SESSION 4A

CROP AND WEED RESISTANCE TO HERBICIDES

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BREEDING FOR HERBICIDE RESISTANCE - SEED COMPANY CONSIDERATIONS

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ABSTRACT

Introduction of herbicide resistance into cultivars by combining classical breeding methods with biotechnological methods opens up completely new possibilities to produced crops environmentally more acceptable and with a lower input. Sugar beet with resistance to glyphosate is used as an example. The considerations are divided into three items: 1. Effect of the trait itself (the gene) - how does it affect living organisms, spread in nature, patents, availability, influence on other traits. 2. The herbicide - what is the effect on environment, price, efficiency, reliability, the product grown, registration for the new crop, possibility of withdrawal of registration. 3. The cultivar - presence in environment, price, efficiency status, the effect on market and price, the plant herbicide safety.

INTRODUCTION

Many strategies are exploited by plant breeders to develop cultivars tolerant or resistant to herbicides. Beside natural herbicide resistance, based on fundamental aspects of metabolism, or different uptake or translocation, the breeders have for many years, deliberately or not, selected for acquired tolerance or resistance due to the selective pressure from continual exposure to a herbicide. Such resistance stems from natural selection of pre-existing resistant plants, or from mutations. Cell and tissue culture methods are widely used to select for acquired resistance. Both the classical breeding and tissue culture approach are mainly used, because they are simple and cheap, are normally not conflicting with patents and give no problems with release of genetically-engineered organisms.

The use of genetic-engineering to introduce acquired resistance is the most elegant approach, as it is relatively predictable and overcomes the normal sexual crossing-barriers, enabling the introduction of genes from all other living organisms, or in the future, to design a resistance gene based upon the exact structure of the target protein, and its precise interaction with the inhibitory herbicide.

This paper reflects the considerations made by a seed company when entering into collaborative research and development with an agrochemical company to develop herbicide resistance. In the actual case it describes the considerations made by Maribo Seed company in the collaboration with Monsanto company, to develop resistance in sugar beet to glyphosate. This collaboration is now so advanced that field testing of transgenic resistant lines and hybrids will start in the spring of 1990.

THE TRAIT (GENE)

Effect on animals and human beings

The gene giving rise to resistance is a mutant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene. EPSPS is involved in biosynthesis or certain aromatic amino-acids. These belong to the so-called essential amino-acids. The biosynthesis of essential amino-acids does not take place in animals and human beings, indicating that there should be no risk to eat or be in contact with plants having the mutated gene introduced.

Spread in nature

New genes in transgenic plants will always, like genes in normal plants, be crossed into cross-compatible crop plants or weeds, if such exist. With regard to sugar beet, they can cross to fodder beet, table-beet and the wild relatives of Beta vulgaris spp. The risk of crossing to normal arable weeds does not exist, but the risk of crossing to wild relatives is there. The wild relatives normally live outside arable land, and an introduced resistance to glyphosate will not confer any competitive advantage to the wild relatives, because glyphosate is not used in this habitat. On the contrary all extra genes normally have a certain cost to the plant.

Influence on other traits

The ideal situation for a new resistance gene is no or very low energy costs to the plant, no epistatic effects, and no associated undesirable characteristic. These aspects are difficult to estimate before all research is done. One can judge the regulatory sequences on the amount of resistance enzyme as % of total extractable protein to confer resistance; and, on model plants, observe whether growth, flowering and seed set is normal. These aspects look positive for the resistant EPSPS-gene.

Availability of the gene and the patents

The introduction of herbicide resistance by genetic-engineering is very costly and the seed company must be sure that the gene available is connected to the right regulatory sequence and that the agreement with an agrochemical company allows normal marketing, is not restricting research on other items, and includes a fair licence system. The patent situation must be very clear to prevent surprises in later stages of the development.

THE HERBICIDE

Toxicity

A herbicide must have a very low acute toxicity to be a candidate for introduced resistance in plants, because there is always the possibility that humans and animals may come in direct contact with the herbicide. Glyphosate has a LD50 of about 5000 mg/kg compared to caffeine 190 mg/kg or salt (NaCl) 3000 mg/kg.

Rate of application

The total application of a herbicide to control weeds should always be kept as low as possible. It is not enough only to apply low rates, it must also be an environmentally acceptable herbicide. For control of all weeds in sugar beets the expected amount of glyphosate to be used is 1200 g a.i./ha compared to about 5000-7000 g a.i./ha of the present used complex of herbicides.

Weed spectrum

To avoid use of additional products, the herbicide must be non-selective or very broad spectrum in activity. The effect should include broad-leaved and grass weeds as well as perennials such as Agropyron repens or Cirsium avense. Glyphosate is a good example of such a herbicide.

Mode of application

From an environmental point of view, it is preferred to use post-emergence applications. The reason is, that a preventative application (pre-em.) must take place without knowing the emergence pattern of the weeds. A pre-em. or very early post-em. application, which is normal in sugar beet, leads to a situation where all weeds are eliminated before emergence or very early post-em., and only the beet remain. This situation forces the different insects, animals or birds, which can be harmful, to feed on the beet, as there is no other choice. With glyphosate the application can wait until the weeds are starting to compete with the beet, thus allowing mammals, birds and insects, which live on arable land, to feed here until other feeding possibilities have developed.

Persistence

A certain persistence of a herbicide can be beneficial, as it reduces the number of applications necessary. For environmental reasons, future herbicides will have to have little persistence. The persistent herbicides disappear very slowly from the soil, thus having the ability to affect the environment over a long period. Persistent herbicides also result in an enhanced danger of development of resistant weeds. Finally persistent herbicides do not allow late germinating weeds to develop. These late germinating weeds can be an important source of food for many living organisms in nature.

Glyphosate, as a non-persistent herbicide, is decomposed microbiological in the soil in about two weeks, into water, carbon dioxide, nitrate and phosphate, all natural components of the soil. There is little risk of weed resistance if a normal rotation of crops and herbicides is used. Glyphosate will allow late emerging weeds to develop once the beet can compete, but these late emerging small weeds are important for feeding partridges and pheasants in the beet field.

Leaching

The risk of leaching of different pesticides to the ground water is becoming increasingly recognised. Especially in water catchment areas, there are strict regulations. Glyphosate with low persistence and good fixing to soil particles is one of the best products on the market to avoid leaching.

Price

The price for the grower to control the weeds means a lot for the success of a herbicide resistant variety. The present cost of weed control in sugar beet is estimated to about 70-200 UK pounds. The estimated cost of using glyphosate is about 45 UK pounds. This price will probably be reduced in the future, as the end of glyphosate's patent protection nears.

Registration

It is important to have a high probability that the herbicide in question will remain registered for quite a long period as it takes five to nine years to develop herbicide resistant varieties. Also the registration for use in the particular crop must have a high probability of success.

THE CULTIVAR

Status quo

Sugar beet are one of the most productive crops in temperate climates, but at the same time one of the poorest competitors to weeds. The poor competitiveness is a combination of rather slow early growth and an extremely low seed rate; only about 100,000 seeds per ha, drilled at a row width of 45-50 cm with about 18-23 cm between seeds. There is a critical period of about six to eight weeks when sugar beet is a poor competitor, and weeds have to be controlled.

Present herbicide programmes involve two to five spray applications with a combination of two to six herbicides, the first application, is applied when the first emerging weeds can hardly be seen. Some of the herbicides used are not especially environmentally acceptable, they are difficult to use, because the effect is very much influenced by temperature, soil and air humidity, amount of light, thickness of wax layer on leaves, and the developmental stage of the plants. Finally the price is high, as already mentioned. The present technique, if successful leaves the soil as a "beet desert" from very early spring until late autumn, thus leaving a relatively poor environment for insects, birds and mammals.

New status

If a non-selective herbicide, environmentally acceptable, with low persistence and low price can be used, such as glyphosate, the beet growers only need two to three spray applications, the first to be done when the weeds are starting to compete with the beets. These applications are little influenced by climate and development of the plants. The last sprays must be applied a little before the sugar beet meet in the row and can compete with weeds. It makes it easy for the farmer; more attractive for the insects, birds and mammals if weeds are present at the beginning of the season and new weeds will be present later in summer and autumn. These late weeds do not harm the sugar beet, due to competition from the crop they will only develop to small plants. The problem of weed beet can also be solved with this technique, if the right strategy is used. Finally the farmer has the possibility to save between 30 and 150 UK pounds per hectare in herbicide costs.

Safety margin

When a herbicide resistance is sold as a special character of a variety, it is extremely important to have a safety margin of at least three times normal dosage, due to overlapping, mistakes etc. If the safety margin is too low, the seed company will too often have complaints from customers, these complaints can be very costly and also have a bad long term effect on image.

Effect on market and seed price

The effect is very difficult to predict, because the market effect is influenced by the overall agronomical competitiveness of the resistant variety, competition from other seed companies and from new beet herbicides. The effect of seed price depends much on the same factors. It is assumed that a part of the benefit made by the beet grower must be shared with the seed company to cover development costs, risks etc.

CONCLUDING REMARKS

As discussed, the main considerations for a seed company are focused on environment, price, agronomy and marketing. In the example used, sugar beet resistant to glyphosate, most of the considerations are positive, thus providing the basis to use considerable amounts of time, money and capacity to develop herbicide resistant cultivars. As the technologies develop and as the understanding of effects on the environment increases, it is important to consider continuously the balance between genetic, chemical, and management based strategies in weed control.

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THE RESISTANCE OF WEEDS TO HERBICIDES: RATIONAL APPROACHES FOR CONTAINMENT OF A GROWING PROBLEM

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ABSTRACT

Since the mid 1960's, 94 weed species are known to have evolved strains that are resistant to herbicides. Recently herbicide cross-resistance has created further problems for growers in some localities. A predictive mathematical model of weed population dynamics is described that illustrates an approach to assessing the effects of herbicide inputs on the frequency of resistant biotypes in weed populations. The model provides a basis for analysing tactics to prevent or delay the evolution of herbicide resistance.

INTRODUCTION

Fifteen years ago weeds that had evolved resistance to herbicides were generally regarded as interesting phenomena that were appropriate subjects for academic research but of little consequence to the agricultural and horticultural industries. At that time several weed species had evolved resistance, in the U.S.A. and Europe, almost entirely to the triazine herbicides. Several species including *Chenopodium album*, *Amaranthus hybridus* and *Senecio vulgaris* had independently evolved resistant biotypes at several different geographic locations and these were becoming widespread (LeBaron & Gressel, 1982).

Agronomists and growers were relatively complacent about the appearance of triazine-resistant weeds since in the great majority of crops, satisfactory control was achieved by other alternative herbicides, albeit at a higher economic cost in most instances (Delaney & Moon, 1984; Putwain, 1982). However, during the 1970's and 1980's there was a relentless, progressive increase, not only in the number of weed species evolving resistance to herbicides (Fig. 1) but also in the increasing variety of herbicide chemistries to which resistance has evolved. Another recent problem is the appearance of cross-resistance in which selection by one class of herbicide confers resistance to other herbicides with similar or different biochemical modes of action (Heap & Knight, 1986; Moss, 1987; Putwain & Collin, 1989). With the advent of resistance to some of the newer classes of herbicides and significant cases of cross-resistance, there appears to be a general re-evaluation of the economic significance of evolved herbicide-resistance in weeds by agronomists and the agrochemical industry. Many organisations are engaged in devising management tactics to prevent or delay the evolution of resistant weeds but less attention has been given to the long-term management of resistant weed infestations. However one major company has published an advisory booklet for growers entitled "Questions and Answers - About Herbicide Resistant Weeds and The Best Way to Manage Them" (Anon, 1989).

The objectives of our paper are two-fold. First to analyse the scientific basis of tactics for preventing or delaying the evolution of herbicide resistant weeds. Second to present a methodology for predicting rates of change (increase or decrease) in the frequency of resistant phenotypes in response to different herbicide management regimes (=different selection pressures). In this paper we use the concept of 'herbicide-resistance' that was suggested by Putwain (1989). It is helpful to recognise 'evolved resistance' where there is evidence that a resistant strain of weed evolved as a direct response to selection by repeated application of a herbicide.

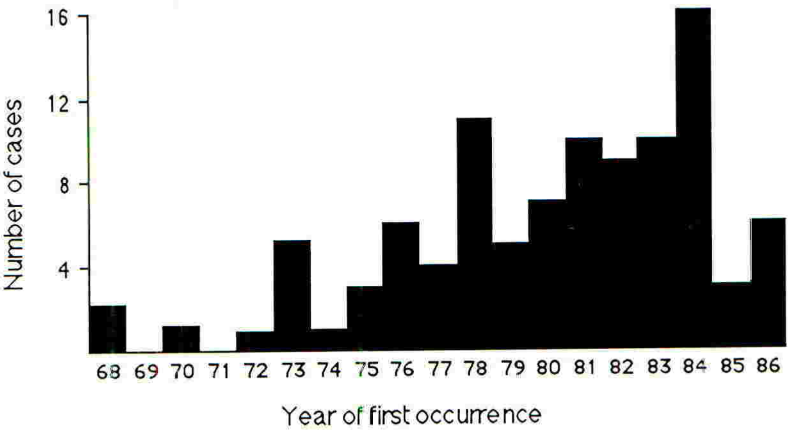

Fig. 1. The number of new cases of scientifically proven herbicide-resistant biotypes occurring each year since 1968. Each case is a species/herbicide/ combination recorded in a different country, not previously recorded.

THE GLOBAL OCCURRENCE OF HERBICIDE-RESISTANT WEEDS: THE EXTENT OF AN INCREASING PHENOMENON

Worldwide at least 55 species are known currently to have evolved strains that are resistant to triazine herbicides. Fifteen of these species are grasses. The majority of resistant populations have been found within the U.S.A., Canada and Europe, although triazine-resistant weeds also occur in Israel, Australia and New Zealand. Since the first occurrence of a triazine-resistant strain of weed (*S. vulgaris*) in the state of Washington, U.S.A. in 1968, there has been a multitude of occurrences of triazine-resistant weeds in widely separated locations within a few years. The majority of these cases must have involved independent selection of a mutant gene at a particular geographic location.

Resistance to herbicides other than triazines has been of more intermittent occurrence but nevertheless there has been a progressive increase during the 1980's in the number of classes of herbicide to which resistance has evolved, in the number of resistant strains of different weed species and in the number of geographic locations of resistant strains. Currently more than 35 species are known to have evolved strains resistant to non-triazine herbicides.

Resistance to the bipyridyl herbicides paraquat and/or diquat has evolved in 11 species despite the lack of soil residual phytotoxicity. In some cases there was repeated application of paraquat several times per year. Growers caused a high selection pressure and consequently resistant biotypes evolved, as in the case of *Poa annua* in a plant nursery and hops (U.K.) *Epilobium adenocaulon* in orchard experimental plots (U.K.) (Clay, 1987) and also in Belgium; *Conyza (Erigeron) bonariensis* in citrus plantations (Egypt); *C. philadelphicus* in mulberry plantations (Japan)(Watanabe *et al.*, 1982) and also *C. canadensis* and *C. sumatrensis* (Japan), (LeBaron pers. comm.,1989). In Australia repeated annual applications of paraquat and diquat in lucerne over a period of 24 years as the only method of weed control resulted in the evolution of resistant strains of *Hordeum glaucum* (Powles, 1986), *H. leporinum* (Powles pers. comm., 1989) and *Arctotheca calendula* (Powles *et al.*, 1989). Paraquat and diquat resistance will never become a widespread and serious problem because resistant strains evolve only in response to very high selection pressure imposed by growers through repeated application of the herbicides. Also in Australia, populations of *Lolium rigidum* resistant to diclofop-methyl were reported in 1982 (Heap & Knight, 1982). These resistant populations occur in many different geographic locations, presumably resulting from independent concurrent evolution. There is considerable cross-resistance with other herbicide classes (Heap & Knight, 1986).

In the U.K. there are now many populations of *Alopecurus myosuroides* that have evolved enhanced resistance to chlorotoluron, involving 20 different farms. The most resistant populations occurred at Peldon in Essex and Faringdon Oxfordshire, although other moderately resistant populations occur in Buckinghamshire, Cambridgeshire, Lincolnshire, Suffolk and Warwickshire (Moss, 1987; Moss & Cussans, 1987). The chlorotoluron-resistant biotype is also cross-resistant to several other classes of herbicide and there is also evidence over a few years of a gradual increase in relative resistance in some populations. Chlorotoluron-resistant biotypes of *A. myosuroides* may therefore become a more widespread and more difficult problem in the future if existing extensive usage of substituted ureas in winter cereals is maintained.

Apart from reports of enhanced resistance to 2,4-D in Canadian biotypes of *D. carota* and moderately enhanced resistance to MCPA in *Matricaria perforata* populations in the U.K., there was little evidence of evolution of resistance to phenoxy herbicides until quite recently. Lutman & Snow (1987) reported that populations of *Stellaria media* resistant to normal field rates of the phenoxy-alkanoic acid herbicide, mecoprop, occur in an area north of Bath UK.Now there is evidence of MCPA-resistant strains of *Ranunculus acris* and *Carduus nutans* in New Zealand (Bourdot *et al.* ,1989), that are insensitive to normal rates of application. Some resistant populations of *C. nutans* had received repeated annual application of 2,4-D for periods between 15 and 30 years.

A new and potentially serious problem of resistant weed biotypes was first reported in 1987 involving evolution of resistance to sulphonylurea herbicides and other acetolactate synthase (ALS) inhibitors such as imidazolinones. *Lactuca serriola* biotypes resistant to sulphonylurea herbicides occurred in winter wheat in Idaho after four years of recurrent herbicide application. In addition sulphonylurea resistant biotypes of *Kochia scoparia* and *Salsola iberica* have evolved in northern and south western U.S.A. A resistant biotype of *Stellaria media* evolved in Alberta, Canada and ALS- resistant *K.scoparia* occurs in Saskatchewan. Resistance to sulphonylurea and other ALS inhibitor herbicides is a potentially serious evolutionary occurrence in N. America that also may endanger weed control in cereals in Europe, Australia and South Africa.

