as auxin induced growth and lipid metabolism (Shimabukuro *et al.*, 1978; Hoppe, 1980, 1981; Gronwald, 1986). In addition, the aryloxyphenoxypropanoates were shown to depolarize membrane potentials (Lucas *et al.*, 1984; Wright and Shimabukuro, 1987).



Haloxyfop

Tralkoxydim

Figure 1. The structures of haloxyfop (2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy] propanoic acid) and tralkoxydim (2-[2-ethoxyimino)propyl]-3-hydroxy-5-mesitylcyclohex-2-enone).

Our immediate goal was to identify the specific site of action for these herbicides. Based upon published information, principally by Hoppe and colleagues (Hoppe, 1980, 1981, 1985; Hoppe and Zacher, 1985) for the aryloxyphenoxypropanoates and by Lichtenthaler and colleagues (Lichtenthaler and Meier, 1984; Lichtenthaler *et al.*, 1987) for the cyclohexanediones, there was sufficient reason for us to believe that both classes of herbicides acted by disrupting the lipid biosynthetic pathway. For example, it was reported that diclofop-methyl (methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate) inhibited acetate incorporation into fatty acids in chloroplasts of susceptible but not tolerant species (Hoppe, 1985). Similar results were reported for the cyclohexanedione sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy--2-cyclohexen-1-one) (Lichtenthaler *et al.*, 1987). Although these studies strongly suggested that *de novo* fatty acid biosynthesis was inhibited, no specific site of action was identified. One report had stated the the site of action was at a step after malonyl biosynthesis, thus, suggesting that ACCase was not the site of action (Hoppe and Zacher, 1982).

IDENTIFYING THE SITE OF ACTION

Before concentrating on the site of action, we first had to be reasonably certain that we knew what physiological process was being affected by these compounds. Our strategy was first to confirm that these compounds disrupted lipid biosynthesis, then determine where in the pathway the disruption was occurring, and finally testing specific sites. To accomplish this, we chose to use leaf discs for experimental material because they are easy to prepare, would reduce the effects of herbicide uptake and translocation, and because we had considerable experience with them from other physiological studies (Secor, 1987). Two very simple criteria served as requirements for identifying the site of action: 1) fast (within minutes) response to the herbicides, and 2) sensitive to low concentrations (μM).

To test whether the inhibition of lipid biosynthesis satisfied our two criteria, we incubated maize leaf discs in a solution containing ^{14}C acetate and 1.4 μM of an aryloxyphenoxypropanoate, haloxyfop (Figure 1). Discs were removed at various times and ^{14}C incorporation into lipids was measured. Lipid biosynthesis was inhibited by about 50% by a low concentration of either the free acid or methyl ester of haloxyfop (Figure 2). The cyclohexanedione tralkoxydim, (Figure 1), also inhibited acetate incorporation into lipids and inhibition was apparent for both classes of herbicides within 20 min (Figure 3). To our knowledge, this was the fastest physiological response response reported for these herbicides *in situ*.

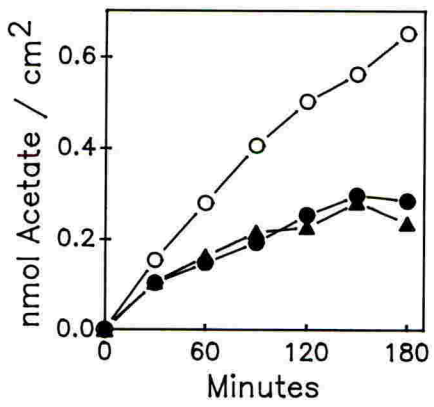


Figure 2. The incorporation of ^{14}C-acetate into lipids in maize leaf discs. The treatments are: control (o), 1.4 μM haloxyfop methyl ester(●), and 1.4 μM haloxyfop free acid (▲).

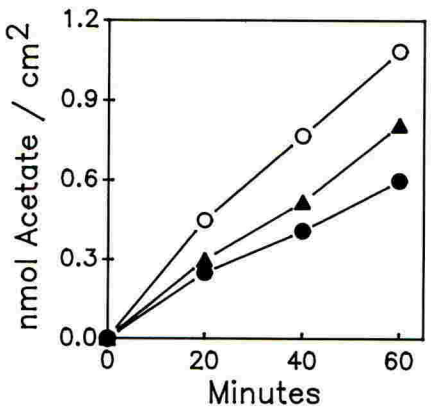


Figure 3. The incorporation of ^{14}C-acetate into lipids of maize leaf discs. The treatments are: control (O), 1.4 μM tralkoxydim (●), and 1.4 μM haloxyfop free acid (▲).

To be certain that the effects that we observed were not peculiar to maize, we conducted a similar experiment using leaf discs from another susceptible species, barley. In addition, we included a tolerant species, soybean. As shown in Table 1, 1.4 μM of haloxyfop reduced ^{14}C acetate

incorporation into lipids by 34% in barley, but had no inhibitory effect in soybean leaf discs.

Table 1. Effect of haloxyfop on ^{14}C-acetate incorporation into lipids in soybean and barley leaf discs after a 40 minute incubation period.

	Acetate Incorporation (nmol/cm^2)	
Treatment	Barley	Soybean
Control	3.10	1.41
1.4 μM Haloxyfop	2.04	1.63

Our next step was to determine where in the lipid biosynthetic pathway these compounds were acting. Maize leaf discs were incubated for 40 min in ^{14}C acetate in the presence or absence of the herbicides. Fatty acids were extracted from the leaf discs and the amount of ^{14}C incorporated into palmitic (16:0) acid was determined. The results of this experiment showed that both tralkoxydim and haloxyfop reduced acetate incorporation into palmitic acid (Table 2). Thus, these compounds were affecting *de novo* fatty acid biosynthesis.

Table 2. The effect of tralkoxydim and haloxyfop on ^{14}C-acetate incorporation into palmitic acid in maize leaf discs.

Treatment	^{14}C Incorporation (dpm ± S.E.M.)
Control	619 ± 95
1.4 μM Tralkoxydim	144 ± 13
1.4 μM Haloxyfop	297 ± 49

THE DISCOVERY THAT ACCase WAS INHIBITED BY THESE HERBICIDES

Rationale for testing ACCase

The next step was to decide which reaction in fatty acid biosynthesis was most likely being inhibited by these compounds. There are at least nine enzymes involved in the pathway from acetate to the palmitic acid-acyl carrier protein complex. We chose initially to select ACCase as the target for several reasons. ACCase catalyzes the first committed step in fatty acid biosynthesis and, thus, inhibiting that reaction should lead to plant death. More importantly, it had been reported that an aryloxyphenoxypropanoate herbicide can act as a hypolipidemic drug by reducing serum cholesterol and triacylglyerol levels in animals (Granzer and Nahm, 1973). Some hypolipidemic drugs act by inhibiting ACCase activity (Beyen and Geelen, 1982). Thus, it was possible that aryloxyphenoxypropanoates acted in an analogous manner by inhibiting ACCase activity in plants. This is in contrast to a conclusion made by Hoppe and Zacher (1982), who had suggested that ACCase was not the site of action of diclofop. Their reasoning was

based on the observation that diclofop inhibited malonate incorporation into polar lipids in maize root tips. They assumed that malonate was esterified to malonyl CoA, which entered the fatty acid biosynthetic pathway. This reaction requires a thioesterase which, in many species, is less active than is a malonate decarboxylase, which converts malonic acid to acetic acid (Hatch and Stumpf, 1962). Even if a malonate decarboxylase was not active, malonic acid would not necessarily be converted directly into malonyl CoA nor would malonyl CoA obligatorily be converted into fatty acids because there are several biosynthetic pathways utilizing malonyl CoA (Hatch and Stumpf, 1962; Nikolau *et al.*, 1984). There is even a question whether malonate is used at all as a precursor for fatty acid biosynthesis (Roughan *et al.*, 1978). For these reasons, we decided to test aryloxyphenoxypropanoates and cyclohexanediones on ACCase activity.

Inhibition of ACCase

Both tralkoxydim and haloxyfop acid inhibited ACCase activity in a concentration dependant manner (Figure 4). The concentration that inhibited activity by 50% (I_{50}) was about 1 μM for both compounds. These values are similar to those recently reported by others (Burton *et al.*, 1987; Kobek *et al.*, 1988). The methyl ester of haloxyfop was more than 100 fold less inhibitory than the free acid (Figure 4), which suggests that the methyl ester is deesterified in the plant. The data presented in Figure 2 suggest that the deesterification occurs rapidly in leaf tissue.

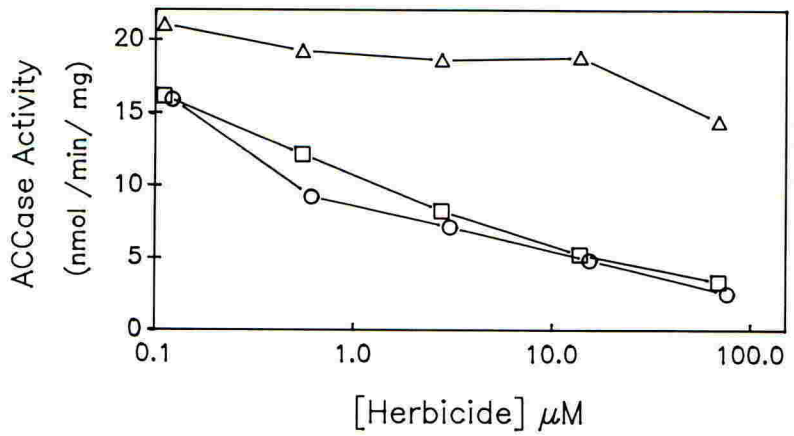


Figure 4. Effect of haloxyfop methyl ester (△), haloxyfop free acid (○), and tralkoxydim (□) on acetyl CoA carboxylase activity from maize.

CONFIRMING THAT ACCase WAS THE SITE OF ACTION

Providing direct evidence that ACCase was the target would be difficult for several reasons. For example, since the product of the ACCase reaction, malonyl CoA, does not pass through the chloroplast membrane, it was not possible to do experiments in which the product of the inhibited reaction was added back to the system. In addition, it would be technically very difficult to measure and compare acetyl CoA and malonyl CoA

levels in chloroplasts of treated and untreated plants. Thus, we had to rely on circumstantial evidence to show that we had identified the site of action.

The herbicidal enantiomer of haloxyfop inhibits ACCase

An important piece of circumstantial evidence came from exploiting the fact that the aryloxyphenoxypropanoates have a chiral carbon at the 2 position of the propanoate moiety of the molecule (Figure 1). The R(+) enantiomer is herbicidally active (Dicks *et al.*, 1985; Sakata *et al.*, 1985) and has been shown to be more effective than the (S-) form in reducing acetate incorporation into free fatty acids in isolated maize chloroplasts (Hoppe, 1985). We showed that ACCase activity is inhibited by R(+) haloxyfop acid (98% enantiomeric excess) but not by the S(-) enantiomer (94% enantiomeric excess) (Figure 5). The inhibition caused by the S(-) enantiomer could be accounted for by the 3% contamination in the S(-) preparation by the R(+) enantiomer. Others have shown similar results for the enantiomers of fluazifop (Walker *et al.*, 1988).

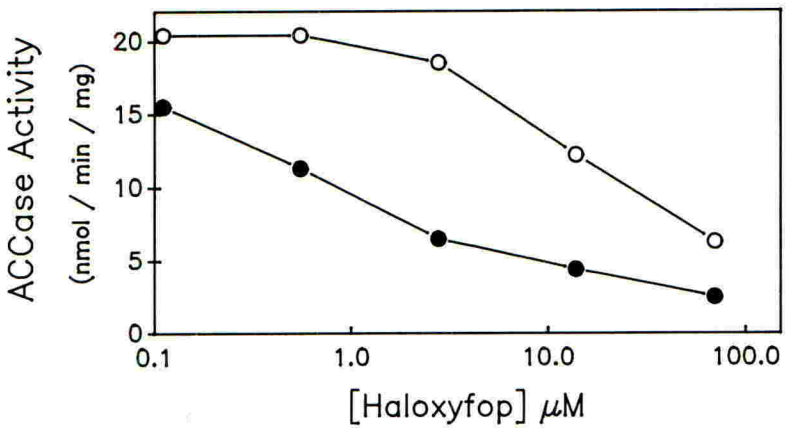
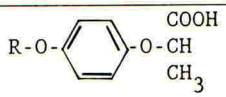


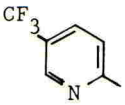
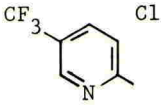
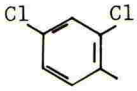
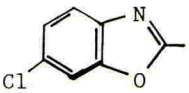
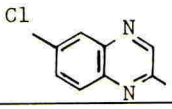
Figure 5. Effect of the S(-) enantiomer (0) and the R(+) enantiomer (•) on acetyl CoA carboxylase activity from maize.

ACCase inhibition by five aryloxyphenoxypropanoate herbicides

Further supportive circumstantial evidence resulted from a comparison of whole plant activity of several related herbicides with their *in vitro* activity. A comparison of five aryloxyphenoxypropanoate herbicides shows that they are very active inhibitors of maize ACCase (Table 3). The I_{50} values range from less than 25 nM for quizalofop to about 3 μM for fluazifop. The I_{50} values are generally well correlated with whole plant activity. The notable exception was diclofop, which has a higher recommended use rate than does haloxyfop, yet diclofop is about three times more active at the enzyme level. A reason for this discrepancy is that the outer benzene ring of diclofop can be readily hydroxylated in plant tissue to form a herbicidally less active molecule (Gorecka *et al.*, 1981).