Cases of evolution of resistance to other herbicides not previously mentioned, are currently only of minor significance. These include biotypes of *Eleusine indica* (Alabama, South Carolina, U.S.A.) and *Setaria viridis* (Manitoba, Canada) resistant to trifluralin and other dinitroaniline herbicides, *C. album* resistant to chloridazon (Hungary) and *P. annua* resistant to aminotriazole (Belgium). In broad terms, there is no doubt that if existing cropping practices and associated application of herbicides are continued unmodified, there will be a steady increase in the number of new cases of evolved resistance in many more plant species, in an increasing number of geographic locations. The economic significance of resistance to herbicides will surely increase in importance in the future.

NEW POTENTIAL PROBLEMS POSED BY CROSS-RESISTANCE

Cross-resistance (evolution of a weed biotype to one class of herbicide that confers resistance to herbicides with different biochemical modes of action) only rarely occurred in triazine-resistant weeds (e.g. Rubin *et al.*, 1985). However cross-resistance involving non-triazine herbicides may become a more serious problem in some situations.

The cropping of wheat in Australia is threatened by the evolution of many resistant strains of *Lolium rigidum* (more than 30 populations) and *Avena fatua* that are resistant to diclofop-methyl. The geographic distribution of resistant populations is widespread encompassing Western Australia, South Australia, Victoria and New South Wales. Resistant strains of *L. rigidum* exhibit cross-resistance to several other herbicides that differ in mode of action. These herbicides include alloxydim-sodium, chlorsulfuron, fluazifop-butyl, haloxyfop-methyl, and sethoxydim. Levels of resistance differed in populations between herbicides and could not be related to the histories of herbicide treatment (Heap, 1988). Different mechanisms of cross-resistance may be involved, although Heap (1988) suggested that P450 mixed function oxidases were involved in degradation and detoxification of diclofop-methyl in resistant biotypes of *L. rigidum*. Control of diclofop-methyl resistant *L. rigidum* will be difficult, since the options available for using alternative post-emergence herbicides are very limited. More emphasis on rotations and cultural methods of weed control will increase economic costs of production.

There is stronger evidence that P450 mixed function oxidases are responsible for detoxification and degradation of chlorotoluron in populations of *A. myosuroides* that are resistant to this herbicide (Kemp & Caseley, 1987). The P450 mixed function oxidase inhibitor l-aminobenzo-triazole (ABT) synergised chlorotoluron phytoxicity in a resistant population but did not do so in a susceptible one. Enhanced oxidase enzyme activity might also be involved in conferred cross-resistance to herbicides with different modes of action to chlorotoluron. These herbicides include chlorsulfuron, diclofop-methyl, imazamethabenz and pendimethalin (Moss & Cussans, 1987).

Herbicide cross-resistance in *A. myosuroides* is potentially serious since all herbicides that are effective in the control of *A. myosuroides* in winter cereals can be detoxified by P450 mixed function oxidases. In order to overcome this problem growers will have to introduce more non-cereal winter crops into rotations, increase use of mechanical cultivation including ploughing for deep burial of seeds of *A. myosuroides* and use non-selective herbicides to kill seedlings emerging in early autumn before sowing cereals. In the longer term other tactics may be feasible to overcome problems caused by cross-resistances. For example, Kemp & Caseley (1987) have suggested that application of species specific oxidase inhibitors that do not affect cereals. (ABT is not suitable since synergy occurs in herbicide treated wheat) may provide a means of overcoming the resistances imparted by enzyme degradation.

The strains of weeds that are resistant to the sulphonylurea herbicides demonstrate cross-resistance to other ALS inhibitor herbicides, in particular imidazolinones and triazolopyrimidines. Commercial research and development programmes for the production and marketing of transgenic crops that are resistant to imidazolinones or sulphylureas pose serious potential problems for weed control in the long-term. Cultivars of soyabean and maize that incorporate germplasm that imparts resistance to ALS inhibitor herbicides will probably be marketed in the 1990's. If resistant cultivars were used in the same rotation (which is highly probable), the selection pressure for evolution of resistant strains of weeds imposed by sulphonylurea-resistant soyabean, would endanger crops of imidazolinone-resistant maize and vice-versa. Competing chemical companies will have to develop joint strategies to reduce the probability of evolution of resistant weeds or risk substantial economic losses if resistant crop cultivars had to be withdrawn from the market place.

EVOLUTION OF HERBICIDE RESISTANCE : POPULATION DYNAMICS

In order to gain a complete understanding of the processes controlling the rate of evolution of herbicide-resistant strains of weeds, much fundamental scientific investigation is required. It will be necessary to measure the amount of natural genetic variability present in weed populations. The equilibrium frequency of resistance alleles depends on a balance between the mutation frequency and the rate of loss of mutant alleles from populations. Natural genetic variation for resistance may not be present in some weed populations and they will not respond to selection by herbicides. In addition to this, the mode of genetic inheritance of resistance and the selection pressure imposed by a herbicide(partly dependent on herbicide persistence) will be important in determining the rate of evolution of resistance. Finally it is necessary to have a detailed understanding of the dynamics of resistant and susceptible phenotypes (above ground plants and seeds) and their relative ecological fitnesses, in order to determine the potential for evolution of resistant strains in any particular cropping practice.

Predicting changes in the relative abundance of resistant and susceptible biotypes requires an understanding of the intensity of both density-dependent and independent forces regulating the population sizes of biotypes in mixture. It is the interaction of these two types of regulatory force that governs the finite rate of increase of individual biotypes and whether or not both will persist in mixture. For many weeds the phasic nature of crop production systems selects weed species whose populations have discrete generations. A suitable difference equation model describing changes over generations is

$$X_{n+1} = \lambda \mathbf{g} X_n (1 + \mathbf{a} \mathbf{g} X_n)^{-\mathbf{s}} + \mathbf{b} X_n \tag{1}$$

where X_n and X_{n+1} are the respective seed population sizes of each biotype at census points n and n+1 spanning a generation ; **g** is the fraction of the population of seeds that produce seed bearing plants and **b** the proportion of seeds persisting in the buried seed bank. λ is the seed yield of an isolated plant under uncrowded conditions ; **a** and **s** are parameters governing the response to increasing density. Equation 1 may be extended to include the competitive influence of a second biotype Y_n on the first as

$$X_{n+1} = \lambda \mathbf{g} X_n (1 + \mathbf{a} (\mathbf{g} X_n + \alpha \mathbf{g}' Y_n)^{-\mathbf{s}} + \mathbf{b} X_n \tag{2}$$

where α is a coefficient describing the competitive effect of an individual of Y in 'equivalent' terms of X and $\mathbf{g}'Y_n$ is the density of adult Y plants. A complementary equation to (2) exists for Y_n. This empirical deterministic model has been found to adequately describe rates of change in populations of annual weeds under a range of cropping practices and to qualitatively predict relative abundance (Sutton, 1988). It is applicable to mixtures of competing biotypes of herbicide resistant and susceptible weeds where resistance alleles do not segregate, as in the case of maternally inherited triazine resistance.

Table 1. Estimated mean parameter values for biotypes of *S. vulgaris* according to equation 2. Data taken from Watson (1987) and Hadfield (unpublished).

Parameter	Biotype	
	Susceptible	Resistant
g	0.350	0.280
b	0.507	0.429
a	0.022	0.002
s	0.744	0.775
λ	4610.240	1229.850
Equivalence coefficient	0.575	2.710
Coefficient of determination	0.97	0.93

Parameterisation of such models involves the measurement of per capita rates of increase of biotypes over a wide range of densities and frequencies, including estimation of the proportion of the population persisting as viable seeds in the buried seed bank. Parameter values for input into the model were obtained from field and pot experiments undertaken at Liverpool University. The field experiments, simazine management treatments and the seed bank dynamics of *S. vulgaris* were described by Watson *et al.* (1987). Analysis of above ground dynamics (Watson,

1987) and biotype competition (Hadfield, unpublished) provided estimates of the parameter values and the equivalence coefficients. Table 1 illustrates the values obtained for parameters for simazine- resistant and susceptible biotypes of *S. vulgaris* grown in the absence of simazine. These biotypes showed significant differences in all fitness components except the power term **s.** and the resistance has been shown to be maternally inherited (Scott and Putwain, 1983).

Whilst the fitness advantage of the susceptible biotype over the resistant is noticeable in the achene (seed) yield of isolated plants and reflected within the equivalence coefficients, the outcome of competition amongst biotypes over generations can only be assessed by simulation. Fig 2a) illustrates that in mixtures of biotypes, initially at equal frequency at low density (2 plants per m^2) with no simazine (control), the susceptible biotype rapidly (in 5 generations) approaches an equilibrium population size. On the other hand, the resistant biotype whilst initially increasing in abundance, (over three generations), ultimately declines at a decay rate governed by the decay rate of the buried achene population. Simazine application to these mixtures results in mortality of seedlings of the susceptible biotype. At recommended application rates this mortality is density- independent with a mean value of 98%. Under such management the resistant biotype rapidly becomes the main component of the mixture. The intensity of selection arising through simazine applications will clearly affect the competitive advantage that the susceptible biotype has over the resistant, in the absence of simazine. This intensity is determined by the level of mortality and the frequency of application. Fig 2b) - d) illustrates the consequences of relaxing the selection pressure. Application of simazine every other or every three years delays the elimination of the susceptible biotype but it is only with infrequent simazine application and lowered mortality that a stable equilibrium mixture is approached (Fig 2d). With even more infrequent application of simazine the susceptible biotype would become the majority component of the mixture.

Mathematical simulation using realistic parameters derived from field studies will give the best possible prediction of the outcome of herbicide management regimes. The present model is deficient in one important component. We have no accurate measure of field selection against the resistant biotype that would be imposed by the use of alternative non-photosystem II inhibiting herbicides, in the years when simazine was not used. Gressel & Segel (1989) describe this as negative-cross resistance and give evidence of fitness differentials (based on fresh weight) ranging from 0.27 to 0.46 against the resistant biotype. Clay (1987) showed that a triazine-resistant strain of *Epilobium ciliatum* was considerably more susceptible to oxyfluorfen and paraquat. The fitness differentials (based on shoot fresh weight) were 0.43 and 0.20 respectively. Putwain *et al.* (1983) demonstrated that soil disturbance (hand weeding) reduced the differential fitness (based on seed output per plant) of the triazine-resistant biotype of *S. vulgaris* to 0.63 . The more violent disturbance caused by mechanical cultivation would probably cause an even greater reduction in the fitness differential against the resistant biotype. Other factors that may select against the resistant biotype in the absence of herbicide are increased susceptibility to plant pathogens, viruses and herbivorous invertebrates (Gressel & Segel, 1989). Thus the number of no-simazine years in a rotation could be reduced whilst maintaining a trajectory moving to an equilibrium dominated by the susceptible biotype.

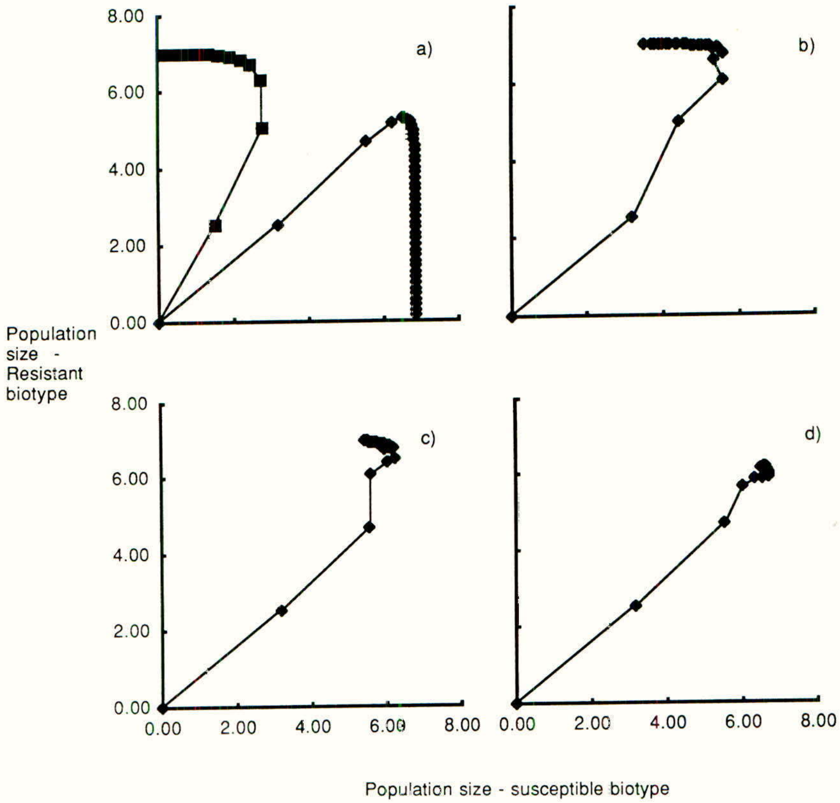

Fig. 2. Simulated trajectories of competing biotypes of *Senecio vulgaris* under differing management regimes a) simazine every season (squares); control (diamonds); b) simazine every alternate year 98% mortality; c) simazine every third year - 95% mortality; d) simazine every third year - 80% mortality. Population sizes are achenes (seeds) per square metre on log $_{10}$ scales. See text for details.

CONCLUSIONS

Until the population model has been further developed incorporating genetic components, and much more field data is available to provide realistic parameters for the model, we cannot make accurate predictions for non-triazine herbicides. Therefore it is possible to draw only some general conclusions concerning the tactics that may substantially reduce the rate of evolution of resistance or even prevent the field occurrence of herbicide-resistant strains of weeds. Rational use of herbicides in the management of weed populations to avoid the evolution of resistant strains or at least delay their appearance has been discussed by Putwain (1982) and in the context of recent events by Gressel & Segel (1989) and LeBaron (1989).

Key concepts that have emerged from studies of farming systems combined with basic biochemical, genetical and ecological data suggest that the following tactics should be utilised to delay the appearance of herbicide resistance, applied in a commonsense fashion; a) in a rotation, herbicides with different sites of action should always be used, b) whenever possible mechanical cultivations should be used to provide differential selection against resistant biotypes, c) where possible herbicides that induced negative cross-resistance should be included in a rotation where resistance to a particular herbicide is expected to evolve, or has already done so, d) farmers should act promptly to have weed populations tested for resistance, where it is a suspected cause of a failure in weed control, so that containment measures can be implemented. Longer-term strategies that should be seriously considered include a) retention of older herbicides for use in rotations to provide for containment of any strains of weeds that evolve resistance to the new herbicides; thus many older herbicides should not be de-registered, b) transgenic crop cultivars should not be used in the same rotation where they are resistant to herbicides with the same site of action; this stricture will apply in particular to cultivars resistant to ALS inhibitor herbicides. Since the evolution of herbicide-resistance is now an increasing problem, there is a pressing requirement for genuine cooperation between industrial companies, professional agronomists and advisors to growers, supported by scientific investigations at Research Institutes and Universities. Is this a naive expectation?

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RESISTANCE IN WEEDS TO SULFONYLUREA HERBICIDES

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ABSTRACT

Following the application of chlorsulfuron or chlorsulfuron/metsulfuron-methyl premix in a way that exhibited considerable residual activity to monoculture wheat fields in the United States for five consecutive years, resistant biotypes of Prickly Lettuce (Lactuca serriola), Kochia (Kochia scoparia) and Russian Thistle (Salsola iberica) have been isolated. Similar applications of chlorsulfuron have resulted in some instances of resistant Chickweed (Stellaria media) in Canada. Repeated applications of sulfometuron-methyl, a non-crop weed control agent, has also resulted in resistant biotypes of Kochia and Russian Thistle. These resistant biotypes are cross resistant to other ALS- or acetolactate synthase- (also know as acetohydroxyacid synthase) inhibiting herbicides including sulfonylureas and imidazolinones. The degree of resistance varies with the biotype and herbicide. The mechanism of resistance is due to the selection of weed biotypes with a modified form of the ALS enzyme which is less sensitive to the herbicide inhibition but still functional. The contributory factors include the continuous use of a single long residual product with no crop rotation and where no other effective herbicide treatment was used, either sequentially or in tank-mix, along with little or no tillage. Appropriate management strategies are discussed.

INTRODUCTION

The phenomenon of resistance in pest management is not new; it has occurred following the use of a variety of insecticides, fungicides and herbicides (Georghiou, 1986). Herbicide resistance is most widespread with the triazines although it has appeared following the use of a variety of other classes as well (Gressel, 1987). One approach to overcoming resistance is to continuously develop new types of pesticides that exhibit no cross-resistance; however, this tactic is proving to be too costly and difficult for the agricultural community to depend upon.

One of the newer types of herbicides is the class known as sulfonylureas. This highly active group of compounds is very effective at controlling a variety of weeds while imposing little added risk to man and his environment (Beyer et al., 1988). The mode of action of these compounds has been demonstrated to be the inhibition of acetolaclate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), an enzyme required for the synthesis of the branched chain amino acids, leucine, isoleucine and valine (Chaleff & Mauvais, 1984; Ray, 1984). This is also the target site for some other classes of herbicides such as the imidazolinones (Shaner et al., 1984). The degradation of sulfonylurea to non-phytotoxic products is an important mechanism of crop selectivity as is evidenced by the more rapid metabolism of chlorsulfuron by the tolerant crop, wheat, than in the more susceptible weeds that are controlled by the product (Sweetser et al., 1982). Plants can, however, demonstrate resistance to this type of compound by way of an insensitive site of action (Haughn et al., 1988).