Table 3. Effect of five aryloxyphenoxypropanoate herbicides on maize ACCase activity.



Herbicide	R	I_{50} (nM)
Fluazifop		2820
Haloxyfop		309
Diclofop		130
Fenoxaprop		108
Quizalofop		24

Whole plant and enzyme comparisons among susceptible and tolerant species

A comparison of herbicide effect on susceptible and tolerant plants and on ACCase extracted from those plants was conducted to determine whether tolerance was conferred by an altered target site. Other mechanisms for tolerance can be due to reduced uptake, translocation or metabolic detoxification. The effects of haloxyfop and tralkoxydim were tested on five species: one dicotyledoneous species, soybean, which is tolerant to both herbicides, and four monocotyledoneous species (1) *Festuca rubra*, which is tolerant to both herbicides, (2) *Festuca arundi nacea*, which is susceptible to both herbicides, (3) wheat, which is tolerant to tralkoxydim but susceptible to haloxyfop, and (4) maize, which is susceptible to both herbicides.

Whole plants were sprayed with a range of concentrations of the herbicides to determine the concentration needed to reduce growth by 50% (GR_{50}) two weeks after application. As expected, soybean and *F. rubra* plants were tolerant to both herbicides, wheat was resistant to tralkoxydim but not to haloxyfop, and the other species were susceptible (Table 4). The I_{50} data for ACCase inhibition reflected well the whole plant data. Soybean was most tolerant to the herbicides at both the whole plant and enzyme levels whereas maize was the most susceptible at both levels. The only notable exception was for wheat which, as expected, was tolerant to tralkoxydim but its ACCase was sensitive to inhibition. Thus, wheat tolerance is not due to insensitivity of its ACCase.

Table 4. Effect of haloxyfop and tralkoxydim on whole plant growth and ACCase inhibition in five species.

	GR_{50} (μM)		I_{50} (μM)	
Species	Haloxyfop	Tralkoxydim	Haloxyfop	Tralkoxydim
Maize	19	18	0.50	0.52
Wheat	83	>760	1.22	0.91
Festuca arundi nacea	133	225	0.94	0.40
Festuca rubra	1250	>6000	23.32	13.83
Soybean	>6000	>6000	138.50	516.72

THE INHIBITION OF OTHER REACTIONS

The number of biotin containing carboxylases in plants is not known, however, it is believed that ACCase is the predominant one (Nikolau *et al.*, 1984). We were interested in learning whether the aryloxyphenoxypropanoates and cyclohexanediones inhibited the carboxylation of other acyl CoA substrates. Maize extract was partly purified on a Sephacryl gel filtration column and subsequently assayed for carboxylation of several acyl CoA substrates. The results in Table 5 are a summary of several experiments. Acetyl CoA was the preferred substrate among the five aliphatic acyl CoA substrates. Both classes of herbicides inhibited to the same extent the carboxylation of both acetyl and n-propionyl CoA. The unsaturated CoA ester, methylcrotonyl CoA, was also carboxylated by this protein preparation, but neither herbicide inhibited the reaction. Thus, it seems that this partly purified extract contains at least two biotin containing carboxylases, one of which is inhibited by these herbicides and the other is insensitive. Another possibility, which is highly improbable, is that there is one carboxylase that is differentially sensitive to the herbicides depending upon the substrates of the reaction.

Table 5. Carboxylation and inhibition of several acyl CoA esters by a partly purified extract from maize. Data are in relative reaction rates in which the carboxylation of acetyl CoA = 100.

Substrate	Control	1.4 μM Haloxyfop	1.4μM Tralkoxydim
		Relative Reaction Rate	
Acetyl CoA	100	35	28
n-Propionyl CoA	33	19	8
n-Butyl CoA	14	nd[1]	nd
Isobutyl CoA	0	nd	nd
Valeryl CoA	0	nd	nd
Methylcrotonyl CoA	19	18	19

[1]nd = not determined

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THE PROPERTIES AND IMPORTANCE OF ACETYL-COENZYME A CARBOXYLASE IN PLANTS

J.L. HARWOOD

Department of Biochemistry, University of Wales College of Cardiff, P.O. Box 903, Cardiff, CF1 1ST, U.K.

ABSTRACT

Acetyl-coenzyme A (CoA) carboxylase catalyses the ATP dependent formation of malonyl-CoA from acetyl-CoA and bicarbonate. This reaction is the first committed step for fatty acid synthesis de novo. In addition, the malonyl-CoA product is used for fatty acid elongation and for other syntheses such as flavonoid production. The enzyme reaction involves biotin which is itself probably carboxylated in a two-step reaction. The carboxylated biotin then serves as the donor to acetyl-CoA and it is this transcarboxylation which is specific to the acetyl-CoA carboxylase. In contrast, there is considerable homology, especially in the biotin carboxyl carrier protein (BCCP) moiety, between different biotin-containing enzymes. In plants there has been considerable controversy about the nature of acetyl-CoA carboxylase i.e. whether it is a high molecular mass multifunctional protein or a dissociable multi-enzyme complex. For seed tissues, it is clear that the enzyme is a multifunctional protein of 200-240KDa but in leaves the evidence is somewhat less convincing. The plant acetyl-CoA carboxylase is not subject to regulation by tricarboxylic acids but is affected by adenine nucleotide concentrations. In many physiological situations its activity seems to be regulated mainly by transcriptional control.

SOURCE OF ACETYL-CoA FOR FATTY ACID SYNTHESIS

Ultimately, photosynthesis can be regarded as the source of carbon for fatty acid formation. On the other hand, acetyl-CoA is a more direct precursor. This acetyl-CoA is generated by the action of pyruvate dehydrogenase. However, until recently it was whether this enzyme complex had sufficient activity in chloroplasts to supply the necessary carbon. Recent work has shown that pyruvate dehydrogenase activity occurs in carefully-isolated chloroplasts (e.g. Camp and Randall, 1985; Treede and Heise, 1985). Nevertheless, there may still be a difficulty in the simple utilisation of pyruvate dehydrogenase to generate acetyl-CoA in that an uninterrupted glycolytic pathway from 3-phosphoglycerate seems to be missing in many plant chloroplasts (Stitt and ap Rees, 1979).

An alternative source of acetyl-CoA involves the use of a mitochondrial pyruvate dehydrogenase complex coupled to acetyl-CoA hydrolase which generates free acetate. Acetate has been shown to move freely into chloroplasts where an active acetyl-CoA synthetase is present. This enzyme also accounts for the ability of isolated chloroplasts to utilise ^{14}C-acetate very efficiently for fatty acid synthesis in vitro (Harwood, 1988).

As a generalisation, it seems that chloroplast acetyl-CoA can be supplied by both of the above routes. The relative importance of the two alternatives probably depends on the tissue source (Heinz and Treede, 1983) although

plastidic pyruvate dehydrogenase would be favoured logically (Liedvogel, 1987). In non-green plastids, however, there seems to be general agreement that acetyl-CoA is generated in situ by pyruvate dehydrogenase (see Harwood, 1988).

THE NATURE OF PLANT ACETYL-CoA CARBOXYLASE

In bacteria such as E. coli, acetyl-CoA carboxylase is a multienzyme complex containing three separable proteins - biotin carboxylase, biotin carboxyl carrier protein (BCCP) and BCCP: acetyl-CoA transcarboxylase. By contrast, the mammalian enzyme is a high molecular mass multifunctional protein. Early experiments with acetyl-CoA carboxylases from plants suggested that these enzymes were similar to the bacterial multienzyme complexes or, alternatively, had an intermediate form (see Stumpf, 1980). However, it now seems that the results were due to proteinolysis and that plant acetyl-CoA carboxylase, like that from mammals, is a multifunctional protein.

Biotin is a cofactor essential for the function of a number of enzymes which have key roles in metabolic regulation (see Dakshinamurti and Bhagavan, 1985). In all such enzymes a single carbon is transferred as a carboxyl group from one substrate (donor) to another (acceptor). Biotin functions as an intermediate carrier of the carboxyl group which is attached to the N-1' position. This function of biotin is needed for the activity of transcarboxylases and decarboxylases as well as carboxylases (see Moss and Lane, 1971).

All biotin-containing carboxylases carry out an identical first reaction in which the carboxyl group is donated from the bicarbonate anion and ATP hydrolysis is used to allow formation of the N-carboxyl bond in the carboxy-biotin intermediate. Recent work has shown that biotin carboxylation occurs in two steps with a phosphorylated intermediate (Knowles, 1989). On the other hand, the acceptor of the carboxyl group is unique in each carboxylase. In the case of acetyl-CoA carboxylase, for example, alternative substrates often have very poor activity.

The structure of biotinyl enzymes has been studied extensively (see Knowles, 1989). These experiments have indicated an evolutionary relationship (Samols et al., 1988) which is well illustrated for acetyl-CoA carboxylase. As mentioned above, in E. coli a multienzyme complex (3 proteins) has been described while in yeast (Mishina et al., 1976) and in vertebrates (Song and Kim, 1981) multifunctional proteins have been found. In the nematode Turbatrix aceti (Meyer et al., 1978) and in Streptomyces erythreus (Hunaiti and Kolattukudy, 1982) the biotin carboxylase and BCCP components are on a single peptide while the transcarboxylase forms a separate protein. This situation is much as was originally suggested for the wheat germ enzyme (see Stumpf, 1980).

Biotin is attached to the epsilon amino-group of a lysyl residue on BCCP. With the purification of enzymes to homogeneity and the availability of DNA sequences coding for some of the carboxylases, decarboxylases or transcarboxylases, it has become clear that there is considerable sequence homology for BCCP. In all enzymes examined, with the exception of the animal acetyl-CoA carboxylases a sequence of:

ala - met - lys - met

is formed around the lysyl residue of the biotin attachment site. (Animal acetyl-CoA carboxylases contain a val-met-lys-met sequence; e.g. Lopez-Cassilas et al., 1988). Further work using oligonucleotide-directed mutagenesis has shown that

two methionine residues which flank the lysine are not for the recognition of the lysyl residue by the biotinylation enzyme but rather they are needed for catalytic activity (e.g. Samols et al., 1988).

Egin-Buhler et al. (1980) were the first to employ a proteinase inhibitor, phenylmethylsulphonyl fluoride (PMSF), in their plant acetyl-CoA carboxylase purification protocol. They found that the enzymes purified thus from wheat germ or parsley cells were composed mainly of high-molecular-mass polypeptides of 240 KDa and 210 KDa, respectively. Their protocol was simplified and improved by the use of affinity chromatography on monomeric avidin - Sepharose 4B. Employing this step allowed the isolation of a native enzyme of 420 KDa and a subunit mass of 220 KDa for the parsley acetyl-CoA carboxylase (Egin-Buhler and Ebel, 1983). The purified enzyme had 60% of the acetyl-CoA activity with propionyl-CoA and 10% with butyryl-CoA and was not subject to regulation by nitrate.

Similar purification methods have been used by Slabas and associates who have purified several plant acetyl-CoA carboxylases to homogeneity. The enzyme from oil-seed rape has a molecular mass by SDS-PAGE under reducing conditions of 220 KDa (Slabas and Hellyer, 1985). The kinetic constants were determined and, in general, were of comparative values to those for other plant acetyl-CoA carboxylases (Table I).

TABLE 1. Properties of acetyl-CoA carboxylases from different plant sources.

	Castor bean	Maize	Rape	Parsley
pH optimum	8.0	8.4	8.5	8.0
Km acetyl-CoA	50uM	100uM	74uM	150uM
Km ATP	100uM	*var.	38uM	70uM
Km HCO_3^-	3mM	2mM	3mM	1mM

For detailed references see Harwood (1988). * depended on other incubation conditions.

The first successful purification of a leaf acetyl-CoA carboxylase to any degree was for the enzyme from maize. The native enzyme had a molecular mass of about 500 KDa but all the functional activity of the carboxylase seemed localised in a subunit peptide of 60 KDa (Nikolau and Hawke, 1984). Biotin enzymes are very strongly inhibited by the egg protein avidin (K_f 10^{-15}M). The availability of an ^{125}I-derivative of the related molecule streptavidin allowed Nikolau et al. (1984) to probe for biotin-containing proteins by Western blotting following SDS-PAGE. They examined extracts from two C3-plant and two C4-plant leaves. All extracts contained bands of about 62 KDa that were suggested to correspond to acetyl-CoA carboxylase and no high molecular mass proteins were detected. However, acetyl-CoA carboxylases purified from oil-seed rape leaves, maize leaves (Hellyer et al., 1986) and soya bean leaves (Charles and Cherry, 1986) had molecular masses in the range 220-240 KDa. Moreover, in somatic carrot embryos a 200 KDa biotin-containing protein was detected and the amount of this peptide increased during development when there was also an increase in acetyl-CoA carboxylase activity (Nikolau et al., 1987).

Acetyl-CoA carboxylase is often considered to be the only biotin-containing protein in plants. However, the demonstration of multiple biotinylated peptides in plant extracts (e.g. Nikolau et al., 1984, 1987) suggested that other biotin-enzymes were also present. Indeed, pyruvate, propionyl-CoA and 3-methylcrotonyl-CoA carboxylases have now been found in a variety of plant tissues. This situation complicates the interpretation of (^{125}I)-streptavidin probed gels.

In summary, the best available evidence points to plant acetyl-CoA carboxylase being a high molecular mass multifunctional protein. The data seems clear for seed tissue but is still somewhat equivocal for leaves. Moreover, the differential inhibition of different acetyl-CoA carboxylases by graminaceous herbicides (e.g. Walker et al., 1989) in itself means that significant structural variations must be present.

REGULATION OF ACETYL-CoA CARBOXYLASE

It is well known that acetyl-CoA carboxylase activity is carefully regulated in bacteria, yeasts and animals. In E. coli acetyl-CoA carboxylase activity (and, hence, fatty acid synthesis) is regulated by the product of the relaxes(rel) locus (Polakis et al., 1973) the nucleotide (ppGpp) (Harwood and Russell, 1984). Increased concentrations of ppGpp allosterically inhibit the carboxyltransferase component of acetyl-CoA carboxylase. By contrast, in yeasts fed exogenous fatty acids, endogenous fatty acid synthesis is lowered by a reduction in the level of translatable mRNA which codes for acetyl-CoA carboxylase (Kamiryo et al., 1979). In vertebrates also the levels of acetyl-CoA carboxylase mRNA allow long term regulation of the enzyme's activity such as is caused by dietary changes. In addition, short-term regulation by allosteric effectors such as citrate or by reversable enzyme phosphorylation (Hardie et al., 1989) is important.

TABLE 2. Regulation of acetyl-CoA carboxylase activity in vitro.

Kinetic constants (Eastwell and Stumpf, 1983)

	Wheat germ	Spinach	Swiss chard
Km ATP	0.13	0.04	0.39
Ki ADP	0.14	0.04	0.31
Ki AMP	8.20	0.69	0.36

Changes mimicking those on illumination
(Nikolau and Hawke, 1984)

pH change	7.1	8.0	Activity increase	3.1x
Mg^{++} change	2	5mM	(Maize enzyme)	2.6x
ADP change	1	0.6mM		1.9x
ATP change	0.5	1.1mM		1.7x

For plant acetyl-CoA carboxylase short-term regulation has been shown for concentration changes in cellular me tabolites. Two examples are given in Table 2. Thus, leaf acetyl-CoA carboxylase activity has been found to be low in the dark and to increase about 20-fold upon illumination (Hellyer et al., 1986). The enzyme from maize was shown to be stimulated by the sort of changes

found in the light. Thus, physiologically relevant increases in pH and in Mg^{++} and ATP concentrations and decreases in ADP concentration caused a combined increase of 24-fold in vitro (Table 2; Nikolau and Hawke, 1984). Moreover, wheat germ acetyl-CoA carboxylase has also been shown to be tightly controlled in vitro through its requirement for ATP and its inhibition by ADP and AMP (Eastwell and Stumpf, 1983). Although adenine nucleotides are potent effectors in these two cases it would be unwise to apply such results to all plant acetyl-CoA carboxylase (see Harwood, 1987). Thus, the soya bean enzyme was unaffected by a pre-incubation with ATP even though this nucleotide protected against loss of activity during purification (Charles and Cherry, 1986).

Because mammalian acetyl-CoA carboxylase is stimulated by tricarboxylic acids, many workers have tested plant enzymes likewise. However, no significant effects have been noted (Charles and Cherry, 1986, Finlayson and Dennis, 1983; Mohan and Kekwick, 1980) nor is there any evidence for phosphorylation/dephosphorylation.

Long term changes in plant acetyl-CoA carboxylase occur in response to environmental signals. Upon illumination of dark-grown plants (Reitzel and Nielsen, 1976) and cells in culture (Ebel and Hahlbrock, 1977) activity of the enzyme increases. Such an increase correlates with changes in the biosynthesis of fatty acids (e.g. Ohnishi and Yamada, 1980) and flavonoids (Ebel and Hahlbrook, 1977). Indeed, in parsley cells the increase in acetyl-CoA carboxylase activity is coordinate with rises in that of other enzymes of the general phenylpropanoid pathway (Ebel and Hahlbrook, 1977). The increase in activity of one of the latter, phenylalanine ammonia lyase, is due to accelerated transcription of its gene (Kuhn et al., 1984). Moreover, changes in acetyl-CoA carboxylase activity in leaves are also due to changes in protein concentration. Specifically, the concentration of acetyl-CoA carboxylase active site biotin increases during cellular development (Hawke and Leech, 1987).

Triacylglycerol accumulates in oil-seeds mainly during the second of the three phases of ripening (see Gurr, 1980). Acetyl-CoA carboxylase activity rises rapidly at the start of this accumulation phase in both soya bean (Charles et al., 1986) and oil-seed rape (Turnham and Northcote, 1983). Carboxylase activity subsequently decreases to low levels in the mature seed. The maximal activity of acetyl-CoA carboxylase is coincident with maximal lipid accumulation.

During the development of somatic embryos of carrot, from embryogenic cell clusters through globular, heart, torpedo and germinating embryos about a 10-fold rise in acetyl-CoA carboxylase activity has been noted (Nikolau et al., 1987). Simultaneous to this rise are increases in amounts of several biotin-containing proteins. This includes a 200 KDa peptide which increases about 5-fold and which may be, as discussed above, acetyl-CoA carboxylase.

THE IMPORTANCE OF ACETYL-CoA CARBOXYLASE IN PLANTS

Acetyl-CoA carboxylase plays a vital role in supplying malonyl-CoA for fatty acid synthesis de novo and for elongation (Harwood, 1988). Moreover, the enzyme lies at a branch-point in metabolism and is at a very suitable locus for regulatory effects such as discussed above. Although no definitive experiments have yet been conducted, it would seem likely that the enzyme could exert a controlling influence on the flux of carbon to membrane or storage lipids.

It is of interest to note in connection with the question of whether isozymes of acetyl-CoA carboxylase exist in plants, that malonyl-CoA generation for fatty acid elongation is relatively insensitive to graminaceous herbicides such as fluazifop (Walker et al., 1988). Because the elongation reactions take place outside the chloroplast (Harwood, 1988) this result may suggest a fluazifop-resistant acetyl-CoA carboxylase which supplies malonyl-CoA for elongation but not de novo synthesis.

It should also be noted that malonyl-CoA has other functions in plants apart from being used for fatty acid synthesis. Thus, malonyl-CoA is needed for the synthesis of anthocyanins and various flavonoids (Hahlbrock, 1981), malonic acid (Stumpf and Barris, 1981), malonyl derivatives of D-amino acids (Su et al., 1984) or aminocyclopropane carboxylic acid (Amrheim and Kionka, 1983), stilbenoids (Rupprich and Kindl, 1978) and certain quinones (Packter, 1980). The existence of isozymes of acetyl-CoA carboxylase might also be expected to aid the regulation of malonyl-CoA supply for these diverse fates.

Although the main role of acetyl-CoA carboxylase is to supply malonyl-CoA for fatty acid synthesis, the utilisation of this compound for other pathways can assume significance in certain tissues. However, it is the key (and possibly regulatory) role of the enzyme in fatty acid synthesis and, hence, membrane lipid synthesis that makes acetyl-CoA carboxylase such an effective target for herbicides. Knowledge of the detailed structure of different plant acetyl-CoA carboxylases is urgently needed to explain the species selectivity of the cyclohexanediones and aryloxyphenoxy propionates and to permit the rational development of new herbicide molecules.

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KINETICS OF INHIBITION OF ACETYL-COENZYME A CARBOXYLASE BY THE ARYLOXYPHENOXYPROPIONATE AND CYCLOHEXANEDIONE GRAMINICIDES

A.R. RENDINA, J.D. BEAUDOIN, A.C. CRAIG-KENNARD, M.K. BREEN

Inventive Research, Agricultural Chemicals Division,
Chevron Chemical Company, Richmond, California 94804, USA

ABSTRACT

The selective grass herbicides diclofop (aryloxyphenoxypropionic acid) and clethodim (cyclohexanedione) are potent, reversible inhibitors of acetyl-coenzyme A carboxylase (ACCase) partially purified from wheat leaves. Although inhibition of the wheat enzyme by clethodim and diclofop is noncompetitive versus each of the substrates adenosine triphosphate (ATP), bicarbonate and acetyl-coenzyme A (CoA), diclofop and clethodim are nearly competitive versus acetyl-CoA since the level of inhibition is most sensitive to the acetyl-CoA concentration. To conclusively show whether the herbicides interact at the biotin carboxylation or carboxyltransfer site of the enzyme, the inhibition of isotope exchange reactions and partial reactions catalysed at each site was studied with the wheat enzyme. Only the [^{14}C]acetyl-CoA - malonyl-CoA exchange and decarboxylation of [^{14}C]malonyl-CoA reactions are strongly inhibited by clethodim and diclofop suggesting that both herbicide classes interfere with the carboxyltransfer site rather than the biotin carboxylation site of the enzyme. Double inhibition studies suggest that the cyclohexanediones and aryloxyphenoxypropionates may bind to the same region of the enzyme.

INTRODUCTION

Various aryloxyphenoxypropionic acids, e.g., diclofop, and substituted 1,3-cyclohexanediones, e.g., clethodim, are two distinct chemical classes (Figure 1) of postemergence herbicides that are used for the control of annual and perennial grasses in a large variety of broadleaved crop plants (Nestler, 1982; Ishikawa et al., 1985). Both classes of graminicides have been shown to inhibit ACCase (Burton et al., 1987; Focke & Lichtenthaler, 1987; Rendina & Felts, 1988; Secor & Cseke, 1988; Rendina et al., 1988; Kobeck et al., 1988; Walker et al., 1988), a key enzyme in plants leading to the de novo biosynthesis of fatty acids, flavonoids, cuticular waxes, stilbenoids, anthroquinones, napthoquinones, N-malonyl amino cyclopropane-1-carboxylic acid and malonic acid (Stumpf, 1987). These studies showed that ACCase from susceptible grassy species was strongly inhibited by representatives of each class of herbicide while the enzyme isolated from tolerant broadleaved species was much less sensitive. The potent inhibition of ACCase from grassy plants and the relative insensitivity of ACCase from broadleaved plants is likely to explain the mechanism of action of the two herbi-

cide classes and their selectivity against monocots.

In all species ACCase contains covalently bound d-biotin which serves as a carboxyl carrier between two physically and kinetically distinct catalytic sites. At one site the enzyme catalyses the ATP and divalent metal ion dependent carboxylation of enzyme bound biotin with bicarbonate (equation 1). At the second site the enzyme catalyses the transfer of the carboxyl group from the enzyme bound biotin to acetyl-CoA (equation 2).

$$\text{E-biotin} + \text{MgATP} + HCO_3^- \rightarrow \text{E-biotin-}CO_2^- + \text{MgADP} + P_i \quad (1)$$

$$\text{E-biotin-}CO_2^- + \text{acetyl-CoA} \rightarrow \text{E-biotin} + \text{malonyl-CoA} \quad (2)$$

The kinetic mechanism for biotin-dependent carboxylases (Wood & Barden, 1977), including ACCase from rat liver (Hashimoto & Numa, 1971), castor oil seeds (Finlayson & Dennis, 1983) and wheat leaves (Rendina, unpublished observations) is two site "ping pong", in which the products of the first site may be released before or after the addition of the substrates at the second site. The catalytic sites can be distinguished by studying isotope exchange reactions and other partial reactions that are unique to the separate half reactions (Moss & Lane, 1971).

In this report we investigate the kinetic interaction of representatives of each herbicidal class with the normal substrates for wheat ACCase. The inhibition of partial reactions catalysed by the enzyme was studied to determine which catalytic site was affected by the herbicides. Multiple inhibition studies were also conducted to learn whether the two distinct chemical classes of inhibitors shared a common binding site and to study the overlap of the binding site for these compounds with the coenzyme A binding site.

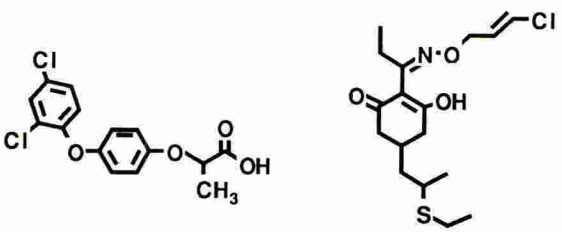

FIGURE 1. Structures of diclofop and clethodim.

MATERIALS AND METHODS

The commercial herbicides, radiochemicals and other reagents were prepared or obtained as described elsewhere (Rendina et al., 1988; Rendina & Felts, 1988; Rendina et al., 1989). Sources of plant materials, radiochemical and spectrophotometric assays for ACCase, and partial purification of the enzyme from wheat have been described previously (Rendina & Felts, 1988; Rendina et al., 1988). The assays for the ATP-[^{32}P]phosphate

isotope exchange, the carboxylation of free biotin, the malonyl-CoA -[^{14}C]acetyl-CoA isotope exchange, and the decarboxylation of malonyl-CoA were described in Rendina et al., 1989. The determination of I_{50} values and kinetic constants for inhibitors and Michaelis constants for substrates was described previously (Rendina et al., 1988). The analysis of multiple inhibition studies was described in Rendina et al., 1989.

RESULTS AND DISCUSSION

Kinetic Characterisation of the Inhibition

The families of intersecting lines in Figures 2-4 show that representatives of both classes of graminicides are linear, non-competitive inhibitors versus each of the substrates of wheat ACCase. The inhibition constants obtained from the slopes of the lines shown in Figure 2 (K_{is} values) are 200 to 600 times lower than the K_M value for acetyl-CoA or the K_{is} value for the product inhibitor, malonyl-CoA, indicating the potency of these compounds. Inhibition by clethodim and diclofop is most sensitive to the level of acetyl-CoA (the K_{is} value is less than the intercept inhibition constant, K_{ii}) and is much less sensitive to the level of either ATP or bicarbonate ($K_{is} \approx K_{ii}$). When acetyl-CoA concentrations are varied, the K_{is} value is nearly 10 times lower than when ATP or bicarbonate concentrations are varied at 150 μM acetyl-CoA (10 times K_M). Although the inhibition data fit best to the equation for noncompetitive inhibition, clethodim and diclofop are nearly competitive versus acetyl-CoA (the family of lines almost intersect on the y-axis in Figure 2), and are nearly uncompetitive versus bicarbonate (the lines in Figure 4 are nearly parallel).