Following the repeated use of chlorsulfuron or chlorsulfuron/-metsulfuron-methyl premix for five or more consecutive years in continuous wheat in a way that exhibited considerable residual activity with no other effective herbicide treatment, either sequentially or in tank-mix, and where little or no tillage was involved, some control failures were noted in the United States and Canada. These control failures were later determined to involve sulfonylurea-resistant weed biotypes. In the U.S., these included Prickly Lettuce, Lactuca serriola (Mallory et al., 1989), Kochia, Kochia scoparia (Primiani et al., 1989) and Russian thistle, Salsola iberica. Similar applications of chlorsulfuron resulted in biotypes of resistant chickweed, Stellaria media, appearing in a few fields in Canada. Applications of sulfometuron-methyl, used for non-crop weed control, also resulted in some resistant biotypes of Kochia and Russian Thistle. In order to prevent this phenomenon from decreasing the herbicidal utility of this type of compound, studies were conducted to determine the mechanism of resistance in order to enable the development, recommendation and implementation of appropriate management strategies.

METHODS AND MATERIALS

Kochia seeds and live plant material were collected from a variety of sites throughout the United States where control failure with chlorsufuron or sulfometuron-methyl had occurred, for comparison with susceptible plant material. The phytotoxic response of selected herbicides was measured by spraying Kochia plants at 3-5 cm height with a belt-type laboratory sprayer (Primiani et al., 1989). The plants were maintained in a greenhouse and visual observations of growth reduction were made at 21 days after treatment. From this, GR_{50} and/or GR_{90} values were calculated using a logit procedure (Bliss, 1935).

ALS enzyme assays were performed using ALS isolated from shoots of 20-25 cm kochia plants (Saari et al., 1989). The I_{50} values were the average of at least three determinations. Metabolism studies were conducted using ^{14}C chlorsulfuron and shoots from 6 week old Kochia plants (Saari et al., 1989). Uptake and translocation studies were conducted in growth chambers where ^{14}C chlorsulfuron was applied to kochia leaves followed by harvesting at 1, 3, 6,24 and 48 hours after treatment. The percent of applied radioactivity absorbed by the plant, and of that which was absorbed, the percent translocated out of the treated leaf, were determined (Saari et al., 1989).

RESULTS AND DISCUSSION

The greenhouse data on the comparative herbicidal effects of selected ALS inhibitors indicate that there is resistance at the whole plant level when comparing the GR_{90}'s for susceptible and resistant Kochia (Table 1). When comparing the rates of metabolism of chlorsulfuron by excised shoots of resistant and susceptible Kochia with that of metabolically tolerant wheat, it was apparent that degradation did not contribute to the observed resistance (Table 2). When comparing the rates of uptake and translocation (Table 3) of chlorsulfuron by susceptible and resistant Kochia, it was apparent that there also were no meaningful differences.

TABLE 1. Post-emergent rates (g/ha) required for 90% control of susceptible and resistant Kochia.

	Kochia Biotype	
Compound	Resistant	Susceptible
chlorsulfuron	>62	4
sulfometuron-methyl	>62	2
metsulfuron-methyl	46	2
triasulfuron	23	2
Imazapyr	>250	39

TABLE 2. Metabolism of ^{14}C chlorsulfuron in susceptible and resistant Kochia.

	Chlorsulfuron Remaining (%)		
Time (h)	Wheat	Susceptible Kochia	Resistant Kochia
0	65	98	99
4	19	97	96
21	1	99	94

TABLE 3. Uptake and translocation of ^{14}C chlorsulfuron by susceptible and resistant Kochia.

	Uptake (%)		Translocation (%)	
Time (h)	Sus.	Res.	Sus.	Res.
1	30	36	3	2
3	43	30	7	8
6	40	34	15	11
24	46	70	40	32
48	65	71	29	43

The data comparing the ALS enzyme inhibition between a susceptible and a resistant biotype of Kochia showed a range of differences for four sulfonylureas: chlorsulfuron, sulfometuron-methyl, triasulfuron, and metsulfuron-methyl and an imidazolinone, imazapyr (Table 4). These differences were not consistent among biotypes with different biotypes showing varying ratios of insensitivity to these herbicides (Table 5). Looking at a comparison of herbicidal effects and enzyme inhibition for one biotype, there was also a clear relationship between whole plant response and relative enzyme inhibition (Table 4). Here it was apparent that where the ratio of GR_{50}'s is high, so is the ratio of I_{50}'s.

TABLE 4. *In vitro* ALS enzyme inhibition and treated whole plant responses for susceptible and resistant Kochia.

Compound	I_{50} (nM) Sus.	Res.	Ratio R/S	GR_{50} Ratio R/S
chlorsulfuron	22	400	18	25
metsulfuron-methyl	26	130	5	7
sulfometuron-methyl	10	280	28	10
triasulfuron	40	460	12	---
Imazapyr	6000	38000	6	6

TABLE 5. Variation in ALS insensitivity with different resistant Kochia biotypes.

Biotype	I_{50} Ratio, R/S Chlorsulfuron	Metsulfuron-methyl	Triasulfuron
#1	118	37	28
#2	74	10	18
#3	21	10	25
#4	89	42	54

From these data,it was concluded that the mechanism of resistance for the Kochia biotype tested was the selection of a biotype with a modified form of the ALS enzyme which was less sensitive to the herbicide inhibition but was functional in producing precursors for select branched chain amino acid biosynthesis. It was also apparent from these data that cross-resistance to the imidazolinone, imazapyr, was present, although the resistance in this case was not as great as for chlorsulfuron or sulfometuron-methyl but about the same as for metsulfuron-methyl.

In order to better understand the input of the various contributory factors to the observed resistance, a model was utilized (Gressel & Segel, 1982):

$$N_n = N_o\ (1 + f\alpha/b)^n$$

Where N_o, N_n = proportion of resistant weeds after 0 and n years, respectively.

f = overall fitness of resistant biotype relative to susceptible biotype

α = selection pressure

b = average lifetime in soil seed bank

n = number of years of continuous use

The sulfonylurea resistant phenotype, with resistance due to a sulfonylurea insensitive ALS, is dominantly or semi-dominantly inherited in tobacco (Chaleff & Ray, 1984). Assuming this type of inheritance has occurred in kochia, then the initial frequency of the resistant ALS gene was assumed to be 1×10^{-6}. The effective kill rate is high for the field application of chlorsulfuron on susceptible Kochia, thus a kill rate of 95% (α=20) was used. The fitness of the resistant phenotype is also quite high, approaching 1, hence the value 0.9. Finally, a large percentage Kochia seeds germinate the season after they mature, such that b = 1.5 years. According to the model and assuming that an N_n value of 10-30% would be readily apparent to the grower as a visible threshold, resistance would be achieved between four and five years of continuous treatment. This correlated well to in the field situation for many of the observed resistance cases to date. However, the majority of fields which have been treated under this scheme have not developed resistance.

With regard to resistance management strategies, some of the contributing factors cannot be changed. The initial gene frequency, the fitness and the lifetime of the soil seed bank are beyond our control. We can, however, impact the selection pressure. Since sulfonylureas have a relatively flat dose/response relationship and since growers expect good weed control when they use an herbicide, small adjustments in dose to lower the effective kill rate are impractical. We can, however, lower the effective kill rate by using shorter residual products where they have soil activity or by shifting to post-emergent products. The effectiveness of this practice will vary with the biology of the weed, depending upon the time frame over which the weed seeds germinate.

Another critical factor in this equation is the number of years of continuous use. Programs that break the continuous use pattern would be of considerable utility. One effective means of doing this is to rotate crops where a different type of weed control agent is used for the intervening crop. Another would be to use sequential herbicides with different modes of action. Tank-mixing herbicides with different modes of action is also highly effective, assuming that an appropriate partner and an effective rate are used.

In addition, using an herbicide less frequently would delay the onset of resistance. One would not anticipate, however, getting any more total applications of the herbicide by using this method alone. Proper cultural techniques, including tillage, are also critical for breaking the continuous selection cycle. Finally, if resistance does occur, it is also important to act quickly to prevent the spread of the resistant weed seeds.

To date, resistance to the sulfonylureas has appeared only where long residual products were used at relatively high dosage rates continuously for several years with no crop rotation. In addition, no effective tank mix/sequential/alternating herbicides were employed and little or no tillage was involved. This does not mean that in situations not meeting all of these critera, we can assume that resistance will never occur. Sooner or later, resistance can occur wherever a population is subjected to selection pressure. We must also avoid panic in concluding that the value of ALS inhibiting herbicides as useful weed control agents must necessarily be diminished. It is essential, therefore, that we keep all this in proper perspective and utilize the proper management techniques at our disposal as discussed in this paper such that we can be confident that the utility of these herbicides will be preserved.

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THE DISTRIBUTION AND CONTROL OF HERBICIDE RESISTANT *ALOPECURUS MYOSUROIDES* (BLACK-GRASS) IN CENTRAL AND EASTERN ENGLAND

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ABSTRACT

One hundred and forty seven seed samples of *Alopecurus myosuroides* (black-grass) were tested for resistance to chlorotoluron. Resistant populations were identified for the first time on ten more farms, bringing the total identified since 1982 to 33 fields on 20 farms. Field trials were conducted on two farms at Peldon, Essex, aimed at controlling herbicide resistant populations. In the cultivation trial ploughing, used in conjunction with an intensive herbicide regime, reduced *A. myosuroides* populations substantially compared with minimum tillage. In the herbicide evaluation trials, none of the 14 herbicides tested gave better than 75% control of *A. myosuroides*. Herbicides giving the best control included carbetamide, ethofumesate, fluazifop-P-butyl, propyzamide, tri-allate and trifluralin. In the sequences trials, applications of three or four herbicides were insufficient to achieve satisfactory control. Integrated strategies are suggested for the control of resistant *A. myosuroides*.

INTRODUCTION

Alopecurus myosuroides (black-grass) showing partial resistance to chlorotoluron was first detected in the UK at Faringdon, Oxfordshire, in 1982. More pronounced resistance was found at Peldon, Essex, in 1984 (Moss & Cussans, 1985). By 1987, resistant, or partially resistant, *A. myosuroides* had been found on a total of ten farms in England (Moss & Cussans, 1987; Moss, 1987; Moss & Orson, 1988; Orson & Livingston, 1987).

Field experimentation aimed at controlling resistant populations at Peldon commenced in autumn 1985 (Orson & Livingston, 1987). During the first two years of these experiments, ploughing improved the control of *A. myosuroides*. This was due partly to the burial of weed seeds, which resulted in lower plant populations, and partly to the burial of the adsorptive surface layer, containing burnt straw residues, which had been shown to be partially responsible for the poor activity of soil acting herbicides at Peldon (Moss, 1987). However, the activity of herbicides tested in the field at Peldon was generally inadequate.

In outdoor container experiments, resistance to chlorotoluron has been associated with cross-resistance to many other herbicides with differing modes of action (Moss, 1987). Some herbicides, however, such as ethofumesate, propyzamide and trifluralin, have been as effective on the Peldon population as on a standard susceptible stock.

This paper describes further studies into the distribution of resistant A. myosuroides and field experiments aimed at determining how best to control resistant populations.

TESTING SEED SAMPLES FOR RESISTANCE TO CHLOROTOLURON

Materials and methods

Seed samples were collected in July 1988 from winter cereal fields and tested either at ADAS, Cambridge, or at Long Ashton Research Station. The test procedure at both sites was that described by Moss and Orson (1988) in which seeds were sown in pots of soil and plants sprayed at the 2-leaf stage with 2.0-2.5 kg/ha (ADAS tests) or 3.5 kg/ha (LARS test) of chlorotoluron. This chemical was used as a representative of the substituted-urea group of herbicides which includes isoproturon. Three standard reference stocks were included in all tests: Rothamsted (susceptible); Faringdon (partially resistant); Peldon Al (resistant). There were five replicates. The % reduction in foliage fresh weights was calculated by relating weights in treated and untreated pots for each stock.

ADAS samples

A total of 105 samples was tested. Seeds were collected from 20 fields, selected at random, surveyed as part of the ADAS Winter Wheat Disease Survey of England and Wales. A further 59 samples were collected as part of a Home-Grown Cereals Authority (H-GCA) funded random survey of fields within 50 miles of Peldon, Essex. In addition, 26 other samples were tested. These were collected either from ADAS trial sites or from fields where clients had requested a test for resistance.

Long Ashton samples

A total of 42 samples was tested. Fifteen of these were from fields at Peldon (7), South Essex A (5), Tiptree A (1) and Oxford A (2); areas where resistance had previously been detected (Moss & Orson, 1988). These were included to confirm resistance in these fields. Twenty-seven other samples were tested.

Results and discussion

The following arbitrary classification has been adopted to identify different degrees of resistance, based on a comparison with the % reduction values of the three standard stocks:

```
Rothamsted              Faringdon                               Peldon
   value                  value                                  value
s   |   s     |      *       |     **     |  ***   |   ****   |    *****
susceptible    need further     partially  ----- increasingly resistant --->
    |         |evaluation    |  resistant|         |          |
```

The mean % reductions in fresh weight for the three standard stocks were: Rothamsted 93%; Faringdon 78%; Peldon 33%. The mid point value between Rothamsted and Faringdon was used to separate those stocks deemed to be susceptible and those requiring further evaluation to determine whether they exhibit a low level of resistance. Similarly, the stocks in the Faringdon to Peldon range were separated into three categories. Populations were only termed 'resistant' (** or more) if they showed greater resistance than the Faringdon population, which has shown partial resistance to chlorotoluron, not only in many pot experiments, but also in simulated field conditions (Moss, 1987).

Results were analysed statistically but it was not thought appropriate to classify resistance groupings using statistical parameters. In a series of notionally identical tests, the standard errors will be influenced by variability, the level of herbicide activity, and the proportion of resistant to susceptible stocks included. Therefore the assignation of resistance groupings could change between tests. In addition, because resistance is of a quantitative nature, any dividing line placed across the continuum of varying response must be arbitrary.

TABLE 1. Results of 1988 seed collections by county (105 ADAS and 27 LARS samples collected for the first time in 1988).

County	Number of samples	S	*	**	***	****
				------ Resistant ------		
Bedfordshire	5	3	2	0	0	0
Buckinghamshire	3	2	0	0	0	1
Cambridgeshire	30	21	8	1	0	0
Essex	33	30	1	1	1	0
Hertfordshire	5	5	0	0	0	0
Lincolnshire	6	4	1	1	0	0
Norfolk	5	5	0	0	0	0
Oxfordshire	8	7	0	0	1	0
Suffolk	27	21	3	2	0	1
Warwickshire	1	0	0	1	0	0
Other	9	9	0	0	0	0
Total	132	107	15	6	2	2

Ten of the 132 populations, collected for the first time in 1988, showed resistance to chlorotoluron (** or more). These populations came from a wide geographical area (Table 1). Two of the populations, one from Buckinghamshire and one from Suffolk, showed pronounced resistance (****).

The results from the 79 ADAS random survey samples showed that 68 were in the S category (86%), 7 in * (9%), 3 in ** (4%) and 1 in **** (1%).

Resistance was confirmed in all the fields identified as containing resistant A. myosuroides in previous tests at LARS. The rating for these fields was: Peldon (****-*****); South Essex A (**-****); Oxford A (**-****); Tiptree A (**).

The "others" category in Table 1 comprised the counties of Berkshire, Gloucestershire, Hampshire, Northamptonshire, Wiltshire and Worcestershire. Only one or two samples were collected from each of these counties and all were in the susceptible (S) category. Samples, classed as *, showed small reductions in fresh weight which were usually significantly lower ($P \leqslant 0.05$) than the susceptible standard stock (Rothamsted). These marginal levels of resistance might result in reduced field performance, especially under adverse conditions for herbicide activity, and need further evaluation.

Since 1982, a total of 296 samples of A. myosuroides have been tested for resistance to chlorotoluron. Resistance has been detected in 33 fields on 20 farms in England. These farms are located in seven counties: Buckinghamshire (1 farm), Cambridgeshire (2), Essex (9), Lincolnshire (1), Oxfordshire (3), Suffolk (3), Warwickshire (1). The most severe cases of resistance occur at Peldon, Essex, but other populations, in Suffolk and Buckinghamshire, with a comparable degree of resistance were detected for the first time in 1988. We conclude that the occurrence of severe resistance is low, but the incidence of more marginal resistance is disturbing in view of the potential for further evolution.

FIELD TRIALS

Materials and methods

Since autumn 1985 three types of field experiment in winter wheat have been carried out by ADAS on two farms at Peldon. All experiments comprised randomised block designs with three replicates. Where applicable, herbicide treatments were applied with a modified van der Weij pressurised knapsack sprayer in 200 l water/ha using F80-02 nozzles at 200 kPa. The full treatment list of each trial is included in the results tables (2-4). Where shown growth stages refer to the size of weed.

Cultivation trial

The first two years results of the cultivation trial have been reported previously (Orson & Livingston, 1987). In autumn 1987 and 1988 the range of herbicide treatments were replaced by applying herbicide according to the normal farm practice to the entire 24 m x 18 m cultivation plots. This included sequences of at least three herbicides active against A. myosuroides (similar to the most intensive programme in the sequences trial).

Individual herbicide evaluation trials

Two herbicide evaluation trials (plot size 6 m x 3 m) were conducted to evaluate, in the field, single applications of herbicides which have shown promising results in experiments at LARS. Some of these treatments are not selective in wheat. The 1987/8 trial was conducted in the absence of any crop.

Sequences trials

The most useful herbicides selective in cereals from the individual herbicide evaluation trial were also included in two herbicide sequences trials (plot size 6 m x 3 m) at one pre-em. and two post-em. timings.

Results

Cultivation trial

The cultivation trial continued to show the benefit of ploughing (Table 2). Although in the second year the density of A. myosuroides after two years ploughing was similar to that after ploughing followed by minimum cultivation, the continued ploughing in the third and fourth years resulted in significant ($P \leqslant 0.05$) reductions in A. myosuroides head densities. It is suggested that the unexpectedly high figure on the annually ploughed treatment in 1987 was due to the return to the surface of seed buried by the first ploughing. In 1986 and 1987 herbicide performance was shown to be improved by ploughing (Orson & Livingston, 1987). This is also likely to have contributed to the benefit shown by ploughing in 1988 and 1989.

TABLE 2. Cultivation trial, Twitch Field, Peldon Hall.

Autumn treatments				A. myosuroides heads/m^2 (July)			
1985	1986	1987	1988	1986	1987	1988	1989
Plough 25 cm (P)	P	P	P	107	1280	44	10
Plough 25 cm	mc	mc	mc	107	1394	883	114
Mincult 5 cm (mc)	mc	mc	mc	915	3667	2162	378
	S.E.D. (6 d.f.) +/-			147.3	549.1	125.0	59.1

Note: 1986 and 1987 counts refer to untreated plots, 1988 and 1989 refer to treatment as farm practice.

Individual herbicide evaluation trials

The maximum control achieved in the herbicide evaluation trials (Table 3) was 75%. Trifluralin, ethofumesate, propyzamide, fluazifop-P-butyl, carbetamide and tri-allate gave the best levels of control. For the 1987/8 results, when there was no crop and for products which are non-selective in cereals (such as carbetamide, fluazifop-P-butyl, ethofumesate and propyzamide) level of control is hindered in these trials by lack of crop competition. For these treatments especially, % reduction of plant numbers is more likely to reflect their capabilities.

Sequences trials

In the herbicide sequences trials higher levels of control were achieved in 1988/9 than the previous year (Table 4). However despite the equivalent of using four chemical treatments the highest level of control was only 86% (pre-em. tri-allate followed by early post-em. isoproturon/trifluralin followed by isoproturon). Both years trials demonstrate the benefit of a pre-em. treatment. The 1987/8 trial demonstrated a benefit from applying tralkoxydim rather than isoproturon as the late post-em. spray, but this was not repeated in 1988/9.

TABLE 3. Individual herbicide evaluation trials.

	1987/88 Church Field Brickhouse Farm, Peldon		1988/89 Lower 16 Acre Peldon Hall	
Cultivation method	Ploughed		Minimum cultivated	
Kd (chlorotoluron)	5.3		5.3	
	% Reduction *A. myosuroides*		% Reduction *A. myosuroides*	
Herbicide (rate/ha)	Plants	Heads	Plants	Heads
Pre-emergence	20/10/87		30/9/88	
trifluralin (1.1 kg)	48	62	53	33
chlorsulfuron (15 g) + metsulfuron-methyl (5 g)	35	55	-	-
tri-allate (2.25 kg) (c)	42	39	68	33
pendimethalin (2.0 kg)	50	38	29	50
Early post-em. (GS 12/13)	14/12/87		24/10/88	
isoproturon (2.5 kg)	26	38	34	31
chlorotoluron (3.5 kg)	5	27	31	43
diclofop-methyl (1.14 kg)	19	35	25	29
tralkoxydim (200 g) (a)	1	31	32	38
SMY 1500 (1.6 kg) (b)	34	45	33	35
isoproturon (1.95 kg) + trifluralin (1.3 kg)	-	-	31	29
carbetamide (2.1 kg)	57	62	51	30
fluazifop-P-butyl (187 g) (a)	75	48	57	31
ethofumesate (2.0 kg)	70	62	55	22
propyzamide (700 kg)	69	53	65	24
barban (312 g)	46	57	-	-
Late post-em. (GS 25-29)	18/4/88			
fluazifop-P-butyl (187 g) (a)	34	53	-	-
tralkoxydim (350 g) (a)	7	23	-	-
S.E.D. +/-	19	18	13	13
d.f.	32	32	36	36
(Untreated population/m^2)	(20.3)	(362)	(958)	(1247)

(a) tank-mixed with 0.1% non-ionic wetter ('Agral')
(b) 4-amino-6-tert-butyl-3-ethylio-1,2,4-triazin-5(4H)-one in formulation UK 220.
(c) granular formulation

TABLE 4. Sequences trials.

1987/8 Twitch Field, Peldon Hall (minimum cultivated
Kd (chlorotoluron) 8.7)

Early post-em. 9/3/88 (GS 11/13)	Late post-em. 18/4/88 (GS 23/25)	Pre-em. applied 10/11/87 Nil	chlorsulf./m-methyl
		% A. myosuroides control (heads)	
IPU	IPU	21	25
IPU	IPU/tralkoxydim	19	12
IPU	tralkoxydim	35	45
IPU/tralkoxydim	IPU	0	18
IPU/tralkoxydim	IPU/tralkoxydim	0	26
IPU/tralkoxydim	tralkoxydim	20	39
tralkoxydim	IPU	0	24
tralkoxydim	IPU/tralkoxydim	20	19
tralkoxydim	tralkoxydim	21	31

S.E.D. +/- 12.5 (60 d.f.)
(Untreated population 1773 heads/m^2)

1988/9 Melondowns Field, Brickhouse Farm, Peldon (minimum cultivated
Kd (chlorotoluron) 11.4)

Early post-em. 2/11/88 (GS 11/13)	Late post-em. 24/1/89 (GS 22)	Pre-em. applied 14/10/88 Nil	trifluralin	tri-allate
		% A. myosuroides control (heads)		
IPU	IPU	56	60	79
IPU	tralkoxydim	51	69	69
IPU/trifluralin	IPU	55	77	86
IPU/trifluralin	tralkoxydim	73	72	81
IPU/tralkoxydim	IPU	64	78	74
IPU/tralkoxydim	tralkoxydim	47	78	77

S.E.D. +/- 13.4 (42 d.f.)
(Untreated population 2206 heads/m^2)

Abbreviations and rates of active ingredient/ha

IPU = isoproturon (2.5 kg except 1989 when 2.1 kg late post-em); tralkoxydim (200 g); chlorsulf./m-methyl = chlorsulfuron (15 g)/metsulfuron-methyl (5 g), trifluralin (1.1 kg); tri-allate granules (2.25 kg); Nil = no pre-em. herbicide applied.

Discussion

These field trials confirm the poor control achieved by herbicides alone. In any one season as the number or frequency of active ingredients applied increased, the level of control improved slightly. However, even sequences of four herbicides, costing at least £85/ha, were insufficient to achieve acceptable levels of control. More consistent was the effect of ploughing which gave more than ten fold reductions in A. myosuroides head number when compared with minimum cultivation. Some control can be achieved by the use of products which are not selective in cereals but this would mean a change of cropping. The most sensible strategy to control resistant A. myosuroides is likely to be a combination of ploughing and the inclusion in the arable rotation of crops on which fluazifop-P-butyl, propyzamide, ethofumesate or carbetamide can be used. Delayed drilling and growing spring or other crops which discourage A. myosuroides must also be considered.

The results from the tests on seed samples indicate that resistant A. myosuroides is more widespread than previously reported. With the increasing incidence of resistance, strategies are required to contain existing resistant stocks. The field trials indicate however, that despite the use of an integrated system, control of resistant A. myosuroides will be unreliable and expensive in winter cereal dominated rotations. Strategies need to be developed to prevent resistance evolving elsewhere.

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THE APPEARANCE OF PHENOXY-HERBICIDE RESISTANCE IN NEW ZEALAND PASTURE WEEDS

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ABSTRACT

Investigations into the appearance in New Zealand of MCPA-resistant *Ranunculus acris* and *Carduus nutans* in dairy and sheep pastures respectively are reviewed. Extreme differences in sensitivity between susceptible and resistant populations of *R. acris* and *C. nutans* of 4.9 and 6.7-fold respectively, render normal rates of MCPA ineffective for the control of the "resistant" populations. Populations with intermediate levels of sensitivity to MCPA occur in both species. Resistance in both species was correlated with historical exposure to phenoxys, supporting the hypothesis that it is the result of herbicide selection pressure. In *C. nutans*, cross resistance occurs between 2,4-D, MCPA and MCPB.

INTRODUCTION

The purpose of this paper is to review New Zealand research into MCPA-resistance in *Ranunculus acris* (giant buttercup) and *Carduus nutans* (nodding thistle). *R. acris* is a perennial weed of dairy pastures receiving high annual rainfall. It occurs widely in the North Island (NI) on land of high fertility (Tuckett, 1961) and is a problem in the South Island (SI) at Takaka and Karamea. *C. nutans* is a biennial occurring mainly in sheep pastures under low rainfall and is most common in summer-droughted high-fertility areas in Hawkes Bay (NI) and Canterbury (SI).

INITIAL EXPERIMENTS IN THE FIELD AND GLASSHOUSE

Field trials in the 1950s in Taranaki showed that MCPA and MCPB gave "very satisfactory" control of *R. acris* (Tuckett, 1961), but in the late 1970s there were reports of poor control from these herbicides. Genetically based resistance was not suspected and trials were conducted in the NI to improve on the previous recommendation (Popay *et al.,* 1984; Popay *et al.,* 1989). This work confirmed late winter applications of MCPA were effective but also revealed the level of control to be highly variable between populations 10 km to 160 km apart. Field trials with MCPA at Takaka from 1984 until 1988, detected differences in control between sites within a radius of 1 km (Bourdot, unpub.). The extremes of this variation occurred on the Jones and Silcock farms. The reduction in ground cover of the Jones population by MCPA at the usual field rate of 1.0 kg/ha, was 93%, but only 11% for the Silcock population. Both populations were treated and assessed under identical conditions. Both sites supported permanent pastures rotationally grazed by dairy cattle and there appeared to be no managerial or environmental differences between them that could explain such a large difference in the efficacy of MCPA. However to eliminate possible environmental effects, a comparison of seedling progeny of the Jones and Silcock populations, was made under uniform conditions in a glasshouse at Lincoln (Bourdot & Hurrell, 1988). For both populations, 128 plants with 6 to 7 expanded leaves were sprayed with a logarithmic series of doses of MCPA from 0.198 to 3.375 kg/ha. Probit curves fitted to the mortality data (Fig.1a) showed the LD_{50s} of the Silcock and Jones populations to be respectively 1.51 and 0.31 kg MCPA/ha. The Silcock population was 4.9 times more tolerant to MCPA than Jones, confirming the existence of a genetic basis for the observed field differences in response to MCPA.

In the late 1970s almost simultaneously with the *R. acris* reports, a sheep farmer from Argyll in Hawkes Bay, reported that 2,4-D no longer gave adequate control of *C. nutans*. In 1981 the susceptibility of *C. nutans* at Argyll was compared with that of another population on a farm at Matapiro, some 30 km further north where no control problems had been experienced (Harrington & Popay, 1987). Nearly 200 plants were sprayed at the two sites with 20 mg/plant of 2,4-D or MCPA. The mortalities of the Argyll and Matapiro populations with 2,4-D were respectively 19% and 90%, and 49% and 96% with MCPA. Seedling progeny from Argyll and Matapiro were subsequently compared in a glasshouse at

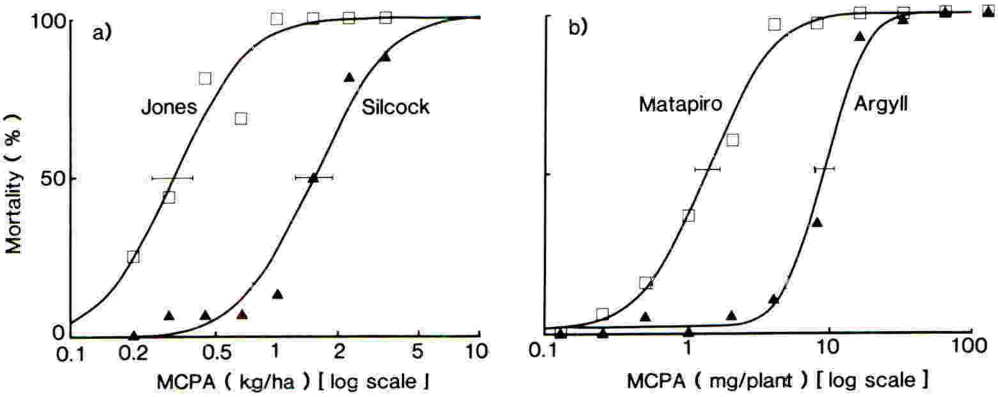

Fig. 1. Dose response curves for MCPA of susceptible and resistant populations of (a) *Ranunculus acris* (From Bourdot & Hurrell, 1988) and (b) *Carduus nutans* (From Harrington *et al.*, 1988). The 95% confidence intervals for the LD_{50}s are given as horizontal bars. The slopes of the probit curves were different (P=.05) for the two populations of *C. nutans,* but there was no evidence of this for *R. acris* for which the curves were constrained to be parallel.

Massey University. This confirmed an inherent difference in susceptibility and suggested the Argyll population could be as much as 30-times more tolerant of MCPA than the Matapiro population (Harrington & Popay, 1987). This first experiment used six rates of MCPA and 150 plants of each population. Comparisons were repeated another two times, and differences between the populations were found each time. In the second experiment only four rates of MCPA and 40 plants of each population were used and the results suggested the Argyll population was five times more tolerant than the Matapiro population (Harrington & Popay, 1987). The third glasshouse comparison used 12 dose rates of MCPA and a total of 260 plants, and this showed a 14-fold difference between the populations (Harrington *et al.,* 1988).

Glasshouse-grown *C. nutans* were stunted compared with field-grown plants, so to avoid the possibility that this may have influenced the results, the comparison of these two populations was repeated in the field. Several months after transplanting 354 Matapiro and 437 Argyll seedlings into a pasture at Massey University, a logarithmic series of doses of MCPA from 0.12 to 128 mg/plant was applied (Harrington *et al.,* 1988). Probit analyses showed 2% "natural" mortality occurred, and while the LD_{50} for the Argyll population was 9.08 mg/plant, the LD_{50} for the Matapiro plants was only 1.35 mg/plant (Fig.1b). Thus the Argyll population was 6.7-times more tolerant at the 50% level of mortality.

INTERMEDIATE LEVELS OF RESISTANCE AND CORRELATIONS WITH SPRAYING HISTORY

Once the existence of genetically-based resistance to MCPA had been confirmed in *R. acris* and *C. nutans*, investigations were begun with both species to find if such resistance occurred at other sites, and to determine if the resistances had resulted from herbicide selection pressure.

Seven populations of *R. acris* from Takaka dairy pastures and one from a roadside in Canterbury were compared in a glasshouse for susceptibility to MCPA. The LD_{50}s varied between the populations indicating intermediate levels of resistance existed. Information on the spraying history for each of these sites was collected by interviewing past and present land managers. The seven sites were ranked according to the intensity of historical spraying. The level of resistance as measured by the LD_{50}s, correlated well with this (Table 1). This suggests that past spraying of MCPA had caused a build-up of resistance in *R. acris.*

Seed from 28 populations of *C. nutans* was collected from various parts of Hawkes Bay, Waikato and Manawatu. The susceptibilities of these populations to MCPA were compared in a glasshouse by applying 3.0 mg MCPA/plant to 30 plants from each population. Differences in mortality were compared using an adjusted chi-square analysis (Harrington unpub; Harrington, 1989). Fourteen sites in Hawkes Bay and Waikato were located where *C. nutans* is more tolerant of MCPA than the Matapiro population. Spraying histories were obtained for seven of the most resistant and seven of the most susceptible populations. All sites had received herbicide in past years but resistance occurs only where 2,4-D has been used every year for many years (Table 2).

Table 1. Responses of the seedling progeny of 8 populations of *Ranunculus acris* to MCPA under uniform conditions in a glasshouse and corresponding MCPA exposure histories. LD_{50}s (kg MCPA/ha) are means of estimates from (n) dose-response experiments. Data from Bourdot & Hurrell (1988) and Bourdot unpub.

Population	Location	LD_{50}	rank[a]	Spraying history
Jones	Takaka	0.36 (3)	0	Not sprayed since 1958, possibly never sprayed with herbicide.
Saltwater Crk.	Canterbury	0.39 (1)	0	Never treated with herbicides.
Stratford	Takaka	0.52 (2)	1	Spot-sprayed rarely with MCPA from 1946 until 1974. Boom-sprayed with MCPB or MCPA occasionally from 1974 until 1984.
Langford t	Takaka	0.67 (1)	2	Sprayed with MCPA from 1946 until 1984 and often from 1979 until 1984.
Fellowes	Takaka	0.72 (2)	1	Occasionally spot-sprayed with MCPA from 1946 until 1984.
Langford b	Takaka	0.83 (1)	2	Sprayed with MCPA from 1946 until 1984 and often since 1979.
Reilly	Takaka	0.94 (1)	-	MCPA has been used but at unknown frequency.
Silcock	Takaka	1.30 (3)	3	Sprayed with MCPA triennially from 1955 until 1966, biennially from 1966 until 1981, annually from 1981 until 1984.

Correlation of "LD_{50}" and "rank"; $r = 0.845^{*}$

(a) Rankings of populations for historical exposure to MCPA based on the anecdotal information on application rates and frequencies. This information was supplied by the farmers in Takaka, and the Hurunui County Council for the Saltwater Creek population.

Table 2. Responses of the seedling progeny of 14 populations of *Carduus nutans* to 3 mg MCPA/plant, under uniform conditions in a glasshouse, and corresponding herbicide spray histories of the populations. Mortality data from Harrington (1989).