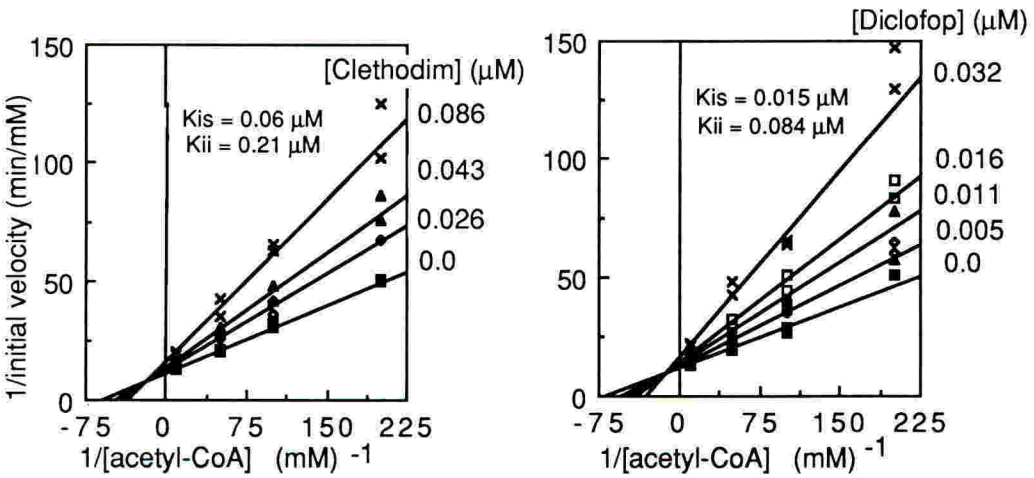

FIGURE 2. Inhibition of wheat ACCase by clethodim and diclofop versus acetyl-CoA at 5 mM $MgSO_4$, 3 mM ATP and 15 mM bicarbonate. The lines in the figure are the theoretical best fit of the data to the equation for noncompetitive inhibition.

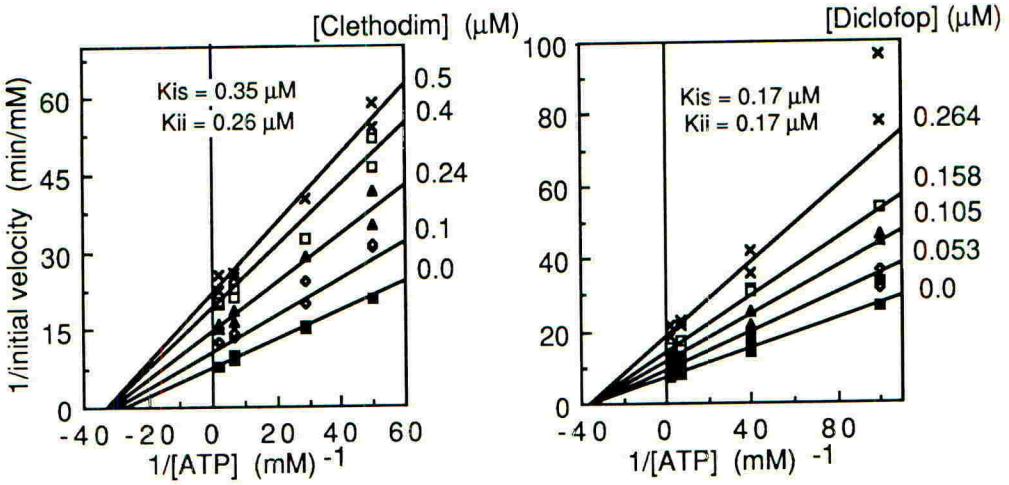

FIGURE 3. Inhibition of wheat ACCase by clethodim and diclofop versus ATP at 150 μM acetyl-CoA, 5 mM $MgSO_4$ and 15 mM bicarbonate. Data analysis was as described in Figure 2.

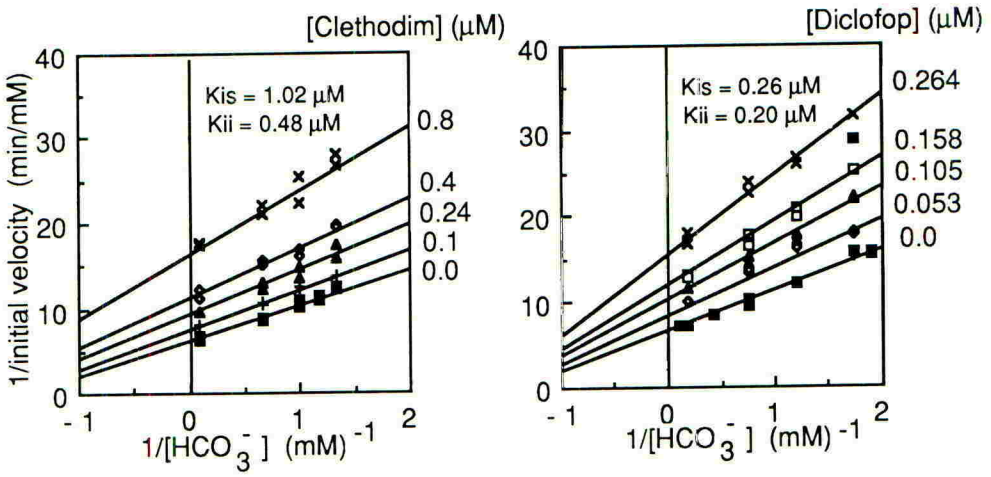

FIGURE 4. Inhibition of wheat ACCase by clethodim and diclofop versus bicarbonate at 150 μM acetyl-CoA, 5 mM $MgSO_4$ and 3 mM ATP. Data were analysed as described in Figure 2.

When malonyl-CoA is varied as a product inhibitor of wheat ACCase, similar patterns of linear, noncompetitive inhibition are obtained versus each of the substrates (data not shown). Like the herbicides, malonyl-CoA is also nearly competitive ver-

sus acetyl-CoA (Figure 5). Noncompetitive inhibition by malonyl-CoA was also observed with ACCase from castor oil seeds (Finlayson & Dennis, 1983) and from rat liver (Hashimoto & Numa, 1971). However, the two-site ping pong kinetic mechanism of biotin-dependent carboxylases predicts that the product, malonyl-CoA, should be a competitive inhibitor versus acetyl-CoA. This apparent contradiction may be explained if the central complexes of the kinetic mechanism (where the chemical steps take place) are partially rate-determining (Cleland, 1973). By analogy to malonyl-CoA, the kinetic data suggest that both classes of herbicides interfere with the carboxylation of acetyl-CoA at the carboxyltransfer site (equation 2) and do not affect the biotin carboxylation site (equation 1).

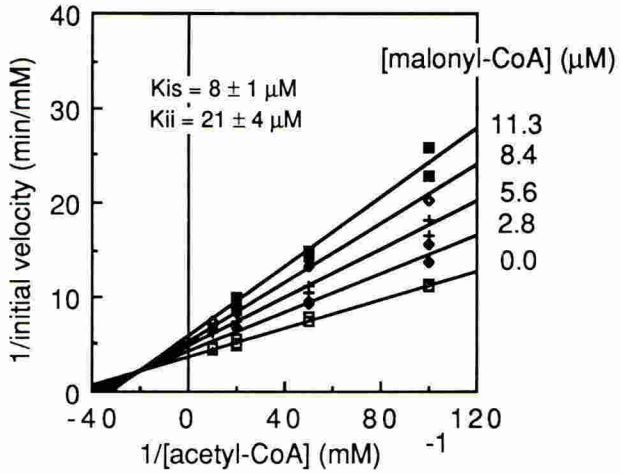

FIGURE 5. Inhibition of wheat ACCase by malonyl-CoA versus acetyl-CoA at 5 mM $MgSO_4$, 3 mM ATP and 15 mM bicarbonate. Data were analysed as described in Figure 2.

Inhibition of isotope exchange and partial reactions

Since the inhibition patterns do not conclusively show that the herbicides are strictly competitive versus the substrates of the carboxyltransfer site, the inhibition of partial reactions that are independently catalysed at the two separate sites was studied. For enzymes having ping pong mechanisms it is possible to observe isotope exchange reactions between the substrates and products of each half reaction in the absence of the substrates of the other half reaction. Wheat ACCase catalyses the exchange of label between ATP and [^{32}P]phosphate in the presence of ADP, Mg^{2+} and bicarbonate, the other substrates of the biotin carboxylation half reaction (equation 1), and in the absence of either acetyl-CoA or malonyl-CoA. The reaction depends on the presence of the bound biotin cofactor since carboxybiotin is required to form the putative carboxyphosphate intermediate (Tipton & Cleland, 1988; Ogita & Knowles, 1988), and is blocked by avidin,

which binds both free and enzyme bound biotin very tightly (Moss & Lane, 1971). Conducting the isotope exchange reaction in the presence or absence of avidin allows the biotin-dependent exchange to be distinguished from identical reactions catalysed by contaminating kinases or ATPases. The biotin carboxylation site can also be studied by following the carboxylation of free biotin in the presence of Mg^{2+}, ATP and $[^{14}C]HCO_3^-$ (equation 3).

$$\text{biotin} + \text{ATP} + [^{14}C]HCO_3^- \rightarrow \text{biotin-}[^{14}C]CO_2^- + \text{ADP} + P_i \quad (3)$$

To distinguish the carboxyltransfer site from the biotin carboxylation site we studied the inhibition of the biotin-dependent isotope exchange between [^{14}C]acetyl-CoA and malonyl-CoA (equation 2). At the carboxyltransfer site the enzyme also catalyses the decarboxylation of [1,3-^{14}C]malonyl-CoA (equation 4).

$$[1,3\text{-}^{14}C]\text{malonyl-CoA} \rightarrow [1\text{-}^{14}C]\text{acetyl-CoA} + [^{14}C]HCO_3^- \quad (4)$$

Each of the isotope exchange reactions and partial reactions that were studied with the wheat enzyme have been demonstrated previously for other biotin-dependent carboxylases (Moss & Lane, 1971; Wood & Barden, 1977).

Tables 1 and 2 are summaries of the inhibition of the isotope exchange reactions and the partial reactions. The I_{50} values for inhibition of the reactions catalysed at the biotin carboxylation site of wheat ACCase by clethodim and diclofop were from 200 to 100,000 times greater than the I_{50} values for inhibition of the complete reaction by these compounds (Table 1). In contrast both clethodim and diclofop were much more potent inhibitors of the reactions catalysed at the carboxyltransfer site. The I_{50} values were only 2 to 20 times greater than the values for the complete reaction (Table 2). Taken together, the data from the kinetic inhibition patterns and the inhibition of the isotope exchange and partial reactions strongly suggest that both herbicide classes interact at the carboxyltransfer site and not at the biotin carboxylation site.

TABLE 1. Inhibition of partial reactions catalysed by wheat ACCase at the biotin carboxylation site.

	I_{50} Values (μM)		
Inhibitor	Complete Reaction	ATP-P_i Exchange	Carboxylation of Free Biotin
clethodim	0.26 ± 0.02	420 ± 170	13,500 ± 1,400
diclofop	0.07 ± 0.01	460 ± 80	7,400 ± 1,300

For the complete reaction assays contained 5 mM $MgSO_4$, 3 mM ATP, 15 mM $NaHCO_3$ and 50 μM acetyl-CoA. For the ATP-P_i exchange reaction assays contained 0.5 mM [^{32}P]P_i (7-10 μCi), 3 mM ATP, 0.6 mM ADP, 12.5 mM $MgSO_4$ and 3 mM $NaHCO_3$. Controls containing 25 μg avidin were subtracted from each sample. The assays for the carboxylation of free biotin contained 7.5 mM [^{14}C]$NaHCO_3$ (2.63 μCi), 0.3 mM ATP, 1.3 mM $MgSO_4$ and 50 mM biotin.

TABLE 2. Inhibition of the partial reactions catalysed by wheat ACCase at the carboxyltransfer site.

Inhibitor	I_{50} Values (μM) Complete Reaction	Acetyl-CoA - Malonyl-CoA Exchg.	Malonyl-CoA Decarboxylation
clethodim	0.26 ± 0.02	4.10 ± 0.40	0.38 ± 0.04
diclofop	0.07 ± 0.01	1.30 ± 0.05	0.14 ± 0.02

Conditions for the complete reaction are the same as in Table 1. For the acetyl-CoA - malonyl-CoA exchange reaction assays contained 100 μM [^{14}C]acetyl-CoA (0.2 μCi) and 200 μM malonyl-CoA. Malonyl-CoA decarboxylation assays contained 6.6 μM [1,3-^{14}C]-malonyl-CoA (0.03 μCi).