Population	Location	Control problem?	% kill[2]	Spraying history[1]
Maungatautari	Waikato	yes	0a	2,4-D annually for the last 15 yrs.
Waotu	Waikato	yes	0a	2,4-D ann. for the last 15 yrs.
Argyll	Hawkes Bay	yes	10ab	2,4-D ann. for the last 25 yrs.
Te Onepu	Hawkes Bay	yes	13ab	2,4-D ann. for the last 20 yrs.
Arohena	Waikato	yes	17b	2,4-D or MCPA ann. for the last 17 yrs.
Glenalvon	Hawkes Bay	yes	20b	2,4-D ann. for the last 30 yrs.
Kia Ora	Hawkes Bay	yes	23b	2,4-D ann. for the last 20 yrs.
Mason Ridge	Hawkes Bay	no	93c	2,4-D or MCPA infrequently for the last 30 yrs.
Limestone Downs	Waikato	no	93c	2,4-D infrequently for the last 10 yrs.
Maraekakaho	Hawkes Bay	no	95c	MCPA ann. for 20 yrs, then infrequently for last 15yrs.
Ohutu	Hawkes Bay	no	97c	2,4-D ann., then infrequently for the last 12 yrs.
Matapiro	Hawkes Bay	no	97c	2,4-D ann., then infrequently for the last 6 yrs.
Colyton	Manawatu	no	100c	2,4-D ann. for 10 yrs, then infrequently for the last 10 yrs.
Hickey Road	Hawkes Bay	no	100c	2,4-D ann. for 10 yrs, then infrequently for the last 8 yrs.

(1) Anecdotal information from farmers; for all populations herbicides may have been applied earlier than indicated, but confirmation was unavailable.

(2) Populations sharing the same letter do not differ in susceptibility (P=0.05).

Although it was resistance to MCPA that was detected in *C. nutans*, the selection pressure has been applied by 2,4-D on almost all farms (Table 2). The presence of cross-resistance in the Argyll population was examined further in a trial where plants from this and the Matapiro population were grown together at Massey University. The plants were grown in the field and received the recommended application rate of 12 commercial herbicide preparations (Harrington, 1989). Argyll plants were resistant to MCPA, MCPB and 2,4-D but not to picloram, clopyralid, dicamba, mecoprop or glyphosate. An unexpected result was a slight but significant (P=0.05) level of tolerance to the sulphonylurea herbicide DPX-L5300.

DISCUSSION

The experiments have shown that resistance to MCPA has developed within populations of both *R. acris* and *C. nutans* in New Zealand pastures. These resistances seem almost certain to have developed due to selection pressure exerted by phenoxy herbicides since for both species, the susceptibility of populations was correlated with their historical exposure to phenoxys.

Although some of the glasshouse experiments suggested very large differences in susceptibility between extreme populations of *C. nutans,* it seems that a 6 to 7-fold difference is the most realistic estimate. This is similar in magnitude to the 5-fold difference found in *R. acris,* and the 6-fold difference in susceptibility to mecoprop found in *Stellaria media* by Lutman and Snow (1987). It is a larger difference than found in *Tripleurospermum inodorum* by Ellis and Kay (1975) where populations never differed in susceptibility to MCPA by any more than 2.5-times. Five or 6-fold differences are not large compared to those reported for triazine herbicides (LeBaron & Gressel, 1982). They are large enough however, to render the least susceptible biotypes of *R. acris* and *C. nutans* "resistant" to MCPA applied at the normal application rate of 1.0 kg/ha.

Species taking many months to produce their first seeds may be more likely to develop resistance to non-persistent herbicides such as phenoxys, than annuals which seed within a short time from germination. Non-persistent herbicides will usually exert a low selection pressure on an annual species when, as is frequently the case, a new cohort of seedlings germinate, develop to maturity and seed after spraying in the same year. In this event both susceptible and resistant genotypes will set seed, thus slowing the build-up of resistance (Gressel & Segel, 1982). By contrast, in a species taking for example, 12 months to produce seed following germination, an application of a non-persistent herbicide needs only to be made once a year to remove all susceptible plants before they flower and seed, no matter when they germinate.

In New Zealand pastures, Popay and Kelly (1986) found that most seedlings of *C. nutans* emerge in late summer and autumn (February through May) and flower in the following summer months. They calculated that 98% of *C. nutans* plants that flower in summer are derived from autumn-germinated seedlings. Thus the usual late-autumn application of 2,4-D or MCPA made when the seedlings finish appearing (Matthews, 1975), will prevent all susceptible plants from flowering in that year. Applications made later in the year, but before seeding, would be similarly effective. The selection pressure on sprayed populations of *C. nutans* in pastures in New Zealand has thus probably been high despite the ephemeral nature of 2,4-D and MCPA.

The selection pressure exerted by MCPA on *R. acris* in New Zealand pastures may also be high. While a detailed demographic study has not been done on *R. acris* in New Zealand, it is likely that germination occurs mainly in autumn and in late winter as it does in the UK (Harper & Sagar, 1953). Field observations in Takaka certainly support this view, and also show seed production is confined to the summer months. Thus the recommended treatment with MCPA in late winter (August) (Popay *et al.*, 1984,1989), with or without additional later spring treatments of MCPA or MCPB (Matthews, 1975), would kill all susceptible plants emerging in a year.

Another factor that may have promoted the build-up of resistance in *C. nutans* and *R. acris* is a lack of genetic buffering. Many weeds produce persistent soil seed banks. The germination of such dormant seed produced by susceptible plants in years prior to the selection pressure being applied, or during years when the herbicide has not been applied, will depress the rate of build-up of resistance in a population (Gressel & Segel, 1982). In this respect the permanent pasture habitats of both species may be of considerable significance. Seeds are more likely to remain dormant if they are buried in the soil rather than left on the surface (Egley & Duke, 1985). Such burial is less likely to occur in a permanent pasture than in arable cropping soil, mitigating against the formation of a persistent seed bank. There will also be less return of any buried seed to the surface where it can germinate, than in cultivated soils.

No study has been made of the seed biology of *R. acris* in New Zealand but Sarukhan (1974) showed that only 1.5% of seeds of this species sown onto pasture in Wales remained viable after one year. While Grime *et al.* (1988) suggest the seeds of *R. acris* may persist in soil, others have failed to find evidence for a persistent seed bank under permanent pasture (Champness & Morris, 1948; Milton, 1936). The evidence indicates that *C. nutans* also may not form a persistent bank of seeds in the soil under a permanent pasture. Popay and Thompson (1979) working in New Zealand, found seeds of *C. nutans* sown within 20 mm of the soil surface disappeared quickly due to germination. As few as 3% remained after one year and none after three years. The seeds persisted only when buried. Similarly Kelly (pers.comm.) found only 7% of seeds of *C. nutans* sown on the soil surface under pasture persisted after one year and only 0.3% after 2 yrs.

Despite the probable existence of high selection pressures and a lack of genetic buffering in *R. acris* and *C. nutans,* it would seem, at least for *C. nutans,* that continued use of phenoxy herbicides over many years is needed to result in resistance in a population. The susceptible populations of *C. nutans* had all been sprayed in the past, but it was only where this pressure had continued every year that resistance occurs today (Table 2). It is possible that resistance had once occurred but disappeared in populations not treated in recent years, due to a fitness differential favouring the susceptible forms (Gressel & Segel 1982). However anecdotal evidence suggests that resistance never occurred at the sites not treated with herbicide in the last 10 to 15 years (Table 2). Studies are being done to determine if resistant forms of *C. nutans* and *R. acris* are less ecologically fit than susceptible forms.

Because phenoxy herbicides have been applied frequently enough to cause resistance to build up in *R. acris* and *C. nutans,* it is reasonable to expect resistance to phenoxys will appear in other pasture weeds in New Zealand. *Carduus pycnocephalus* is already suspected of having developed resistance at one of the farms on which a resistant population of *C. nutans* was found (Harrington, 1989). Preliminary experiments suggest that the resistance to MCPA is of similar magnitude to that in the Argyll *C. nutans* but further comparisons are required to confirm this.

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NEW DEVELOPMENTS IN TRIAZINE AND PARAQUAT RESISTANCE AND CO-RESISTANCE IN WEED SPECIES IN ENGLAND

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ABSTRACT

Biotypes of *Epilobium ciliatum* (American willowherb) and *Poa annua* (annual meadow grass) resistant to either paraquat or simazine have been recorded in fruit crops in England since 1982. Plants taken in 1988 from two hop gardens in Kent treated annually with paraquat and simazine for about 25 years were found to be resistant to these herbicides but showed unusual features. The *P. annua* biotypes were resistant to both herbicides, although the level of resistance to simazine was slight. An *E. ciliatum* biotype from one site was resistant to both paraquat and high doses of atrazine. At the same site triazine-resistant *Chamomilla suaveolens* was also found. These occurrences emphasise the need to use alternative herbicides in hops. Atrazine-resistant biotypes of *Chenopodium album* (fat hen) and *Solanum nigrum* (black nightshade) were discovered in forage maize areas in southwest England and south Wales.

INTRODUCTION

Triazine resistant weeds have been an increasing problem in fruit and ornamental crops in England for the last eight years. Species showing resistance have included *Senecio vulgaris* and *Poa annua* (Putwain, 1982), *Epilobium ciliatum* (Syn. *E. adenocaulon*) (Bailey *et al*.,1982), *Erigeron canadensis* (Clay & West, 1987) and *Chamomilla suaveolens* (Clay, 1987). Paraquat resistance was first documented for *P. annua* in 1982 (Putwain, 1982) and more recently for *E. ciliatum* (Bulcke *et al*., 1987; Clay, 1987). Control of many of these weeds with paraquat and simazine has become difficult in hop gardens in Kent where the herbicides have been used regularly for up to 25 years. The results of tests for resistance of these weeds are presented in this paper. Triazine resistant weeds of maize crops are common in the USA and Europe but none have been recorded previously in the UK. Recently, *Solanum nigrum* and *Chenopodium album* have become difficult to control with atrazine in some forage maize crops in SW. England and S. Wales. Results of tests of their response to atrazine are reported below.

MATERIALS AND METHODS

The locations and their herbicide history from which suspected resistant and susceptible biotypes were taken are shown in Table 1. Several plants of *C. suaveolens*, *E. ciliatum* and *P. annua* taken from two hop gardens near Maidstone, Kent (M1 and M2) in May 1988 were grown on in the glasshouse and their seed collected. Seed was obtained from other glasshouse-grown plants from biotypes selected from previous work as resistant to paraquat or simazine (Clay, 1987 and unpublished data). Seed of C. album and S. nigrum was taken from different parts of affected maize fields and, before use, was washed and soaked in 0.2% KNO_3 for 24 h. For tests of response to pre-emergence herbicides, seed was sown in sandy loam soil (12 per pot) and covered with 2 mm of soil.

TABLE 1. Sources of weed biotypes for testing of herbicide resistance.

Species	Biotype Code	Location	Crop	Herbicide history
C. suaveolens	M1	Maidstone district, Kent	Hops	c 25 yrs annual paraquat & simazine
	R	Luddington, Warks	Fruit	Annual simazine treatment c 10 yrs
	V	Luddington, Warks	None	None
E. ciliatum	E	E.Malling, Kent	Top Fruit	Paraquat c 27 yrs+ simazine c 17 yrs
	L	Long Ashton, Avon	Top Fruit	c 5 yrs annual simazine + paraquat 2-3 times a yr
	M1, M2	Maidstone district	Hops	c 25 yrs annual paraquat & simazine
	U	Luddington, Warks	Top Fruit	2 yrs paraquat
	W	Begbroke, Oxford	Bush Fruit	15 yrs simazine & occasional paraquat
Poa annua	B	Berkshire	None	None
	C	Chard, Somerset	Forest Nursery	c 10 yrs annual simazine
	M1, M2	Maidstone district	Hops	c 25 yrs annual paraquat & simazine
C. album	K	Keynsham, Avon	Forage Maize	5 yrs annual atrazine
	U	Usk, Gwent	Forage Maize	2 yrs annual atrazine
S. nigrum	B	Berkshire	None	None
	C	Chepstow, Gwent	Forage Maize	5 yrs annual atrazine
	E	Exeter district, Devon	Forage Maize	4 yrs annual atrazine
	U	Usk, Gwent	Forage Maize	No recent atrazine

For tests of post-emergence herbicides, seed was sown in trays of peat-based compost and the seedlings transplanted to 8 cm diameter pots of loam-based compost, five per pot for P. annua and C. suaveolens and one per pot for E. ciliatum. The herbicide formulations used were:- atrazine 50% a.i. 'Gesaprim 500FW', paraquat 20% a.i. as 'Gramoxone 100' and simazine 50% a.i. as 'Gesatop 500FW'. In post-emergence applications an adjuvant oil 'Actipron' was added to atrazine treatments at 2.5 l/ha.

Herbicides were applied using a laboratory track sprayer fitted with an 8002 flat fan nozzle giving a volume rate of 373 l/ha at 210 kPa. After spraying, pots were set out in the glasshouse in two to four randomised blocks. Plant condition was assessed at intervals after spraying and foliage fresh weight recorded three to six weeks after treatment.

The atrazine resistance of Solanum nigrum from three sites was also tested using the leaf disc flotation method devised by Hensley (1981). Discs, 4 mm diameter, were cut from the youngest expanded leaves of glasshouse-grown S. nigrum when the plants were 10-15 cm tall, and placed in tubes of buffer solution with 100 mg/l atrazine. Tubes were placed under vacuum until discs sunk, then illuminated and the number of discs floating counted after 15, 30, 45 and 60 min.

RESULTS

Poa annua

Atrazine applied post-emergence did not reduce the growth of the known triazine resistant biotype (C)(Table 2) but was very damaging to the susceptible type (B). The biotypes from the hop gardens (M1, M2) showed some degree of resistance to atrazine but were severely damaged by the highest dose. The only paraquat treatment not killing P. annua biotypes was the lowest dose on M1 and M2.

TABLE 2. Effect of atrazine + oil and of paraquat applied post-emergence to P. annua on 24/10/88[a].

Herbicide dose (kg a.i./ha)	Foliage fresh wt (% untreated) 23/11/88								Untreated fresh wt (g/pot)
	Atrazine				Paraquat				
	0.25	1.0	4.0	16.0	0.25	1.0	4.0	16.0	
Biotype C	77	72	112	101	0	0	0	0	12.9
B	6	22	4	0	0	0	0	0	29.4
M1	91	60	59	30	29	0	0	0	18.8
M2	60	51	44	40	27	0	0	0	18.8

[a] seed sown 25/8/88, soil surface watered, pots free draining.

TABLE 3. Effect of paraquat applied to P. annua biotypes on 13/1/89[a].

Paraquat dose (kg a.i./ha)	Foliage fresh wt (% untreated) 23/2/89									Untreated fresh wt (g/pot)
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40		
Biotype C	93	74	9	0	0	0	0	0	0	22.2
B	119	84	34	16	4	0	0	0	0	26.3
M1	100	87	86	75	77	60	36	32	18	23.1
M2	104	94	83	64	50	23	7	2	5	22.9

[a] seed sown 17/12/88, pots placed on capillary matting after spraying.

TABLE 4. Effect of simazine applied pre-em. to P. annua on 8/6/89[a].

Simazine dose (kg a.i./ha)	Foliage fresh wt (% untreated) 28/6/89 0.025	0.05	0.10	0.20	0.40	Untreated fresh wt (g/pot)
Biotype C	105	94	98	93	99	5.07
B	3	0	0	0	0	4.88
M1	42	4	0	0	0	3.06
M2	60	7	1	0	0	5.16

[a] Pots free draining, receiving light overhead watering.

In a dose-response experiment with paraquat a two to three times larger herbicide dose was required to give an equivalent degree of damage on the M1 and M2 biotypes compared with the standard susceptible type, B (Table 3); the C biotype was slightly more susceptible than the standard. Where simazine was applied pre-emergence the C biotype was not affected by the highest dose (0.4 kg/ha)(Table 4). The susceptible type (B) was virtually killed by the lowest dose (0.025 kg/ha). The doses required for > 95% weight reduction of the M1 biotype was 0.05 kg/ha and for M2 0.1 kg/ha.

Epilobium ciliatum

When atrazine and paraquat were sprayed post-emergence, plants from both hop-garden sites (M1, M2) were resistant to atrazine and that from one site (M1) was also resistant to paraquat as well (Table 5). The standard simazine-resistant biotype (W) showed no leaf damage from atrazine but was killed by all the paraquat doses. Growth of the standard paraquat-resistant biotype from East Malling was inhibited by the higher paraquat doses; the Long Ashton biotype (L) was more resistant. Both biotypes were killed by all atrazine doses. The Luddington biotype (U) was killed by all doses of both herbicides.

TABLE 5. Effect of atrazine + oil and paraquat applied post-emergence to C. suaveolens and E. ciliatum biotypes on 22/9/88[a]

Herbicide	Dose (kg a.i./ha)	Foliage fresh wt (% untreated) 28/10/88 C. suaveolens M1	C. suaveolens R	C. suaveolens V	E. ciliatum E	E. ciliatum L	E. ciliatum M1	E. ciliatum M2	E. ciliatum U	E. ciliatum W
Atrazine	0.25	118	98	0	0	0	105	86	0	44
	1.0	100	104	0	0	0	73	115	0	70
	4.0	112	113	0	0	0	96	90	0	98
Paraquat	0.125	0	0	0	81	104	96	79	0	0
	0.5	0	0	0	66	86	62	0	0	0
	2.0	0	0	0	32	59	58	0	0	0
Untreated (actual value, g/pot)		7.4	8.1	9.2	12.8	5.4	4.3	5.7	10.1	8.3

[a] seed sown 25/8/88, soil surface watered, pots free draining.