Double Inhibition Studies

The nearly identical kinetic behavior of diclofop and clethodim suggested that the two classes of herbicidal inhibitors of ACCase might share a common binding site. To determine whether the binding of the two classes of inhibitors was mutually exclusive, we applied the double inhibition method (Yonetani & Theorell, 1964 as modified by Northrup & Cleland, 1974). This method, in which two inhibitors are varied at fixed, subsaturating levels of the substrate, yields the factor β, which defines the degree of interaction between the inhibitors at the enzyme active site (equation 5). If the inhibitors exclude each other from a common binding site, β becomes infinitely large and the pattern becomes a series of parallel lines described by equation 6, where v is the initial velocity, V is the maximal velocity, I and J are the two inhibitor concentrations and K_I and K_J are their apparent inhibition constants.

$$v = V/(1. + I/K_I + J/K_J + IJ/\beta/K_IK_J) \quad (5)$$

$$v = V/(1. + I/K_I + J/K_J) \quad (6)$$

The data shown in Figure 6 were fitted to both equations and the best fit was to equation 6 for a family of parallel lines. These results for clethodim and diclofop demonstrate that the cyclohexanediones interfere with the binding of the aryloxyphenoxypropionic acids and vice versa, and suggest that the two classes may share some common structural features.

The double inhibition method was also used to study the interaction of the herbicides with the CoA binding site. Since both classes of herbicides interact at the carboxyltransfer site and behave nearly the same as malonyl-CoA in kinetic studies, we expected both clethodim and diclofop to prevent malonyl-CoA from binding. The families of parallel lines shown in Figure 7 confirm this prediction. Clethodim, diclofop and malonyl-CoA can exclude each other from a common binding site.

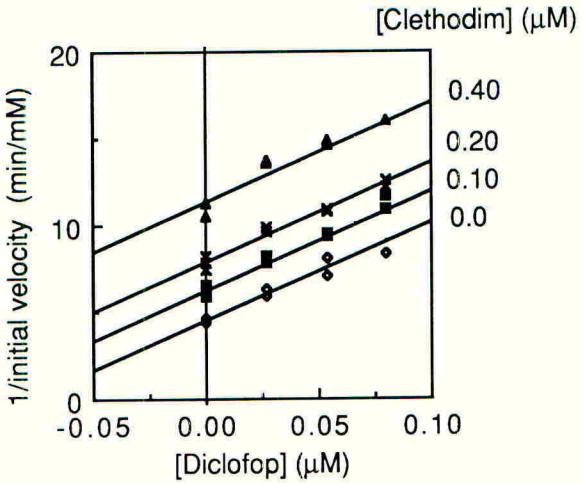

FIGURE 6. Double inhibition or Yonetani-Theorell plot of clethodim versus diclofop at 50 μM acetyl-CoA, 5 mM $MgSO_4$, 3 mM ATP and 15 mM bicarbonate. Data were fitted to equation 6 and the lines shown in the figure are to the theoretical fit.

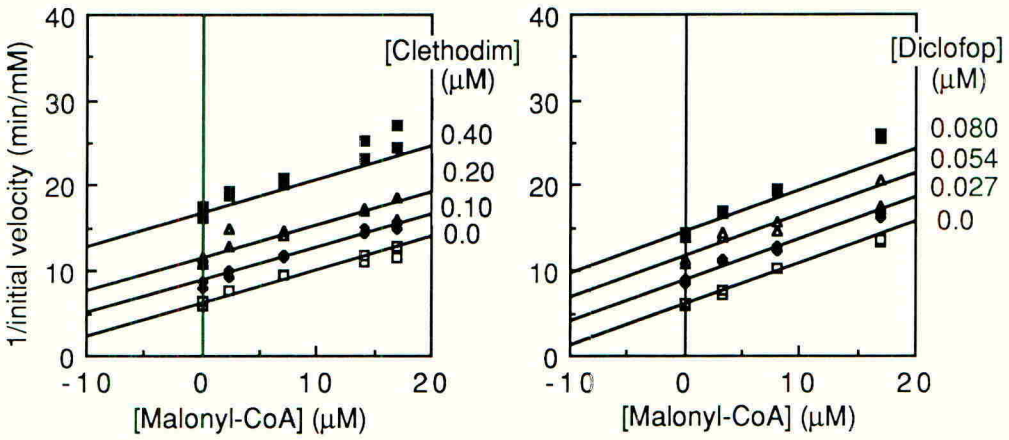

FIGURE 7. Double inhibition plot of clethodim versus malonyl-CoA and diclofop versus malonyl-CoA. Conditions are the same as in Figure 6. The best fit of the data was to equation 6 and the lines shown in the figure are to the theoretical fit.

Not surprisingly, when CoA (a competitive inhibitor versus acetyl-CoA) was varied versus malonyl-CoA, we obtained a family of parallel lines (data not shown). However, to our surprise,

when CoA was varied versus clethodim and diclofop, patterns of intersecting lines were obtained (Figure 8). These results suggest that only the thioester region of malonyl-CoA and acetyl-CoA overlaps with some structural feature of the herbicides. We are currently trying to define those structural features of each herbicide class that are important to the overlap between the different classes and between the herbicides and the substrate.

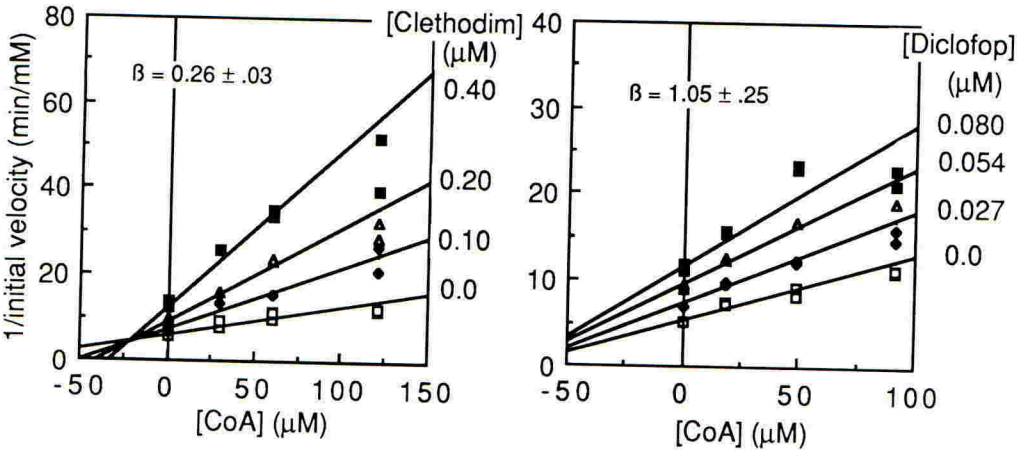

FIGURE 8. Double inhibition plot of clethodim versus coenzyme A and diclofop versus coenzyme A. Conditions are the same as Figure 6. The best fit of the data was to equation 5 and the lines shown in the figure are to the theoretical fit.

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DIFFERENCES IN SENSITIVITY AND TOLERANCE OF MONOCOTYLEDONOUS AND DICOTYLEDONOUS PLANTS TOWARDS INHIBITORS OF ACETYL-COENZYME A CARBOXYLASE

H.K. LICHTENTHALER, K. KOBEK, M. FOCKE

Botanisches Institut (Plant Physiology and Biochemistry), University of Karlsruhe, Kaiserstr. 12, D-7500 Karlsruhe 1, FRG

ABSTRACT

The effect of graminicides such as the cyclohexane-1,3-dione derivatives and aryloxyphenoxypropionic acid herbicides which inhibit acetyl-CoA carboxylase in sensitive species, was investigated in a range of sensitive grasses (oat and other Poaceae) and in resistant/tolerant mono-and dicotyledonous plants. The basis of tolerance of some of the Poaceae species appears to differ from that of the resistant dicotyledonous and monocotyledonous species. In chloroplasts from oat I_{50}-values of 0.1 - 3 µM were obtained for herbicides inhibition of ^{14}C-acetate incorporation into fatty acids. In contrast, most dicotyledonous species appear to be resistant towards these herbicides at the whole plant, isolated chloroplast and isolated enzyme levels. Isolated spinach chloroplasts, however, are slightly sensitive to diclofop and fenoxaprop at high concentrations (100µM). Some tolerant grass species (Festuca rubra, Poa annua) were sensitive at the isolated chloroplasts level. It appears that cytoplasmic properties as well as differences in the sensitivity of acetyl-CoA carboxylase contribute to the tolerance of certain Poaceae. Tolerance of wheat towards diclofop appears to lie solely in the cytoplasmic properties.

INTRODUCTION

Cyclohexane-1,3-diones (e.g. alloxydim, cycloxydim, clethodim, sethoxydim, tralkoxydim) and the aryloxyphenoxypropionic acid derivatives (diclofop, fenoxaprop, fluazifop, haloxyfop, propaquizafop, quizalofop) are two structurally different classes of herbicides which selectively control grass weeds in a variety of dicotyledonous crop plants. Both herbicide groups effectively block chloroplast-located de novo fatty acid biosynthesis in sensitive grasses e.g. diclofop and fenoxaprop (Hoppe, 1985; Hoppe & Zacher, 1982, 1985) and sethoxydim (Burgstahler, 1985; Burgstahler et al., 1985). Subsequent publications show this also applied to the other compounds mentioned above (Bocion et al., 1987; Kobek et al., 1988a,b; Lichtenthaler & Kobek, 1989; Lichtenthaler et al., 1987; Secor et al., 1989; Uchiyama et al., 1986; Walker et al., 1988). In higher plants de novo fatty-acid biosynthesis is bound to chloroplasts (plastids) (Ohlrogge et al., 1979). This is why chloroplasts and etioplasts repesent a very good test-system for inhibitors of de novo fatty-acid biosynthesis (Kobek et al., 1988a; Lichtenthaler & Kobek, 1989). In the case of aryloxyphenoxy-propanoates the R(+)-enantiomers are the active compounds, whereas the S(-) enantiomers show no or little effect (Hoppe & Zacher, 1985; Uchiyama et al., 1986). In 1987, four different laboratories discovered independently that the target enzyme of both classes was acetyl-CoA carboxylase (EC 6.4.1.2) (Burton et al., 1987; Focke & Lichtenthaler, 1987; Kobek et al., 1988a; Rendina & Felts 1988; Secor & Cséke, 1988). The aryloxyphenoxypropanoates were applied as esters which are hydrolysed in the plant to active free acids and only the latter are active

at the target site (Kobek et al., 1988b). The inhibition of de novo fatty-acid biosynthesis by inhibiting acetyl-CoA carboxylase causes an inhibition of thylakoid membrane formation, chloroplast multiplication and biogenesis and subsequently cell division and membrane lipid biosynthesis (Burgstahler, 1985; Burgstahler & Lichtenthaler, 1984 Burgstahler et al., 1986; Lichtenthaler, 1984, 1987; Lichtenthaler & Meier 1984). The inhibition and binding of the two herbicide classes is reversible as was shown with isolated etioplasts as test-system (Kobek et al., 1989).

The tolerance and/or resistance of dicotyledonous plants and the sensitivity of grasses towards both herbicide classes appears to be due to differences in properties of the target enzyme (Kobek et al., 1988b; Rendina et al., 1988). Monocotyledonous plants outside the Poaceae family were found to be tolerant and/or resistant to sethoxydim (Kobek et al., 1988a). There are also some members of the Poaceae family, which are relatively tolerant towards some of the cyclohexanedione and aryloxy- phenoxypropanoate herbicides. Poa annua and Festuca rubra plants are relatively tolerant towards sethoxydim, whereas P. pratensis and F. ovina are sensitive (Kobek et al., 1988a; Weber et al., 1988). However, isolated P. annua chloroplasts were sensitive towards sethoxydim (Kobek et al., 1988a). Festuca rubra is also tolerant towards haloxyfop (Stoltenberg et al., 1988). Wheat is more or less tolerant towards diclofop, which is ring-hydroxylated and glucosylated to an inactive metabolite (Jacobsen et al., 1985). The ring-hydroxylated diclofop is much less effective than diclofop (Kobek et al., 1988b). In some cases the relative tolerance of some members of the Poaceae seems to lie in particular properties of the cytoplasm which modifies the active ingredient to an inactive or less active compound, whereas in others there may be an altered target enzyme (e.g. Stoltenberg et al., 1988). In order to obtain more information on the apparent resistance of dicotyledonous and monocotyledonous plants (except the Poaceae) and on the susceptibility and tolerance of grasses we have studied the effect of cycloxydim and diclofop in a wide range of higher plants. Some of these results with particular emphasis on susceptible and tolerant Poa and Festuca species as well as differences between cycloxydim and diclofop are described in this report.

MATERIAL AND METHODS

Seedlings of oat (cv. Flämingsnova), barley (cv. Alexis) and pea (cv. Kleine Rheinländerin) were cultivated on peat with nutrients (TKS II, Floratorf) in a 14/10 h day/night cycle at ca. 24° C at a light intensity of 1 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. F. rubra (cv. Roland), F. ovina, F. arundinacea (cv. Ludion), Poa annua and Poa pratensis were cultivated hydroponically in the dark for 7d and illuminated for 12 h (1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) before plastid isolation. The other plants used in this investigation were cultivated in the Botanical Garden of the University.