Chamomilla suaveolens

The biotype from one of the hop gardens (M1) was resistant to the highest dose of atrazine (Table 5); one of the biotypes from Luddington (R) was completely resistant, the other (V) was susceptible.

Solanum nigrum

Most of the biotypes from the C, E and U maize sites were found to be relatively resistant to atrazine (Table 6), being unaffected by a dose of 0.4 kg/ha, whereas the susceptible biotype B was killed by the lowest dose (0.05 kg/ha).

TABLE 6. Effect of atrazine applied pre-emergence to S. nigrum biotypes on 8/6/89[a].

Atrazine dose	Foliage fresh wt (% untreated) 6/7/89				Untreated fresh wt
(kg a.i./ha)	0.05	0.40	3.2	25.6	(g/pot)
Biotype B	0	0	0	0	8.4
C	124	82	3	0	6.3
E	122	108	22	0.4	7.8
U	103	84	5	0.2	8.9

[a] pots free draining, receiving light overhead watering.

TABLE 7. Response of leaf discs of S. nigrum to atrazine (100 mg a.i./l).

	No. of leaf discs floating (% total present)		
Illumination time (min)	15	30	45
Biotype B	0	0	0
C	77	90	90
E	100	60	60
U	100	100	100

However, plants were killed or severely damaged by the higher atrazine doses and surviving plants showed typical triazine injury symptoms (leaf margin chlorosis and necrosis). These results were confirmed by the leaf disc test where the numbers of discs from the same C, E and U biotypes floating after treatment was 90-100% whereas none from the susceptible population (B) floated (Table 7).

Chenopodium album

Where atrazine was applied post-emergence one biotype (K) was found to be much more resistant to the herbicide than a biotype from another forage maize site (U) which was killed by 1 kg /ha of atrazine (Table 8).

TABLE 8. Effect of atrazine + oil applied post-emergence to C. album[a] at two dates.

Atrazine dose (kg a.i./ha)	Foliage fresh wt (% untreated) /3/89 1.0	3.0	9.0	Untreated fresh wt (g/pot)
Application 13/1/89[b]				
Biotype K	98[d]	69	41	4.13
U	0	0	0	4.59
Application 6/2/89[c]				
Biotype K	85[e]	90	82	14.5
U	0	0	0	17.1

[a] seed sown 7/12/88 [b] growth stage 1 or 2 pairs true leaves
[c] plants 9-13 cm tall. [d] 10/2/89 [e] 9/3/89

DISCUSSION

The P. annua from the hop garden sites (M1, M2) showed a degree of resistance to paraquat similar to that previously reported (Putwain, 1982), but clearly was susceptible to paraquat in certain conditions. Conditions in the glasshouse experiments were conducive to damage with low light levels, humid growing conditions and, in the second test, small plants. A feature of the response of the biotypes to paraquat in the field has been some foliar damage followed by recovery. This pattern was also found with paraquat resistant E. ciliatum (Clay, 1987). The level of triazine resistance in the hop garden biotypes of P. annua was much lower than that found with the Chard (C) biotype and also in earlier reports (Putwain, 1982). There was, however, a big difference in pre- and post-emergence susceptibility; doses of 0.1 kg/ha of simazine were lethal pre-emergence, while plants recovered from 16 kg/ha of atrazine post-emergence. The mechanism of resistance may, therefore, be different from that normally found in triazine resistant biotypes of this and other species where a modification of the thylakoid membrane binding site confers resistance (Le Baron & Gressel, 1982). With the hop garden biotypes resistance may be due to a detoxification mechanism reducing the amount of herbicide reaching the site of action comparable to that found with Alopecurus myosuroides resistance to chlorotoluron and other herbicides (Kemp et al., 1988). The only previous reports of paraquat and simazine co-resistance are with Erigeron species in Egypt and Hungary (Gressel, 1987; Polos et al, 1987). The failure of simazine to control P. annua in hop gardens even though the degree of resistance is small, may be due to its germination pattern. It is possible that spring-germinated P. annua are controlled, but that seedlings germinating after the hops are harvested in autumn are unaffected by the low levels of simazine remaining in the soil and these become large enough to survive spring-applied simazine and paraquat.

The occurrence in fruit and hop plantations of E. ciliatum biotypes resistant to either simazine or paraquat parallels that found previously in biotypes from fruit (Bulcke et al., 1987; Clay, 1987). A unique feature of the M1 biotype, however, was its resistance to both herbicides, paralleling that found with P. annua at both hop garden sites except that the level of triazine resistance in E. ciliatum was very much greater. The degree of paraquat resistance in M1 was relatively low, comparable to other E. ciliatum biotypes showing paraquat resistance and much less than that shown in paraquat- and triazine-resistant Erigeron species in Egypt and Hungary (Gressel, 1987; Polos et al., 1987). The M1 biotype is morphologically different from the others, being smaller with narrower leaves and branched shoots. However, Bulcke et al., (1987) found no correlation between leaf characteristics and paraquat or triazine resistance. Triazine-resistant C. suaveolens also found at the M1 site showed resistance at a high level similar to that found previously (Clay, 1987). The occurrence of at least three herbicide-resistant species at one hop garden site, two of which were resistant to simazine and paraquat, highlights the need to use herbicide programmes which may avoid such developments. Traditionally, simazine and paraquat have been the only herbicides used in hops along with dinoseb for controlling excess bine growth. After the withdrawal of dinoseb, which had appreciable post-emergence activity, weed problems have increased highlighting the need for alternative herbicides.

Triazine resistant C. album and S. nigrum have not been reported before in England although common overseas. The degree of resistance found in this work was comparable to that found elsewhere (Fuerst et al., 1986) but is less than in other triazine resistant species found in Britain (Clay, 1987). Doses of 3 kg/ha of atrazine on S. nigrum and 9 kg/ha on C. album caused typical triazine symptoms on leaves whereas no leaf symptoms occurred with atrazine at 4 kg/ha on C. suaveolens or E. ciliatum. Atrazine-resistance of S. nigrum was confirmed by the leaf disc flotation technique. This is a rapid and inexpensive method of identifying atrazine-resistant biotypes. The technique was used initially by Hensley (1981) on C. album and more recently on common simazine-resistant weeds in the UK, E. ciliatum, E. canadensis and Senecio vulgaris (Clay & Underwood, 1988).

As in maize overseas, resistant weeds generally occur where atrazine is used as the sole herbicide in a maize mono-culture. Surprisingly; S. nigrum at the Usk site, was resistant even though there had been no recent maize crops. However, the site was given dressings of farmyard manure deriving partly from maize silage from other parts of the farm probably containing S. nigrum seed. Since maize normally gets dressings of FYM from other cropped areas, rotating the sites where it is grown may not prevent resistance developing. Inclusion of effective S. nigrum herbicides in the spray programme is, therefore, essential.

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SESSION 4B

TREE CROPS: NEW PRODUCTION SYSTEMS AND THEIR IMPLICATIONS FOR WEED CONTROL

CHAIRMAN	DR J. D. QUINLAN	
SESSION ORGANISER	DR N. A. HIPPS	
INVITED PAPERS		4B-1 to 4B-3
RESEARCH REPORTS		4B-4 & 4B-5
POSTER		4B-6

EUROPEAN FORESTRY SYSTEMS

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ABSTRACT

Current forestry systems and their future development in Europe are a product of the historical development within an individual country and fiscal measures. In recent years financial aid linked to fiscal measures has been used to direct that development within individual countries and within the European Community.

Recent trends and forecasts for the near future can be grouped into Traditional, Urban and Farm Woodlands with Climatic Hazards straddling all three.

INTRODUCTION

The pattern of land use within the European Community is shown in Table 1. in order to provide background to the later explanation of changes in, and likely future developments in forests and woodlands. The Community is a net importer of wood and wood products and has surplus production on some agricultural products. As the climate throughout the Community is favourable for tree growth of a wide range of species it is a reasonable assumption that an expansion of the area of forest will take place. This is a very generalised statement and the following table illustrates national differences.

TABLE 1. Land Use. EC.

Country	Total Land Area (million ha)	Percentage of Total Area		
		Forestry	Agriculture	Urban and Other
United Kingdom	24.1	10	78	13
Belgium and Lux.	3.3	21	46	33
Denmark	4.2	12	67	21
France	54.6	27	57	16
West Germany	24.4	30	49	21
Greece	13.1	20	70	10
Iceland	6.9	5	83	12
Italy	29.4	22	59	20
Netherlands	3.4	9	60	32
Portugal	9.2	40	36	24
Spain	49.9	31	61	8
EEC Total	222.5	24	60	15
Sweden	41.2	64	9	27

Source UK. CSO Annual Abstract of Statistics 1988.
Elsewhere. F.A.O. Production Year Book Vol 40 1986.
From Forestry Commission Handbook 5 "Urban Forestry Practice" 1989.

rotation".

The phrase "Urban Woodland" could replace Arboriculture.

This is a high profile, high value industry. Bradshaw 1988 estimates the planting stock value at £121M per annum. In 1987/88 the Countryside Commission England/Wales gave £2.6M in grants and in Scotland £.26M was the equivalent sum. (Forestry Commission 1989.) The Department of transport plants over 1 million trees per annum. This is all at the smaller, amenity end of the scale.

On the larger scale two major initiatives have been launched this year. In Scotland the "Central Scotland Woodlands Trust" has been created to draw together public and private finance in order to create a woodland structure on 66,000 hectares of less favoured land in central Scotland between Edinburgh and Glasgow. This is a complex area of low grade agricultural land, derelict industrial sites, reclaimed mineral workings and with low population levels, but fringed by the very high density population zone of the central Scotland Industrial Belt.

In England and Wales a joint project by the Forestry Commission and Department of the Environment was launched in July 1989 "Forestry for the Community". A series of forests are planned in Urban areas to improve amenity and recreation areas. The launch was accompanied by a Forestry Commission Handbook (B Hibberd Ed 1989) that uses as its basis for weed control advice the very successful joint research work by the Forestry Commission and Department of the Environment (R J Davies 1987).

There are similar major schemes throughout Europe in Milan a scheme was started in 1967, "The Wood in the City". From the original small scheme 70 small woods have now been developed in Milan and surrounding villages (Luca Carra 1985).

The diversity of sites and species with the close proximity of man creates a considerable challange for the manager.

FARM FORESTRY

Woodlands

"We have received nearly 1000 applications to plant approximately 7000 ha of woodland over the first three years of the scheme and we expect 75% of this area to be broadleaved trees" (MAFF 1989).

This statement shows the farming response to a Government grant scheme to use trees to take some of the surplus land out of agricultural production. The other scheme "set aside" has not been as successful, as >1000 ha were proposed to go with woodland; 2-3% of the total set aside.

These schemes move higher quality land with easy vehicular access into forestry. Vigorous growth rates for a much wider spectrum of weeds, many of which have lain dormant in seed banks for a considerable number of years and are classed as "rare", are one of the penalties. On this high quality land very high productivity will be expected and great care will be needed to achieve good weed control in those early years. A resurgence of interest in Poplar has followed the availability of good quality land and Poplar is very sensitive to weed competition.

A wider range of tree species will be used, many for amenity or game improvement.

The European Commission published a Forestry Action Plan in the Official Journal of 15 June, seven regulations were adopted. They, amongst other things, increased grants and provided a new forestry premium for farmers in order to encourage farm land to go to forestry. It has brought no change in the UK as grants were in place, but is being considered in Ireland and a suggestion is made in the Timber Trade Journal that the annual planting rate will double to 30,000 ha by 1993. Not all European countries are interested in these schemes. Belgium, France and Germany have no such schemes to date and it will be interesting to see how the plan progresses.

Other possible farm forestry systems have not gone beyond the development stage. Short rotation coppice for energy purposes whilst achieving many headlines has never shown itself to be economical and has no grant support in the UK, but has in N Ireland. Of more interest is short rotation single stem growing of Poplar for use as industrial small roundwood as pulp or fibreboard. Demonstration areas of 250 ha are established in Belgium and a similar area is proposed in France.

Agro-Forestry

Trees can be grown in combination with either a pastoral or arable system and both are regularly used in parts of Europe. In the Po valley of Italy Poplar is grown at wide spacing with maize as an intercrop for 3-5 years of a 10 year rotation. Silvi-pastoral systems are successfully used in New Zealand with *Radiata* pine.

The arguments for agro-forestry as a method of reducing agricultural productivity were discussed and summarised S M Newman 1989. The one of interest to this Conference is that where a monoculture of a tree species that requires wide spacing is planted eg poplar, cricket bat willow and Walnut agro-forestry provide an alternative way (to use of herbicides) for managing the understorey.

Species used in the UK could well be fast growing broadleaves eg Poplar and *Nothofagus*, with the additional problem that grazing animals are expected to be present.

There is as yet no indication that these systems will be accepted for grant purposes in the UK or elsewhere in Europe.

Hazards

It is rather paradoxical that what is misfortune for some brings good fortune to others. I have mentioned the large restocking programme in East England as an aftermath to the 1987 gale damage, which also afflicted parts of Europe, particularly the Western sea board. A hazard much greater in magnitude is that of fires in the Mediterranean countries. The Daily Telegraph of Saturday 9 September 1988 gives the area damaged in France in 1988 as 170,000 ha, this is not journalistic licence as other EEC figures show a regular loss of 50,000 ha per annum. The afflicted countries may wish to use the Forestry Action Plan to replant these areas rather than plant up agricultural land.

Conclusion

The message that effective weed control is a necessity for quick establishment of trees still needs publicity. Forestry Commission Broadleaved Policy Review 1989 "The limited use of herbicides can be interpreted either as an indication of a lack of awareness, or money or long-term commitment on the part of the owner".

In France an investigation has been started into what has happened to the previously fire damaged areas. It is suspected that some is now covered by urban development and even golf courses. Whatever the land use, there appears to be a place for trees and therefore herbicides are needed for weed control.

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USE OF GENETICALLY IMPROVED FOREST TREES AND WEED CONTROL

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ABSTRACT

The tree improvement carried out by the Forestry Commission covers the wide range of coniferous and broadleaved species planted on the diverse sites available for forestry in the UK and managed under different silvicultural systems. The greatest effort is on Sitka spruce (*Picea sitchensis*), because of its commercial importance to the forest industry. A conventional tree breeding programme includes the identification of most suitable seed origins, establishment of clone banks and seed orchards, breeding new cultivars and field testing of progeny. Volume increments at harvest of >10% are confidently predicted from the use of improved stock of Sitka spruce. Increasing attention is being given to the use of vegetative propagation, including *in vitro* methods, which can offer substantial savings in making the genetically improved stock available earlier to the tree grower. The investment in genetically improved trees is likely to be wasted unless it is combined with sound silviculture including efficient weed control. The use of more sophisticated biotechnological methods can be expected in the future.

INTRODUCTION

The function of tree improvement is to provide seed and/or vegetatively propagated material of broadleaved and coniferous forest tree species of improved quality in terms of vigour, timber properties, disease and insect resistance and stability for use in commercial forestry. Commercial forests can be defined as individual scattered trees as in agroforestry, blocks of trees as in a shelter belt, or large areas of forest. In our tree improvement programme therefore we are covering a diverse group of species, forest systems and sites throughout the UK. The degree of effort varies with the species and generally reflects its economic importance or potential for forestry.

The tree improvement programme of the Forestry Commission for Sitka spruce (*Picea sitchensis*) will be briefly described as an example of a conventional tree breeding programme for forest trees. Since there is considerable and increasing interest in clonal forestry, some of the advantages and disadvantages of this will be noted. Both weed control and tree improvement have the common aims of increasing productivity, and profitability. These will be discussed in the general context of improvements in silvicultural methods and areas will be suggested where the two technologies may combine to provide further increases in profitability.

CONVENTIONAL TREE BREEDING PROGRAMME

Sitka spruce is the main forest species planted in Great Britain with current annual planting at 50 to 60% of the total forest area. The popularity of Sitka spruce is due to the tree's vigour, adaptability, form and timber quality (Holmes, 1987). It tolerates a wide variety of poor soils, a high degree of exposure and grows more rapidly than any other conifer in our oceanic climate to produce a timber very highly regarded for paper pulp and a good general purpose white sawn timber.

Sitka spruce is native to the west coast of North America and was introduced into Britain by David Douglas in 1831. Its native range is restricted to humid oceanic conditions from southern Alaska to northern California (Lines, 1987a). In a tree improvement programme the most immediate and greatest gains are obtained from identification and use of the best seed origins. Seed have been collected from throughout its range and experiments established to identify the best seed sources for Britain. The results from these trials form a solid data base allowing differences between seed origins in growth performance and habit to be quantified for our conditions. Queen Charlotte Islands origins were recognised early for the reliably, impressive growth and timber quality. More southern and northern origins are not as suitable, eg trees from California have an extended growing season and are susceptible to early frosts whereas trees from Alaska grow slowly and use only part of the growing season. The selection of provenance within the UK may also vary, ie trees of Washington origin are more suitable for south west England and parts of Wales. Generally for most economic traits, eg vigour, approximately 40% of the total variation is exhibited between origins and 60% of the variation is within an origin (Lee, 1989).

More intensive tree breeding programmes should only be initiated after the best seed origin has been identified. Tree breeding uses techniques of agricultural plant breeding but adjusted for plant size and generation time (Namkoong *et al.*, 1988). Forest crops however are more heterogeneous than agricultural ones and there is less control of the environment in forestry than in agriculture. The rate of improvement is largely controlled by the breeding cycle and by the time taken to obtain reliable data on progeny performances for the commercially important traits selected (Faulkner, 1987). Sitka spruce is selected for growth rate, stem form, branching habit and wood quality, including wood density.

The success of a tree breeding programme depends on the genetic variation in the trait to be selected for and on the effectiveness of choosing parents for breeding. Most tree breeding programmes start with the selection of superior phenotypes, ie trees appearing to have the desired attributes. These trees are chosen as the best individuals from the best stands on a variety of site types throughout the country. The phenotype of an individual is the result both of its genetical constitution or genotype and of the environment in which it was grown. By the end of 1986, 2700 superior phenotypes of Sitka spruce had been selected as potential breeding trees. The majority of these are of British Columbian origins with characteristics similar to those from Queen Charlotte Islands. Separate selections of Washington and Alaskan origins have also been made for southern sites and colder harsher sites respectively.

To determine how good these superior phenotypes really are, progenies of these trees, obtained from wind pollinations or crossing with pollen of known parentage, are grown in replicated experiments on at least three sites. If the progenies from a particular mother tree perform very well, the mother tree is used in future breeding work. Sitka spruce families are considered acceptable for inclusion in the breeding population if they exceed a Queen Charlotte Island control by 15% and the overall family mean by 7% for height at six years (Lee, 1986). Close correlations have been noted been between height at 6 years and height and diameters at 15 and 20 years (Faulkner, 1987). Of the 2700 Sitka spruce selected as superior phenotypes, approximately 2500 are currently being tested and the aim is to have a breeding population of 200 individuals. The best 40 individuals are being used to produce, in so called seed orchards, improved seed through open pollination or by controlled pollination with a mix of pollen from 20 superior parents. Progeny testing is costly to undertake as a range of distinct site types of sufficient uniformity are required to establish well replicated experiments. Preliminary assessments are available after 6 years, but some characters, eg wood density, can only be evaluated later. This requires a longer period of maintenance and also necessitates larger plot sizes with many more progeny. The true potential of a parent tree can only be judged from the results of such long-term and large scale field experimentation. With less than 10% of the original phenotypes providing superior genotypes, standards of selection are demanding.

Since the length of the breeding cycle is a major limitation to the rate of genetic improvement in forest trees, various attempts are being made to shorten it. For example, trees are being raised in large polythene houses and the plant growth regulators, gibberellins 4 and 7, are injected to enhance flowering (Philipson, 1987). Various artificial screening methods, eg frost tolerance (Rook *et al.*, 1980) are under review and efforts are continuing to improve juvenile/adult correlations.

Results from our present programme using seed from seed orchards and multiplying by vegetative propagation show that genetic gains in height growth at age 6 years of 15% have been attained; 10% increases in diameter growth at age 20 years, ie basal area increments of 21%, at age 20 have been measured and >10% increases in volume production at the end of the rotation are confidently predicted (Lee, 1989). Further significant gains from more intensive selections are anticipated, especially from tested specific crosses between known males and females.

HYBRIDS

As for some agricultural crops, the progenies of some interspecific crosses exhibit superior growth when compared to either parent. Interspecific hybrids are important in forestry. Poplar hybrids such as *Populus interamericana* are produced from seed then commercially made available to the grower as cuttings. A major programme is underway to develop improved F1 hybrids of European and Japanese larch (*Larix x eurolepis*). Research is investigating possible methods of multiplying the limited amount of hybrid larch seed by vegetative propagation.

There is also interest in hybrids between Sitka spruce and some of the other north American spruces, particularly *P. glauca*, to provide greater frost resistance and tolerance of drier sites.

IMPROVEMENT USING VEGETATIVE PROPAGATION

Clonal forestry has been practised with tsugi (*Cryptomeria japonica*) for centuries in Japan and for many decades in Europe with poplars (Toda, 1974; Libby, 1987). The gains in yield and greater uniformity of some tree crops have been most impressive, eg the volume of *Populus interamericana* "Unal", a new poplar cultivar, has been estimated as being almost three times that of *P. robusta* at age 9 years (Steenackers, 1984).

In future, vegetative propagation will be increasingly important in forestry, especially in intensively managed, short rotation plantations. The best individuals from a breeding population can be supplied to the forest industry more quickly than going through a conventional tree breeding programme employing seed orchards (Kleinschmit, 1987; Mason, 1989). This approach is also used when seed production is spasmodic and insufficient, due to poor flowering or insect and pathogen attack. Use of rooted cuttings may provide a constant source of material, although this may only be possible when cuttings are taken from physiologically young trees. Leakey and Ladipo (1987) claim for the west African hardwood, *Triplochiton scleroxylon*, that by their selection, multiplication and planting only high yielding clones, it is possible to achieve an 8 fold increase in yield. Improvements in other characters such as form and branching habit are claimed to be equally as impressive (Leakey and Ladipo, 1987). Similar approaches of selecting for branching characteristics are being used in this country for improving oak (Harmer, 1988).

Several *in vitro* propagation methods are being developed for forest tree species and they range from the relatively simple technique of micro-propagation to the more sophisticated techniques of somatic embryogenesis and production of transgenic plants (Hanover and Keathley, 1988). These methods are being developed to allow rapid multiplication of new genotypes (John and Mason, 1987) or manipulation of heritable variation to alter genetically individual trees (Hanover and Keathley, 1988). An example of particular interest is the introduction into poplar of a foreign gene, via the soil microorganism *Agrobacterium tumefaciens* as a vector, which confers tolerance to the herbicide glyphosate (Fillatti *et al.*, 1988).

Currently, the production of planting stock by *in vitro* propagation is substantially more costly than using rooted cuttings and considerably more than seedlings. In addition there are also dangers in using solely vegetative propagation as can be seen from experience with tsugi and poplars. Reduction of genetic variation needs careful consideration and there is a need for broadly based breeding populations to augment clonal forestry. Without variability there is no potential to improve or respond to changing requirements. The new biotechnologies being developed suggest immense gains but they should be seen as supplements to the improvements obtained via traditional breeding methods. Potential improvements obtained using new technologies will need to be thoroughly tested under field conditions.

TREE IMPROVEMENT AND WEED CONTROL

Any tree improvement programme costs money and improved seed can be several times the price of unimproved seed; these costs will be multiplied further if vegetative propagation methods are also employed. Justification for payment of these added costs are based on increased profitability from the gains in yield, improved quality or reduction of costs.

Economic analyses carried out on the use of improved stock emphasise that the greatest returns can be expected on the best sites. On this basis it is not possible to justify the use of improved stock for the poorer, exposed sites in this country (Mason, 1989).

Having made the decision to use improved stock it is important that the other aspects of silviculture are optimised as much as possible. The extra money paid for improved stock is wasted if proper measures are not taken to protect the trees after planting and to encourage rapid growth. McIntosh (1981) observed on experiments with Sitka spruce growing on mineral soils that weed control resulted in greater early height growth than fertilizer applications. In some experiments the trees only responded to P fertilizer applications in the absence of weeds.

It should be noted that the greater protection need not of necessity lead to greater establishment costs. Although the 15% increased height growth which has been measured for 10 year old Sitka spruce from progenies produced by our breeding programme will have tended to reduce the number of weedings required and number of years of protection from fences, the reductions in costs would have been negligible. A 20 to 30% gain in early height growth, ie 20 cm extra height at age 5, could however be important in reducing establishment costs. Positive selection for rapid early height growth on the other hand could downgrade timber quality to be saleable only at reduced prices.

The more common reforestation of forests in this country is providing problems where it might be economically justified to use the best genetical material. Many Sitka spruce stands on being felled regenerate a dense carpet of seedlings. Clearing the site is costly and improved planted stock may be difficult to distinguish from the regenerated seedlings - in addition to running the risk of being smothered by vigorous regeneration growth. In this example the availability of genetically improved Sitka spruce with tolerance to glyphosate or other appropriate herbicide would be a considerable advantage.

Looking further into the future, I presume we can expect the development of other generations of more efficient and selective herbicides or herbicide modes of action. Early screening for tolerance to herbicides of progenies from our breeding programmes could be relatively easily included.

Many trees exhibit allelopathy, ie suppression of germination or growth of neighbouring plants by chemicals which are leached, exuded or volatalised from the plant. Introduction of gene(s) to provide allelopathic control of competing neighbours is an obvious goal for those of us working on tree improvement and weed control. Regrettably it is probably a distant goal as, is the case in most of the new innovative biotechnology, advances are being hampered by the lack of knowledge of the basic physiology and biology of species important in forestry.

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WEED COMPETITION AND CONTROL IN NEW TREE PRODUCTION SYSTEMS

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ABSTRACT

The weed problems encountered in new tree plantings for timber, energy, conservation and amenity uses are reviewed with emphasis on perennial weeds. Weed competition is one of the most important factors leading to failure in establishment. Opportunities for using pre- and post-planting herbicide treatments, and non-chemical control methods, are discussed. There is a need to avoid over-dependence on one herbicide, glyphosate. Greater awareness of the potential benefits and difficulties of herbicide use is required, along with 'user friendly' systems of herbicide selection.

INTRODUCTION

Recent years have seen an upsurge in commercial and public interest in tree crops in the UK. This stems from a number of factors. There is increased public concern for the countryside and the health of the nation's woodlands. Food production is currently in surplus so alternative uses for agricultural land are needed. There is a demand for wood; the UK spends £4-5 billion annually on imported timber and timber products much of which could be supplied by home-grown timber. Wood from fast growing species such as poplar and willow may also be an alternative energy source. Tree planting for amenity and recreational purposes is also increasing, giving greater diversity in associated plant and animal species. These changes broadly determine the locations and characteristics of current tree plantings; surplus agricultural land is available both in upland and lowland areas. A greater variety of species is being planted, with emphasis on broad-leaved trees. Farm forestry normally involves planting in distinct blocks or strips and timber production may be less important than provision of conservation areas or game cover. Agroforestry systems, with either grass or arable intercropping are also being examined. The production of trees for uses other than timber - notably for energy or pulp - is also being tested; short rotation coppice of willow or poplar or single stem poplars are the most promising systems. Finally, there is the recently announced national commitment to urban forestry - development of woodlands on the fringes of major conurbations primarily for aesthetic and recreational reasons. With all these systems there is an overriding need for successful and rapid tree establishment - either to permit harvesting and, therefore, return on investment as early as possible, or to create the desired visual impact or game cover in minimum time. Weeds present one of the main obstacles to achieving these objectives - they threaten the survival or growth of newly planted trees in all systems and situations. The nature of this threat, the opportunities for overcoming it and the problems still awaiting resolution are reviewed in this paper.

THE WEED PROBLEMS OF NEW PRODUCTION SYSTEMS

The weed species causing problems are initially more likely to arise from characteristics of the sites than of the growing system. An example of this is the use of natural regeneration of trees to form new forests where the first and most difficult requirement is control of unwanted trees of the same species or habit. The main areas for new plantings are in low-grade agricultural land and perennial weeds will usually be the dominant problem both in upland and lowland areas. The extent and frequency of these weeds is often not apparent while land remains in pasture or arable crops, but removal of competition from a ground cover crop enables a rapid build-up of perennial weeds. In upland areas, perennial grasses, Pteridium aquilinum (bracken) and Cirsium arvense (creeping thistle) will dominate, while in lowland areas Convolvulus arvensis (field bindweed) and Equisetum arvense (field horsetail) present severe problems. The weed flora resulting from arable land will differ from that in pasture. Cirsium arvense, Convolvulus arvensis and Elymus repens (common couch) are frequent in the former but certain annuals, notably Polygonum species also cause particular difficulties because of their resistance to many residual herbicides. Where land has been under pasture, Cirsium arvense is the most common problem but Trifolium repens (clover) and Ranunculus species (buttercups) occur frequently. Maintenance of bare soil under trees with herbicides is the commonest weed control system practiced and in this situation, perennial weeds such as Cirsium arvense, Convolvulus arvensis and Elymus repens spread rapidly, patches can extend by several metres a year even when cut or sprayed with herbicides.

While the basic problems result from past history of the land planted, their severity depends greatly on the type of crop grown. The biomass crops poplar and willow are particularly vulnerable if grown from cuttings because they are rapidly smothered by uncontrolled weeds. The vegetation management system appropriate to urban fringe plantings may also be influenced by the problem of tree death, from vandalism and fire (Boylan, 1988); these problems can be mitigated by rapid establishment and canopy closure of high density plantings maintained with bare soil.

Agroforestry crops, particularly those grazed by stock, present problems of re-invasion of tree base areas from the reservoir of perennial weeds often present in the adjacent pasture. This will necessitate repeated treatment with herbicides during establishment. A particular problem of silvo-arable systems is tree damage from the herbicides applied to arable crops e.g. glyphosate applied to desiccate arable crops may drift onto trees which are particularly susceptible in late summer. With sylvo-pastoral systems an additional difficulty is restriction of stock access following application of certain herbicides.

In farm and urban fringe forestry, certain climbing weeds can quickly smother young trees (Hibberd, 1989). Species occurrence depends on soil type and condition but Tamus communis (black bryony), Calystegia sepium (hedge bindweed), Lonicera periclymenum (honeysuckle), Clematis vitalba (old man's beard) and Humulus lupulus (wild hop) occur commonly. However, in urban situations some of these species may be regarded as desirable.

WEED COMPETITION

The competitive effects of weeds and the need to weed have no doubt been recognised by careful growers since tree culture began, but documented evidence is comparatively recent and sparse. Davies (1985) reported results of Forestry Commission experiments carried out 60 years ago in which hoeing around young broadleaf trees improved growth considerably compared with repeated cutting of vegetation. The introduction of herbicides has allowed the effects of weed growth to be assessed in the absence of the soil disturbance and root damage inevitable with cultivations. White and Holloway (1967) demonstrated the competitiveness of annual weeds in tree fruit establishment while Atkinson and White (1976) showed the competitive effects of grassed alleys on growth of young fruit trees and that water was the main limiting factor. Davison and Bailey (1980) showed that growth of newly-planted *Acer platanoides* was severely restricted by uncontrolled annual weeds, the main competitive period being May and June.

In forestry results of weed competition experiments have differed according to geographical area. Newly-planted conifers in upland areas of the UK have not generally shown major growth increases from complete weed removal particularly on deep or peaty soils (McIntosh, 1980; Tabbush, 1984) so cutting weeds that compete for light is often the only control measure. In some experiments growth increases were recorded only where fertilizer addition accompanied weed control, suggesting that, on infertile soils weeds will prevent trees benefitting from additional nutrients. Although experimental evidence of significant weed competition for water is lacking it is accepted that it can restrict growth in well drained soils in dry seasons.

In a range of sites in the English lowlands Davies (1985) clearly demonstrated the competitive effect of uncontrolled weed growth on newly planted broad-leaved trees. The work showed the greater inhibitory effect on tree growth of a mown grass sward compared with uncut grass due to greater water use by the mown grass. This has obvious implications for sylvo-pastoral agroforestry systems where a grazed sward will compete for moisture with the young trees. The size of the weed free area below young trees is also important for survival and growth. Forestry Commission experiments indicate that at least 1 m diameter is needed below transplants and 1.5 m diameter below standard trees (Davies, 1987).

How long is weed control needed after planting? Davison & Bailey (1980) found no growth reduction of *A. platanoides* from annual weed growth in the second growing season, trees having been kept weed free in the planting year. However, Davies (1987) reported large increases in growth of 10 year old Ash trees once weed competition was removed. It is likely that tree species will differ in their ability to withstand weed competition but there is little published information on this aspect. The inhibitory effect of *Calluna vulgaris* (Heather) on Sitka spruce root mycorhizae and consequent inhibition of nitrogen uptake is an extreme example of competition (Hendley, 1963) and also indicates the possibility of allelopathic effects of weeds on tree growth.

Weed competition also has severe effects in short rotation coppice where rapid even establishment is essential for productivity. Stott (1980) found that in willow uncontrolled weeds reduced willow stool survival by 50% in season after planting and shoot weight was reduced by 88%. Weed competition is unlikely to be severe after canopy closure but development of woody weeds, particularly *Rubus fruticosus* (bramble) and *Rosa canina* (dog-rose) can seriously interfere with harvesting.

The effects of uncontrolled weeds are most obvious in amenity plantings such as roadsides where it is accepted that up to 50% of trees planted will fail to establish (Patch, personal communication). The advantages of an effective weed control policy in urban planting have been demonstrated in Milton Keynes, where use of contact and residual herbicides and bark mulches has reduced the time for canopy closure from 10 years to 3-5 years (Salter & Darke, 1988).

WEED CONTROL OPPORTUNITIES

Pre-planting treatments

These are most useful where the perennial weeds present a good target for spraying. This, often, is prior to harvesting the previous crop e.g. where glyphosate is sprayed pre-harvest in cereals for control of E. repens, Cirsium arvense and Convolvulus arvensis (O'Keefe, 1980), spraying vigorous Cirsium arvense in pasture, and control of Rhododendron ponticum prior to felling trees, (Williamson & Mason, 1989). Where land is not ploughed, immediate pre-planting treatments are essential to aid rapid establishment. There is some evidence from sylvo-pastoral experiments that autumn spraying of glyphosate on swards is less satisfactory than spring spraying in that it allows more weed to develop during the growing season and leads to reduced tree growth (Sibbald, personal communication). Where perennial weeds are growing poorly in a preceding crop due to senescence, grazing or shielding by other weed growth, pre-planting use of glyphosate has given poor control. Thus the need to get perennial weeds into the right condition for spraying is paramount when their control in the subsequent crop is risky. Crops which may be at particular risk from herbicide damage include nursery transplant lines and cuttings. In these cases, a fallow may be justified in which annual weeds are controlled by a short-lived residual, herbicide and the perennial weeds sprayed during the growing season with the most effective translocated herbicide. Cultivated fallows are most successful for the control of E. repens and some perennial broad-leaved weeds if the growing season is dry, permitting the desiccation of rhizome fragments. Where the tree growing system permits use of translocated herbicides on perennial weeds after planting, e.g. trees in shelters, there may be no advantage in using a pre-planting herbicide treatment.