Chloroplasts from the Festuca and Poa species were isolated using an medium containing 0.4 M sorbitol, 50 mM Tris/HCl (pH 9.2), 1 mM $MgCl_2$ and 0.2 % BSA. The isolation medium used for the other plants contained 0.4 M sorbitol, 50 mM phosphate buffer (pH 8.0), 2 mM $MgCl_2$ and 0.2 % BSA. All steps of isolation were carried out at 4° C. 20-30 g leaves (primary leaves) were homogenized, filtered, centrifuged and the chloroplasts resuspended in isolation medium (pH 8) without BSA. This chloroplast suspension was used for the incorporation studies of ^{14}C-acetate into the total fatty-acid fraction. Incubation (20 min) of chloroplasts with 1-^{14}C acetate, saponification and

extraction of the fatty acids and determination of incorporated radioactivity was performed as described previously (Kobek et al., 1988a). The direct enzyme assay for acetyl-CoA carboxylase in enriched protein preparations (0-40 % $(NH4)_2SO_4$ precipitation) was performed by studying the heat and acid-stable incorporation of $^{14}C\text{-}HCO_3^-$ into malonyl-CoA; the incorporated radioactivity was expressed in Bequerel (Bq) during a 10 min period.

RESULTS AND DISCUSSION

The cyclohexane-1,3-dione herbicides (CHDs) sethoxydim, cycloxydim and clethodim and the aryloxyphenoxypropionic acids (APPAs) diclofop, fenoxaprop and haloxyfop inhibited de novo fatty-acid biosynthesis of isolated oat chloroplasts from ^{14}C-acetate in a concentration-dependent manner (Figures 1 & 2). The I_{50}-values for inhibition of fatty-acid biosynthesis were low (0.1 - 0.5µM) (Table 1). The esters of the APPAs, generally applied as herbicides to the intact plants, are much less effective in isolated chloroplasts (Kobek et al., 1988b) and on the target enzyme. This relative ineffectiveness of the ester forms at the chloroplast and enzyme level is not due to a lower rate of uptake into the chloroplast as compared to the free acids (Hoppe & Zacher, 1985), but to the fact that the free acid form is the real active component.

In chloroplasts isolated from spinach cycloxydim had no effect on de novo fatty-acid bioynthesis even at very high concentrations (Fig. 1). This has been shown for sethoxydim (Kobek et al., 1988a). The very high I_{50}-value in chloroplasts from dicotyledonous plants for cycloxydim (Table 1: spinach, pea and tobacco) and also from the monocotyledonous plant Chlorophytum comosum (a member of the Liliideae) indicate that these plants are resistant towards the different CHD-herbicides.

Dicotyledonous plants (spinach, tobacco) and monocotyledonous plants outside the Poaceae (C. comosum) also appear to be tolerant or resistant towards the APPA herbicides at the whole plants and the chloroplast levels, whereas oat is sensitive (Figure 3). In chloroplasts of some dicot plants such as spinach fatty acid biosynthesis is inhibited at a high (100µM) concentration of diclofop and fenoxaprop (Figure 2). In fact, the I_{50}-value for diclofop in spinach is considerably lower than that of cycloxydim (Table 1). From this it may be concluded that some dicotyledonous plants (spinach) are not resistant, but only tolerant i.e. they are much less sensitive than the really sensitive Poaceae and can endure a higher concentration of diclofop. The sensitivity of spinach chloroplasts is, however, ca. 1500 times lower than that of oat chloroplasts, which accounts for the tolerance of the spinach plant towards diclofop as compared to oat.

The resistance of spinach and pea towards cycloxydim and a certain sensitivity towards diclofop is not only found at the chloroplast level but also at the target enzyme (acetyl-CoA carboxylase) (Table 2). These results indicate that the two different classes of graminicides (CHDs and APPAs) which inhibit the same target enzyme may also bind to the same binding domain, but possess different subsites. This assumption would explain that dicotyledonous plants appear fully resistant towards CHDs, whereas some of these such as spinach or pea are only tolerant (i.e. sensitive at higher concentrations) to diclofop and certain other APPAs.

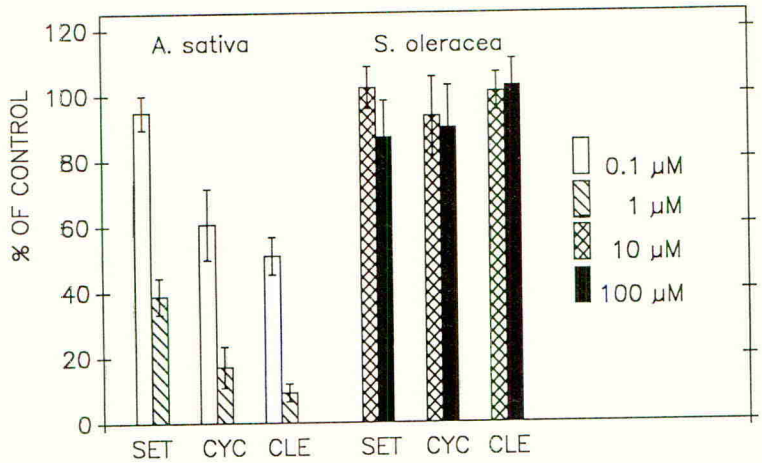

FIGURE 1. Effect of the cyclohexane-1,3-dione herbicides sethoxydim (SET), cycloxydim (CYC) and clethodim (CLE) on de novo fatty acid biosynthesis of chloroplasts isolated from sensitive oat plants and from resistant spinach. Incorporation rates of ^{14}C-acetate of the controls were 12.2 ± 2 (oat) and 22 ± 2.5 kBq per mg a + b (spinach) during a 20 min incubation period. Mean values of 4 determinations from two experiments with standard deviation.

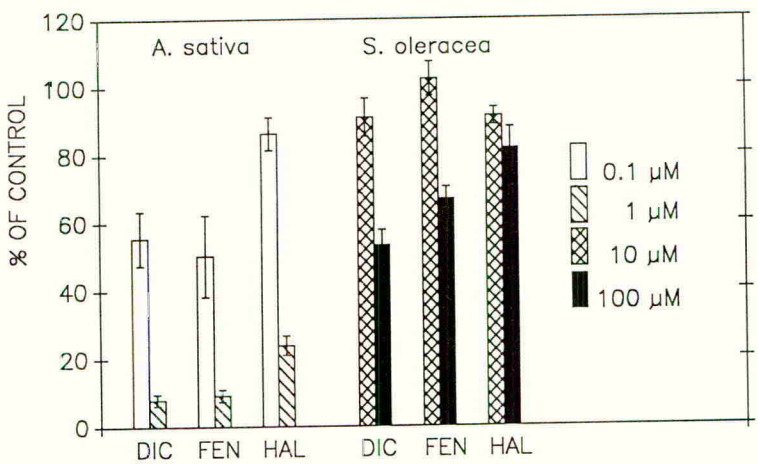

FIGURE 2. Inhibition of de novo fatty acid biosynthesis (^{14}C-acetate incorporation) in chloroplasts isolated from sensitive oat and resistant spinach plants by aryloxyphenoxypropionic acid derivatives diclofop (DIC), fenoxaprop (FEN) and haloxyfop (HAL). The D,L-enantiomers of the three herbicides were used. For incorporation rates see Figure 1.

Within monocotyledonous plants the Poaceae seem to be the only family having sensitivity towards CHD and APPA herbicides (Table 1). About 20 other monocotyledonous plants from different families, which we investigated were resistant or tolerant at the whole plant and isolated chloroplast levels towards cycloxydim and diclofop. Also in chloroplasts from plants, closely related to the Poales (Restionales, Commelinales, Cyperales,Juncales) de novo fatty-acid biosynthesis was not affected by cycloxydim and diclofop (except for some plants at very high concentrations of diclofop).

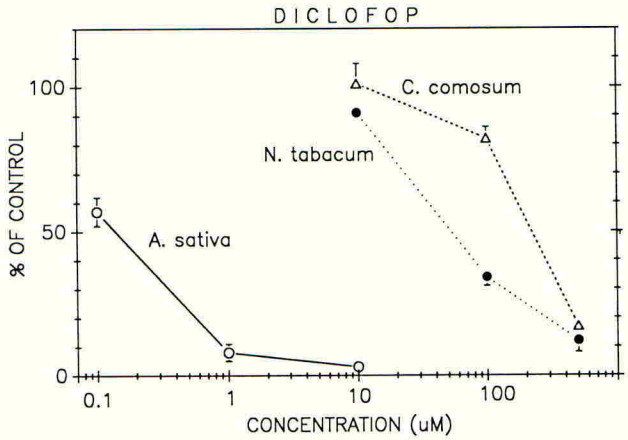

FIGURE 3. Inhibition of de novo fatty-acid biosynthesis of chloroplasts isolated from sensitive oat and tolerant tobacco and C. comosum by D,L-diclofop. (Control incorporation rates were 12 ± 2 kBq per mg a+b for oat, 95 ± 7 kBq per mg a+b for tobacco and 4 kBq per mg a+b for C. comosum during a 20 min incubation period. Mean values of at least 4 determinations with SD).

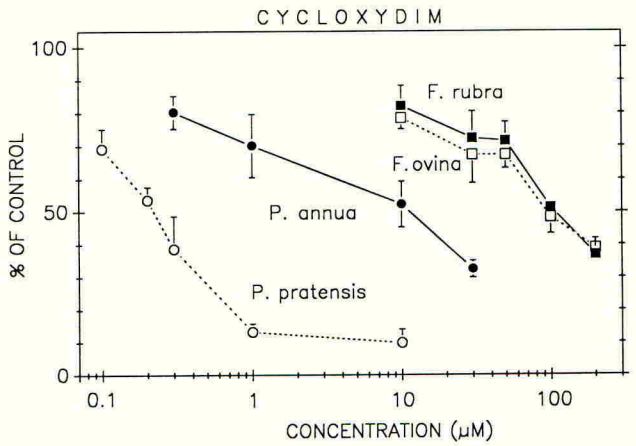

FIGURE 4. Inhibition of fatty-acid biosynthesis in isolated chloroplasts from Festuca and Poa species by cycloxydim. Mean control incorporation rates were 62 (F. rubra), 128 (F. ovina), 65 (P. annua) and 102 (P. pratensis) kBq per mg chlorophyll a+b. Mean of at least 4 determinations with SD.

TABLE 1. I_{50}-values for the inhibition of de novo fatty-acid biosynthesis of chloroplasts (^{14}C-acetate incorporation) isolated from plants with different sensitivity towards cycloxydim and diclofop. Diclofop was a 50 % mixture of the active D(+) and the inactive L(-) enantiomers.

species	I_{50}-value (µM) cycloxydim	diclofop
C. comosum	300	ca. 280
tobacco	300	ca. 250
pea	300	ca. 250
spinach	300	150
F. ovina	100	0.4
F. rubra	100	0.4
P. annua	10	0.2
P. pratensis	0.2	0.1
A. sativa	0.15	0.1
F. arundinacea	0.1	0.1

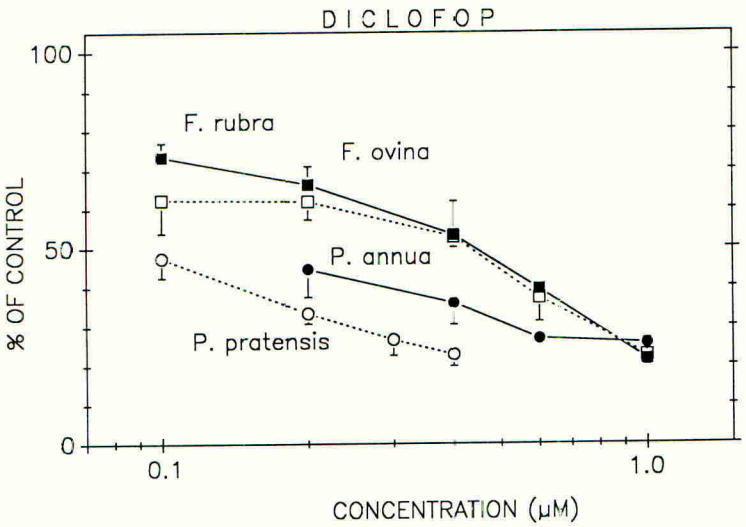

FIGURE 5. Inhibition of ^{14}C-acetate incorporation into the total fatty-acid fraction of chloroplasts isolated from different Festuca and Poa species by D,L-diclofop. The incorporation rates are the same as in Figure 4.

Within the Poaceae there are some species which at the whole plant level are relatively tolerant to CHDs or APPAs. P. annua was found to be tolerant towards cycloxydim, whereas P. pratensis was sensitive. At the level of isolated chloroplasts, the fatty acid biosynthesis of both was, however, inhibited by cycloxydim in a dose-dependent manner (Figure 4). The I_{50}-values of the tolerant species was, however, 50 times higher than in the sensitive P. pratensis (Table 1). The results show that the relative tolerance of these

grasses is not comparable to the apparent resistance of dicotyledonous and certain monocotyledonous plants, where the I_{50}-values of the CHDs are considerably higher than 300 µM. In contrast to cycloxydim, chloroplasts of both Poa species were highly sensitive to diclofop with I_{50}-values of 0.1 - 0.2 µM (Figure 5, Table 1). Thus P. annua belongs to the few grasses, where at the level of isolated chloroplasts large differences exist in the sensitivity towards CHD and APPA herbicides.

Further examples for this phenomenon are the grasses F. rubra and F. ovina. Plants of both species are relatively tolerant to CHDs and APPAs. The ^{14}C-acetate incorporation into fatty acids by chloroplasts, however, is only slightly inhibited by cycloxydim (I_{50}-values of 100µM), whereas the very sensitive F. arundinacea exhibited the expected very low I_{50}-value (Table 1). F. ovina and F. rubra are relatively tolerant to diclofop, but both species are very sensitive on the chloroplast level. The I_{50}-values are much lower than for cycloxydim, but not as low as for the diclofop-sensitive F. arundinacea (Table 1). From the sensitivity at the chloroplast level we conclude that the tolerance of both Festuca species towards diclofop

TABLE 2. Effect of cycloxydim and diclofop on the activity of acetyl-CoA carboxylase (ACC) in enzyme preparations from sensitive oat and tolerant plants (pea, spinach). Mean of 3 values with a SD of the ACC activity of 5 % or less. The latter is expressed as incorporated radioactivity (Bq).

	ACC activity (in Bq)	% inhibition
oat		
control	260	0
10µM cycloxydim	62	76
5µM cycloxydim	87	67
2µM cycloxydim	144	44
5µM diclofop	45	87
2µM diclofop	92	64
1µM diclofop	125	52
pea		
control	174	0
100µM cycloxydim	164	6
10µM cycloxydim	169	3
1µM cycloxydim	172	0
100µM diclofop	35	80
10µM diclofop	94	46
1µM diclofop	153	12
spinach		
control	177	0
100µM cycloxydim	157	12
10µM cycloxydim	164	7
1µM cycloxydim	173	0
100µM diclofop	37	79
10µM diclofop	112	37
1µM diclofop	147	17

may mainly be caused by cytoplasmic properties e.g. metabolism or inactivation of the herbicide, but partially also by altered acetyl-CoA carboxylase in the two tolerant species, since the I_{50}-values are higher than that of the sensitive F. arundinacea.

The situation can be different for other APPA herbicides. In F. rubra only a slight sensitivity was found towards haloxyfop and no sensitivity towards sethoxydim (Stoltenberg et al., 1988), which may be caused by a modification of the target enzyme and also by different subbinding sites for diclofop and haloxyfop. More evidence for this assumption comes from research with Lolium multiflorum, which is resistant to diclofop-methyl, but sensitive to sethoxydim (Gronwald et al., 1989). This difference in sensitivity was also found at the level of acetyl-CoA carboxylase. The authors conclude that this diclofop resistance is due to a modification of the acetyl-CoA carboxylase, which results in a resistance to the APPAs but not to the CHD herbicides. The differential sensitivity of some Poaceae to the CHD and APPA herbicides offers interesting possibilities in the practical control of grass weeds in cereals.

CONCLUSION

From the results it appears likely that cycloxydim and diclofop possess different subsites at the binding domain of the target enzyme acetyl-CoA carboxylase. The tolerance of several members of the Poaceae towards certain CHD and APPA herbicides seems to lie in a combined action of cytoplasmic properties of plants and a lower sensitivity of the target enzyme, whereas the apparent resistance of many dicotyledonous plants seem to be due to a modification of the target enzyme. Among the variety of tested plants there seem to exist fluent transitions between sensitivity, relative tolerance and a clear resistance towards cyclohexanedione (CHDs) and aryloxyphenoxy-propionic acid (APPAs) herbicides.

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ANTI-AUXIN ACTIVITY OF GRAMINICIDES

A.H. COBB, P. BARNWELL

Herbicide Research Group, Department of Life Sciences, Nottingham Polytechnic, Clifton Lane, Nottingham NG11 8NS, UK

ABSTRACT

The interaction between aryloxyphenoxypropionates (diclofop-methyl, DM and quizalofop-ethyl, QE) and auxin-type herbicides (MCPA and clopyralid) has been investigated in glasshouse- and laboratory-based studies. The addition of MCPA to DM or clopyralid to QE significantly reduced graminicide symptom severity in Avena fatua seedlings in a concentration-dependent fashion. This antagonism was further examined in an auxin-sensitive assay of H^+-efflux in which DM, and to a lesser extent QE and FB (fluazifop-butyl), significantly inhibited this rapid response. These observations are consistent with the proposal that graminicides may have an additional site of action at the cell membrane of tissues undergoing active extension growth.

INTRODUCTION

Modern farming practices have ensured a wide diversity of both monocotyledonous and dicotyledonous weeds in major crops. Consequently, the tank mixture of a graminicide with a broad leaf weed herbicide would be advantageous to the farmer, since it would be expected to give a broad spectrum of weed control with a single treatment. Additional benefits could include savings in time, labour and fuel costs as well as less mechanical damage to the crop, for example wheelings. However, it is now well established that these herbicide mixtures are invariably incompatible, such that a tank-mixture will reduce graminicide efficacy and induce crop damage. The most widely reported example of this "graminicide antagonism" has been the mixture of the aryloxyphenoxy-propionate graminicide diclopfop-methyl with the phenoxyalkanoates 2,4-D or MCPA (eg. Köcher, 1984).

The site and mechanism of this antagonism remain obscure with some conflict in the literature. Whilst most authors accept the selective inhibition of acetyl CoA carboxylase as the primary target of graminicides (eg. Secor and Cséke, 1988), it is becoming increasingly evident that diclofop-methyl can also disrupt both cell membrane function and activity (eg. Ratterman and Balke, 1987, 1988). In addition, Fitzsimons et al, (1988) have shown that DM can interfere with auxin activity both through a competition for receptor sites and a non-competitive inhibition of the H^+-efflux mechanism in Avena sativa coleoptiles. Furthermore, the need for a rapid rate of tissue extension has recently been identified as an essential feature for optimal DM activity, such that decreased expansion rates associated with water stress may contribute to reduced DM efficacy (Field and Caseley, 1987; Andrews et al, 1989) and also presumably to antagonism.

This report describes a series of experiments that investigate the

consequences of mixing MCPA with DM and clopyralid with QE on both intact plants and in a rapid and sensitive assay of auxin-induced H^+-efflux, to confirm the anti-auxin activity of these graminicides.

MATERIALS AND METHODS

Glasshouse Study

Avena fatua L. (wild oat, non-dormant strain; Herbiseed, Berkshire, UK) was raised in J Arthur Bowers seed and potting compost in 100 mm diameter pots in a heated glasshouse (20-40°C day; 9-15°C night) at 60-70% relative humidity. A photosynthetic photon flux density (PPFD) of 300-400 µmol/m/sec was provided by natural daylight supplemented with high pressure sodium lamps and a 16h photoperiod maintained throughout the study. Approximately 17 d after sowing the seedlings had reached the 3-leaf stage and were thinned to 10 plants per pot prior to herbicide application.

Diclofop (methyl ester), quizalofop (ethyl ester), MCPA (Na^+/K^+ salt) and clopyralid (monoethylamine salt) herbicide treatments were prepared from commercial formulations and applied immediately after mixing with a Mardrive Laboratory Sprayer (Marine Engineering Company Ltd, Stockport, UK) fitted with a single Lurmark 02 - F80 nozzle, which delivered 250 l/ha spray volume at 3.0 bars pressure. Plants were sprayed at 18-20°C and 50-60% relative humidity, and maintained under these conditions for approx 2h before their return to glasshouse conditions. Three replicate pots, each containing 10 plants, were used for each treatment. Fresh weights were accurately determined 14 days after treatment (dat) and mean values computed.

Laboratory Study

Auxin-induced H^+-efflux by etiolated coleoptile segments from Avena sativa L. (spring oat, cv Maris Tabard) was performed as previously described by Fitzsimons et al, (1987). This method allows the accurate determination of a rapidly induced auxin-mediated effect and has allowed the relative activities of auxin-type herbicides to be described in a model of "hormone-receptor binding kinetics" (Fitzsimons et al, 1988).

All herbicide solutions were prepared from technical samples of greater than 95% purity. MCPA and clopyralid were firstly dissolved in acetone, neutralised with KOH and then diluted with distilled water. DM, FB and QE were dissolved in acetone only and diluted such that the final concentration of acetone in the incubation medium was always less than 1.0%. The graminicides were routinely added to the tissue segments 30 min before the auxin-type herbicides and a time-course of H^+-efflux determined as a drop in bathing medium pH over an 80 min incubation period. Each treatment was repeated four times on separate occasions.

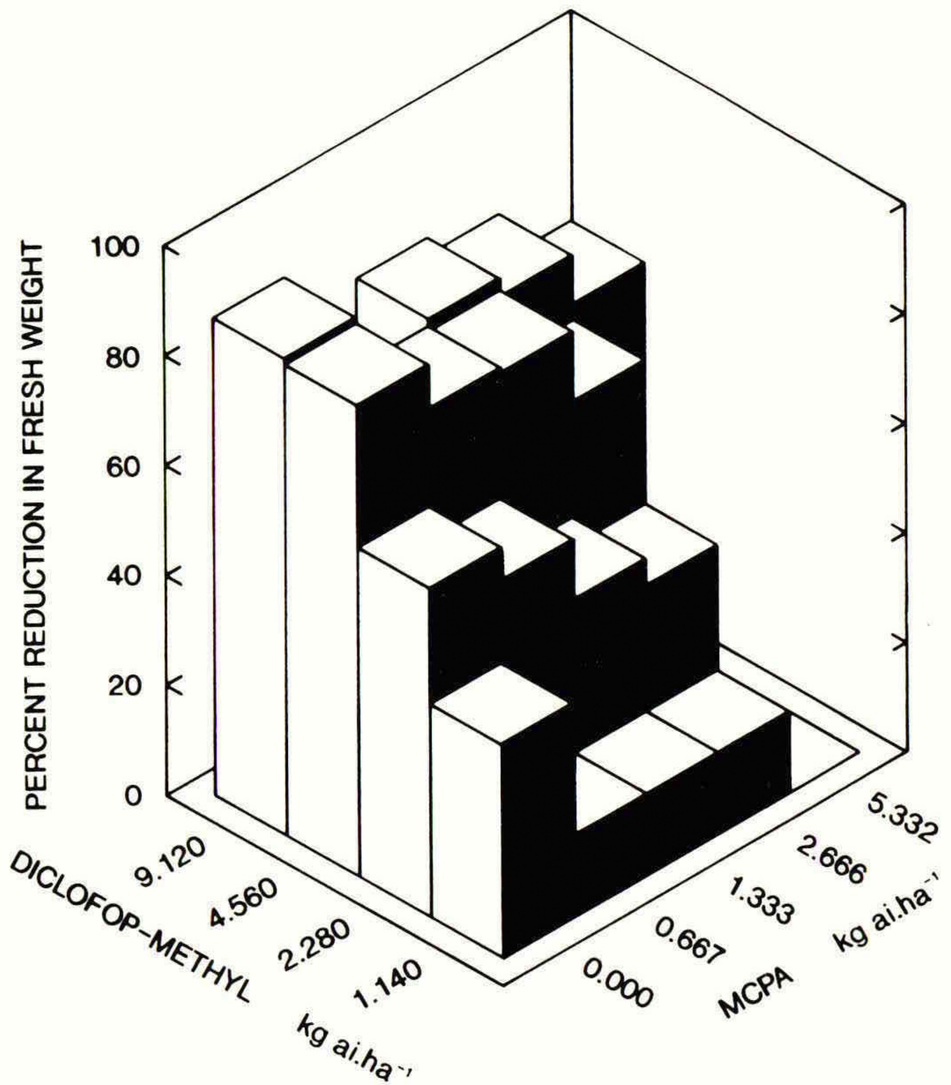

FIGURE 1: Effect of DM alone and in tank-mixture with MCPA on the fresh weight of A. fatua seedlings 14 days after treatment. Data are a mean of 30 plants for each treatment.

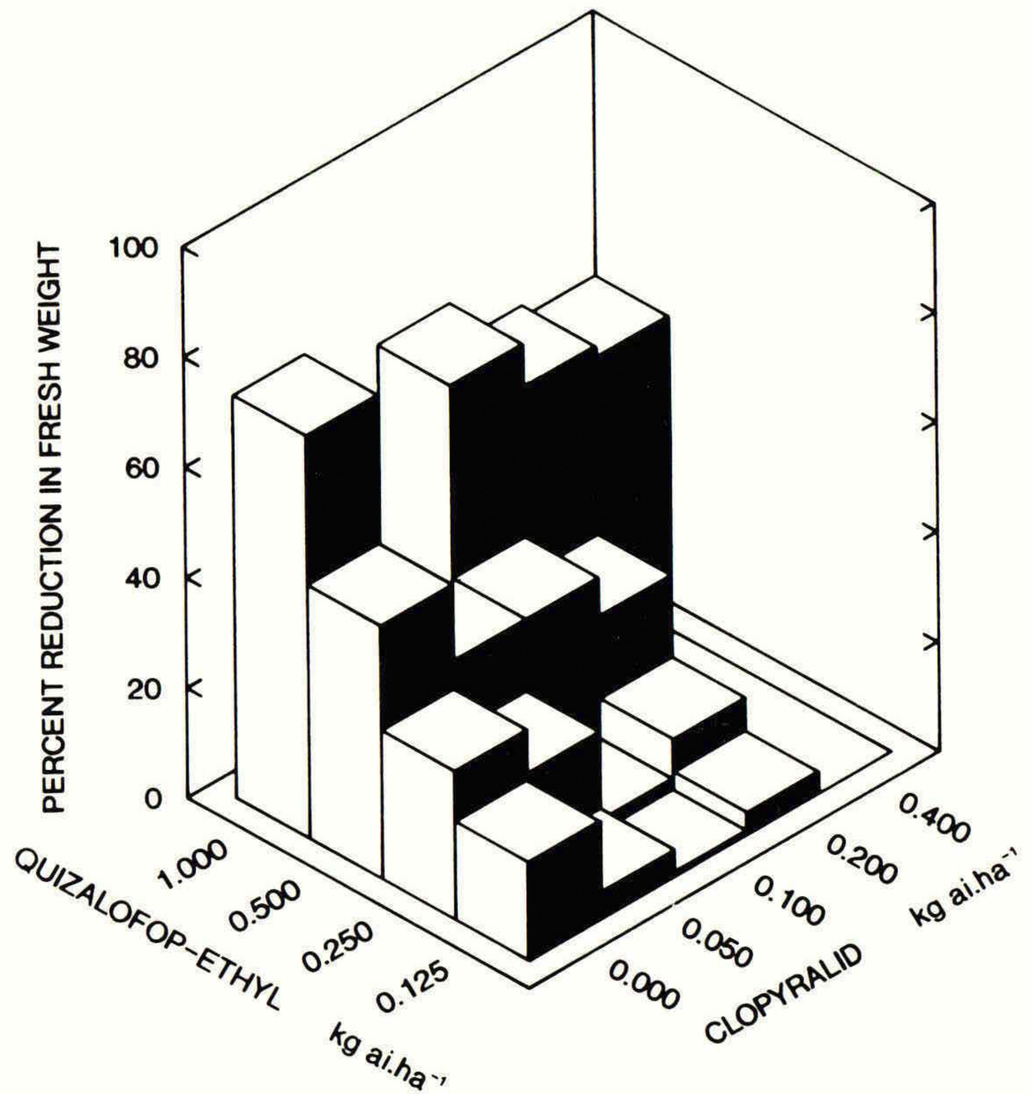

FIGURE 2: Effect of QE alone and in tank-mixture with clopyralid on the fresh weight of A. fatua seedlings 14 days after treatment. Data are a mean of 30 plants for each treatment.

RESULTS

Glasshouse Study

Symptom development in A. fatua followed a similar pattern to both graminicides, although DM appeared more potent than QE at comparable dosage, under the conditions described. Chlorosis of leaf margins and tips was observed 3-4 dat in youngest leaves followed by the necrosis of these regions 3d later. Reduced internode length and seedling stunting were evident 7-10 dat, and by 10-14 dat stem bases had become necrotic. Symptom severity increased with dosage. The addition of MCPA to DM and clopyralid to QE markedly reduced symptom severity, particularly at higher rates of MCPA and clopyralid. Thus, A. fatua plants treated with mixtures were less chlorotic, had longer internodes and generally appeared more vigorous than plants treated with graminicide alone. Plants treated with MCPA or clopyralid only were identical in appearance to untreated controls, with the exception of some stunting at the highest MCPA dose.

Analysis of fresh weights 14 dat (Figures 1 and 2) confirmed these observations of antagonism and the interactions proved to be statistically significant at the 5% level in most instances, ie. graminicide efficacy was invariably reduced, manifest as less reduction in fresh weight, in the presence of the auxin-type herbicide.

Laboratory Study

Figure 3 shows a time-course of H^+-efflux induced by MCPA in A. sativa segments, and indicates the striking sensitivity of this tissue to this synthetic auxin, the speed of the response and its inhibition by diclofop-methyl. The effect of 5-100 µM DM, FB and QE on H^+-efflux caused by saturating concentrations of MCPA (10 µM) and clopyralid (1 mM) are presented in Figure 4.

Statistical analysis revealed that with MCPA-induced H^+-efflux, DM significantly reduced efflux at 20, 50 and 100 µM concentrations, QE had a significant effect at 50 and 100 µM, whilst FB only significantly reduced control rates in the 100 µM treatment (Figure 4a). Whilst the general trend of inhibition was similar with the weaker or less active auxin clopyralid (Figure 4b), lower graminicide concentrations produced a greater relative inhibition, such that both DM and QE significantly inhibited efflux at 20, 50 and 100 µM and FB was also effective at both 50 and 100 µM.

DISCUSSION

The morphological observations reported in this study are in accord with the findings of others. Thus, the chlorotic symptoms of young leaf tips and margins result from structural damage to developing chloroplasts (Brezeanu, Davies and Shimabukuro, 1976) and was followed by progressive leaf and stem necrosis (Hoerauf and Shimabukuro, 1979). Similarly, reductions in internode elongation confirm the findings of the latter authors and Carr (1986), and agree with the general observation that young, rapidly expanding tissues are most sensitive to graminicides (Donald and Shimabukuro, 1980).

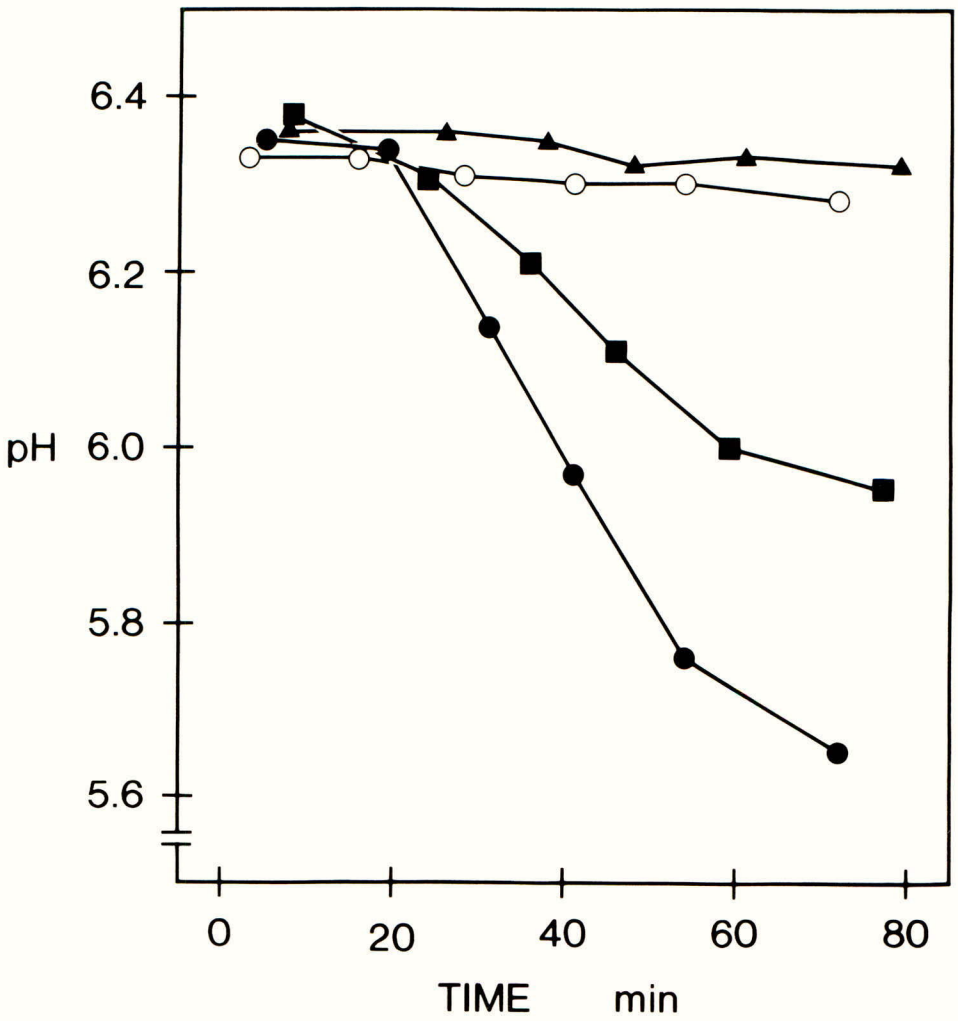

FIGURE 3: Time-course of H^+-efflux induced by 10 µM MCPA in the absence (●) and presence of 50 µM DM (■). ▲ represents 50 µM DM alone and O represents H^+-efflux with 1.0% acetone (v/v) only.

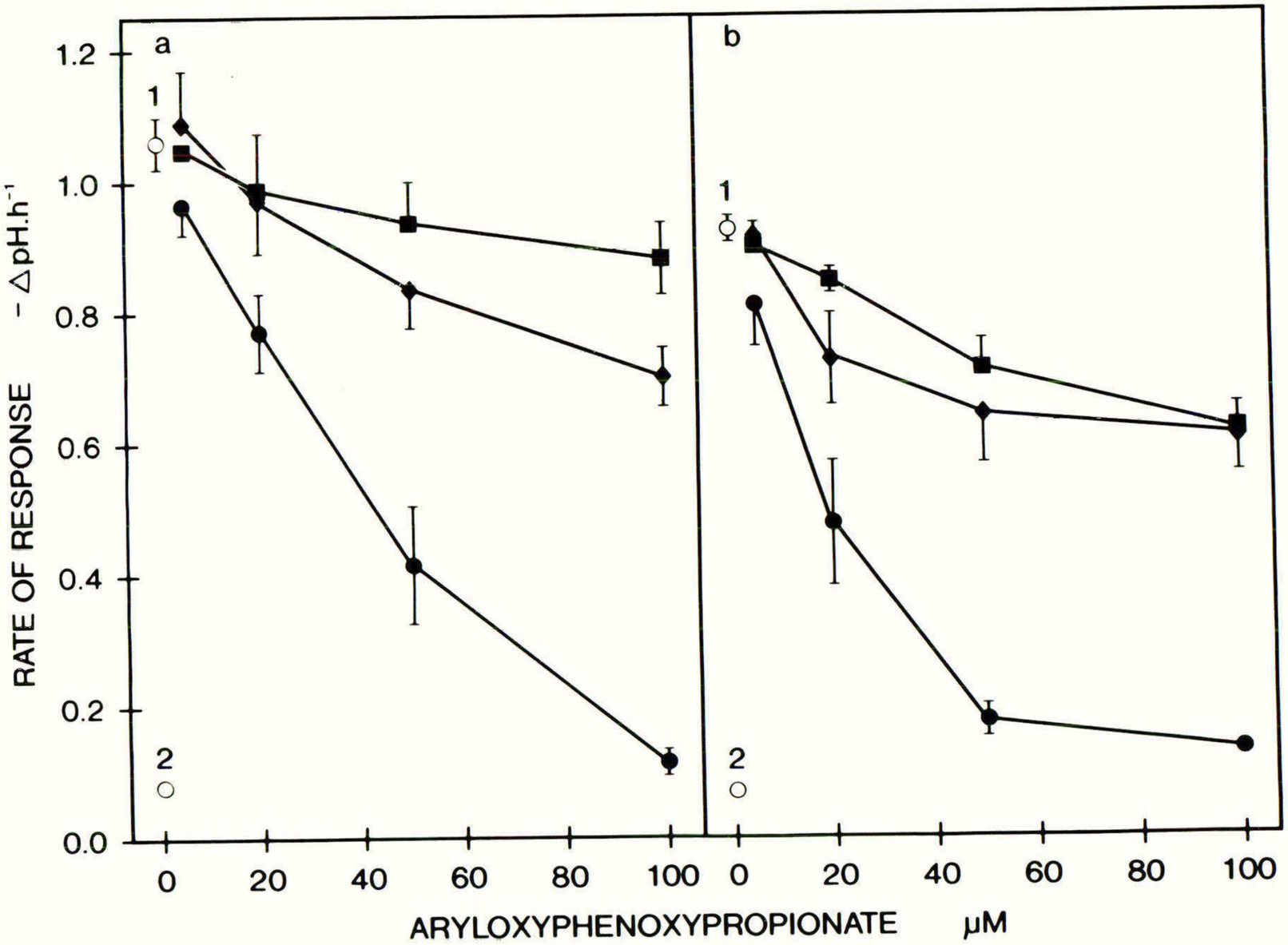

FIGURE 4: Effect of DM (●), FB (■) and QE (◆) concentration on H^+-efflux induced by 4a: 10 µM MCPA (○) 1, 4b: 1 mM clopyralid (○) 1. 02 represents H^+-efflux with 1.0% acetone (v/v) in the absence of MCPA or clopyralid. Data points are a mean of four H^+-efflux measurements and bars represent standard errors.

The addition of MCPA clearly reduced the effectiveness of DM in wild oat control (Fig 1) and Fig 2 extends this observation to include the mixture of QE with clopyralid, an interaction previously unreported in the literature, and another example of graminicide antagonism by auxin-type herbicides. Such a reduction in symptom severity implies that the synthetic auxin may in some way override or overcome the inhibition by the graminicide, which may then be regarded as possessing anti-auxin activity.

This anti-auxin activity is further demonstrated in Figures 3 and 4. In the highly sensitive, specific and rapid auxin assay of H^+-efflux, DM, QE and FB were shown to significantly inhibit auxin activity in a concentration-dependent manner. Clopyralid-induced H^+-efflux proved most sensitive to such inhibition, indicating that the weaker auxin could be most easily antagonised by the graminicides. In both cases, DM was a more active anti-auxin than QE and FB appeared the least potent of the three. More work is needed with other auxins and aryloxyphenoxypropionates to confirm this ranking and establish structure-activity relationships in this experimental system.

Given that the selective inhibition of acetyl CoA carboxylase appears to be the target site for graminicides (see preceding papers), the following questions are therefore important.

(1) Is there a link between the inhibition of both fatty acid biosynthesis and auxin-induced H^+-efflux?
(2) Can these effects be reconciled with the known action of DM at least as an ionophore?
(3) What is the relative contribution of each factor to grass death?

Certainly, the physiological link between auxin-induced H^+-efflux and cell extension is well established (Brummell and Hall, 1987), so that the reduction in internode elongation following graminicide application may relate directly to their anti-auxin activity, that may be overcome by adding auxins to the tank mixture.

Further work is clearly needed to establish the metabolic sequence of events following graminicide application, since the anti-auxin and general membrane activity of graminicides are now too widely documented to be ignored. Furthermore, if the metabolic basis of graminicide antagonism is unravelled then the potential of such tank mixtures may be realised in future.

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