Post-planting herbicides

The use of soil-acting herbicides post-planting can provide effective season-long weed control, given the absence of perennial weeds. Traditionally, simazine or atrazine mixtures have been used on conifer crops, but there is a whole range of possible alternatives (Lawrie & Clay, 1989) which may be safer to broad-leaved crops and, in mixtures, effective on a wider range of weed species. Effective use of these herbicides is particularly important in poplar and willow cuttings and in small trees transplanted without tree shelters. These plantings are particularly vulnerable to spray damage if contact herbicides have to be used after planting. The use of soil-acting herbicides may have disadvantages. The unprotected soil surface is more vulnerable to erosion on sloping sites or poaching by stock in sylvo-pastoral situations. The herbicide itself may be leached laterally on sloping sites in heavy rain. Burrowing by vermin may also be a problem. As was found early on in fruit crops, using residual herbicides on such bare soil systems inevitably leads to a build-up of perennial weeds (Robinson, 1964) which, themselves, require treatment.

Foliar-acting herbicides become essential where annual weeds have not been controlled or where perennial weeds develop. Where tree shelters or unfeathered tree stems are present, herbicides such as glyphosate can be used successfully. There are certain weeds more susceptible to other herbicides, e.g. Equisetum arvense to amitrole (Davison and Bailey, 1982) and Trifolium species to triclopyr. Problems arise where foliar-acting herbicides have to be used near vulnerable trees, e.g. young poplar and willow plantings, where overspraying with glyphosate or paraquat can do severe damage, perhaps greater than that caused by the weed infestation itself. In such situations, careful choice of herbicides, spray timing and application methods is essential. With poplar and willow, clopyralid (effective on Cirsium arvense) appears appreciably safer than glyphosate (Clay et al., 1989); amitrole may also be relatively safe as a directed spray in willows early in the season. Dormant, cut down, stools of poplar and willow may also show sufficient tolerance to amitrole to permit overall spraying where weed growth would, otherwise, get out of control (Parfitt, 1989). Fluazifop-p-butyl is selective on broad-leaved crops and effective in suppressing E. repens (Ivens, 1989). Spot spraying, using tree guards, can also be effective for tree-base treatments.

For many situations, therefore, the annual use of a broad spectrum translocated herbicide, such as glyphosate, in early summer may be a preferable alternative to repeated use of soil-acting herbicides. The presence of dead vegetation can maintain good soil surface conditions and temporarily restrict weed growth as well as permitting vigorous tree growth. In some experiments using tree shelters there has been vigourous weed growth inside the tube but this has not appeared to inhibit tree growth (Sibbald, personal communication). A decision on the right herbicide programme will depend on all factors involved including economics - how quickly is a return on investment on trees required, and is optimising tree growth paramount?

Farm woodlands grown primarily for conservation purposes present special problems, and perhaps opportunities. While some tree-base weed control is essential for establishment, large scale use of broad-spectrum herbicides such as glyphosate is undesirable. With the knowledge now available on the selectivity of residual and foliar-acting herbicides, it should be possible to select those which suppress the most competitive, leaving a variety of 'desirable' species according to the properties of the herbicide used (Watt et al., 1988).

Non-chemical methods

Mulches have been used successfully for tree establishment. Since the value of black polythene mulch was demonstrated in fruit trees and bushes (Davison & Bailey, 1979), where it increased growth above that on weed-free soil plots, effective use has also been shown in trees (Davies, 1985; Potter, 1988), poplar and willow cuttings (Parfitt & Stott, 1984) and in silvo-arable systems (Wainright et al.,1989). In some circumstances, such mulches have disadvantages. Anaerobic conditions can develop on poorly drained sites and reduce growth (Davies, 1985) and they should not be used where perennial weeds occur as these may build up rapidly under mulch. Damage to plastic film also occurs from vermin, including voles and their predators. There have also been reports of damage to young trees after mulch removal, where an application of glyphosate has been taken up by the mass of roots near the soil surface (Pudwell, personal communication). A further drawback is the cost of polythene mulch; the material and laying can cost around £1.00 per tree which is, unacceptable for many types of planting. However it can give valuable long-term weed control in situations where regular herbicide use is not feasible or desirable.

Organic mulches such as straw, wood chip or bark have been used. Where straw was used to mulch willow and poplar cuttings, weed control and crop growth were as good as herbicide-treated bare soil, but growth was less than under polythene mulch (Parfitt & Stott, 1984). Soil temperatures in the growing season were lower under straw than bare soil but soil moisture levels were higher. Wood chip or bark mulches need to be thick to suppress annual weeds effectively, and perennial weeds are not controlled (Stimson, 1989). Both wood chip mulch and straw topped by farmyard manure effectively controlled all weeds in the year of planting in a recent sylvo-pastoral experiment at Long Ashton which was stocked with cattle. However, both these mulches were invaded by vigorous growth of perennial weeds (Cirsium arvense and P. aquilinum) in the second growing season. The possibility of tree growth inhibition due to toxins from fresh bark or nutrient deficiencies from use of organic mulches must also be considered (Davies, 1987).

UNRESOLVED PROBLEMS

The requirement for effective tree base weed control in most of the new production systems is clear, but certain problems likely to restrict progress require action from researchers, advisors, users and 'politicians'. The first is a research/advisory problem. The continuous and exclusive use of glyphosate in many growing systems is a recipe for the development of resistant weeds - either from build-up and spread of poorly controlled perennial weeds, such as Convolvulus arvensis or Trifolium repens, or the selection of resistant biotypes as has happened with the continuous use of triazine and paraquat herbicides in fruit and ornamental crops (Clay, 1989). Even if use lasts for only a few years, resistant types of weed species with wind-borne seed can be transferred to newly-treated areas and selection pressure for resistance is, thus, continued. Wherever possible, alternative treatments should be used in rotation. This underlines the need for new herbicides to be available as alternatives. When glyphosate is applied to weeds at the base of young trees, there is also a need to confirm that transfer of root-secreted herbicide from treated weeds to the trees, with consequent damage, is not a problem. Such damaging secretions have been demonstrated with high doses of glyphosate on herbaceous plants (Coupland & Lutman, 1982).

Secondly, there is a need for 'planners' and growers to be aware of the importance of weed control and its three vital elements: the choice of herbicide, the timing of treatment and careful, drift-free, application. Without these three components any system of tree establishment will fail. With some crops, such as poplar and willow grown from cuttings where infestation with perennial weeds is almost inevitable, there is a need for information on the capacity of the crops to recover from different degrees of overspraying with contact herbicides.

More detailed and easily accessible information is also needed on herbicide suitability in terms of crop tolerance, weed control spectrum, cost etc., than is currently available even from otherwise excellent publications such as 'The Use of Herbicides in the Forest' (Williamson & Lane, 1989.) Collated information from product leaflets such as is available on the 'Herbrex' computer database may be one answer (Lawson, 1989); this currently covers amenity plantings but not forestry.

Finally, anomalies in the implementation of the Control of Pesticides Regulations 1986 need to be resolved so that introduction of effective herbicide treatments in these forestry systems is not curtailed. Under the interim arrangements for minor crops, any Approved herbicide can be used on any non-edible crop coming within the "agriculture and horticulture" field of use but not in "forestry". This, therefore, permits use under trees in amenity areas, on roadsides and parks, but not in commercial forestry, farm woodlands or biomass plantings. For applications in such areas, "Off-label" approval is the only route to legalising use and it is not clear whose responsibility it is to obtain this. Manufacturers are, understandably, not often interested in obtaining label clearance for herbicide use in small market, high value crops, particularly where they may be used in areas accessible to the public with the risk of adverse reactions. This issue needs to be resolved to provide the range of treatments required for successful tree establishment. Without such treatments and their timely use, planting schemes will continue to fail as they have in the past, with detriment to the grower, the environment and waste of public money.

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TOLERANCE OF FORESTRY AND BIOMASS BROAD-LEAVED TREE SPECIES TO SOIL-ACTING HERBICIDES

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ABSTRACT

The tolerance of six broad-leaved forest tree species and five biomass species to eighteen herbicides was tested in pot experiments. Diphenamid, metamitron, napropamide, oxadiazon and pendimethalin were not phytotoxic to most species. Most other herbicides were not damaging at recommended doses but caused either initial damage or long-term growth reductions at higher doses. Imazapyr was very damaging to most species and diuron was damaging to all species except ash, oak and sycamore. Terbuthylazine was more damaging than simazine on many species. The results showed that there were a number of residual herbicides tolerated by the eleven species at relatively high doses and which could be effective components of herbicide programmes.

INTRODUCTION

With increasing interest in the UK in broad-leaved tree planting for timber, energy forestry (biomass) and agroforestry there is a need to find effective residual herbicides to prevent weed competition (Clay, 1989). Four pot experiments were carried out at Long Ashton Research Station (LARS) in 1987 and 1988 to evaluate the potential safety of soil-acting herbicides and mixtures on six broad-leaved forest tree species and five biomass species. The herbicides selected for trial were from those approved by MAFF in the UK for use in other crops and their weed control performance was well known.

MATERIALS AND METHODS

In experiments 1 (1987) and 3 (1988) the six broad-leaved tree species tested were, ash (Fraxinus excelsior), beech (Fagus sylvatica), birch (Betula pendula), cherry (Prunus avium), oak (Quercus robur), all planted as 2-year-old trees and sycamore (Acer pseudoplatanus), as 1-year-old trees. In experiments 2 (1987) and 4 (1988) the biomass species used were red alder (Alnus rubra), as 1-year-old trees, two poplars (Populus trichocarpa), Fritzi pauley and (P. trichocarpa) Rap and two willows (Salix burjatica) Korso and (S. viminalis) Bowles Hybrid planted as 14 to 17 cm long cuttings in 1987 and 12 cm long in 1988.

Trees were potted up in March each year in 20 cm diameter pots in sandy clay loam + 15% V/V sand + 1.7 g l^{-1} Osmocote (1987) and sandy loam soil + 2 g l^{-1} Osmocote (1988). The biomass species were grown in the same soils but in 15 cm diameter pots. Cherries were cut back to 30 cm from the soil surface in 1988. The plants were put outside after potting and watered overhead and herbicides applied to moist soil 1 or 2 days later for forestry trees and one week for biomass species. Herbicide formulation and active ingredient content used were:- atrazine, 50% S.C.; cyanazine + atrazine, 50% S.C.; diphenamid, 50% WP; diuron, 80% WP; imazapyr, 25% a.c.; isoxaben, 50% S.C.; lenacil 80% WP; metamitron, 70% WP; metazachlor, 50% S.C.; metsulfuron-methyl, 20% WG; napropamide, 45% S.C.; oryzalin, 48% S.C.; oxadiazon, 25% EC; pendimethalin, 33% EC; propyzamide, 50% WP; simazine, 50% S.C.; terbuthylazine, 50% S.C.; terbuthylazine + atrazine, 40% + 10% S.C.

Herbicides were applied over three replicate pots of each species at the doses and on the dates shown in Tables 1-4, using a laboratory track sprayer fitted with an 8002E Teejet giving 425 l ha^{-1} at 175 kPa. Imazapyr was applied as a soil drench using 50 ml of solution per pot at the required concentration. After treatment, plants were placed outside in randomized blocks and watered by rain or trickle irrigation as required.

Plant condition was scored at intervals on a 0-7 scale, 0 = plants dead and 7 = healthiest untreated. At the end of the experiments shoot height and fresh weight were recorded.

RESULTS

Experiment 1

Diphenamid, lenacil, metamitron, napropamide and oxadiazon had little or no adverse effect at the recommended dose or at three times recommended dose on most of the six species (Table 1) and the diphenamid + lenacil mixture was not more phytotoxic than the individual components. Propyzamide damaged both cherry and sycamore but was only slightly phytotoxic to other species.

Oak, beech and birch were tolerant to most of the herbicides at nine times the recommended dose; these species were damaged by imazapyr but not as severely as other species. Isoxaben, oryzalin + diphenamid, terbuthylazine and terbuthylazine + atrazine caused growth reduction in most species mainly at the higher application rates. Atrazine and simazine caused growth reductions in ash, birch, cherry and sycamore; beech and oak were little affected. Atrazine was more phytotoxic than simazine.

Experiment 2

Diphenamid, metamitron, napropamide and oxadiazon were not damaging, or caused only a slight reduction in growth, on the five species at three times the recommended dose (Table 2) but there was greater reduction in growth when applied at the highest doses, particularly metamitron on alder. Oxadiazon at 18 kg ha^{-1} caused considerable leaf necrosis on shoots emerging after treatment but this was outgrown. The highest dose of isoxaben and lenacil reduced growth of most species and 9.0 kg ha^{-1} dose of propyzamide reduced growth of alder and willow (Korso). Simazine caused severe damage to all species at 3 kg ha^{-1} or more. Damage from atrazine and terbuthylazine was more severe than simazine particularly on poplars. All doses of imazapyr were very damaging to all species.

TABLE 1. Effects of residual herbicides on forestry species applied on 11 March 87 to ash, beech (Be), birch (Br), cherry (Ch), oak & sycamore (Sc) (Experiment 1)

Herbicide Doses (kg a.i. ha^{-1})	Total shoot fresh wt [a] (% of untreated), 13-14 January 88					
	Ash	Be	Br	Ch	Oak	Sc
Atrazine (1, 3, 9)[b]	59*	93	59*	98	126	59*
Diphenamid (4, 12, 36)	81	83	87	87	122	75
Imazapyr (0.2, 0.8, 3.2)	46*	116	94	23*	81	29*
Isoxaben (0.15, 0.45, 1.35)	44*	89	70	53*	97	76
Lenacil (1, 3, 9)	112	106	107	81	84	61*
Lenacil + Diph. (1+4, 3+12, 9+36)	107	124	104	67	94	83
Metamitron (3, 9, 27)	105	115	98	68	100	91
Napropamide (3, 9, 27)	110	108	95	108	71	73
Oryzalin (3, 9, 27)	80	96	92	77	107	59*
Oryzalin + Diph. (3+4, 9+12, 27+36)	99	122	76	60*	67	67
Oxadiazon (2, 6, 18)	101	107	96	91	98	74
Propyzamide (1, 3, 9)	94	121	106	65	102	68
Simazine (1, 3, 9)	80	89	79	87	143	71
Terbuthylazine (2, 6, 18)	58*	96	35*	26*	111	54*
Terbuth. + atra. (2, 6, 18)	78	96	60*	34*	94	39*
Untreated (fresh wt, g)	(43.9)	(23.9)	(56.2)	(35.8)	(30.4)	(7.42)
SED trt v untrt (df 98)	±18.0	19.7	17.2	17.9	19.8	19.2

* indicates values significantly lower than untreated at $P = 0.05$

[a] values represent means of the three doses

[b] generally lowest dose corresponds to recommended dose

TABLE 2. Effects of residual herbicides on biomass species applied on 16 March 87 to alder, poplar, Fritzi pauley (PF) Rap (PR) and willow, Korso (WA) and Bowles Hybrid (WB) (Experiment 2)

Herbicide Doses (kg a.i. ha^{-1})	Total shoot fresh wt [a] (% of untreated), harvested 22-30 September 1987				
	Alder	PF	PR	WA	WB
Atrazine (1, 3, 9) [b]	51*	15*	26*	49*	47*
Diphenamid (4, 12, 36)	74	108	103	82	100
Imazapyr (0.2, 0.8, 3.2)	25*	6*	0*	0*	20*
Isoxaben (0.15, 0.45, 1.35)	74	92	83	70*	90
Lenacil (1, 3, 9)	63*	102	73	40*	67*
Metamitron (3, 9, 27)	70*	94	94	78	96
Napropamide (2, 6, 18)	76	103	83	113	92
Oryzalin (3, 9, 27)	66*	73*	76	61*	74*
Oxadiazon (2, 6, 18)	88	115	88	106	103
Propyzamide (1, 3, 9)	56*	116	99	86	112
Simazine (1, 3, 9)	75	60*	66*	53*	54*
Terbuthylazine (2, 6, 18)	43*	17*	15*	23*	20*
Untreated (fresh wt, g)	(151)	(28)	(30)	(22)	(48)
SED trt v untrt (df 81)	±14.2	12.9	14.2	14.3	12.8

* indicates values significantly lower than untreated at P = 0.05
[a] values represent means of the three doses
[b] generally lowest dose corresponds to recommended dose

<u>Experiment 3</u>

Pendimethalin and simazine + propyzamide were not damaging in this experiment (Table 3). Cyanazine + atrazine also were relatively safe although some chlorosis occurred on birch three months after treatment with 6.0 kg ha^{-1}. Necrotic symptoms occurred with metazachlor on both beech and oak in the two months after treatment but this was outgrown. Metsulfuron-methyl initially appeared to be non-phytotoxic on all species but later scores and shoot weights indicated that some damage and reduction in growth occurred to both cherry and sycamore. Diuron was the most damaging herbicide in this experiment, the 3 kg ha^{-1} dose reduced growth of beech, birch and cherry. However, ash, oak and sycamore were unaffected.

TABLE 3 Effects of residual herbicides on forest species applied on 10 March 88 to ash, beech (Be), birch (Br), cherry (Ch), oak and sycamore (Sc) (Experiment 3)

		Score of condition and total shoot fresh wt (% of untreated)											
	Dose	Ash		Be		Br		Ch		Oak		Sc	
Herbicide	(kg a.i. ha^{-1})	S	W	S	W	S	W	S	W	S	W	S	W
Cyanazine +	2.0[a]	5.7	122	6.0	93	6.7	108	6.3	102	6.3	96	6.7	99
atrazine	6.0	5.3	81	7.0	162	7.0	130	6.3	104	7.0	121	5.7	154
Diuron	1.0	5.7	97	7.0	106	6.3	117	6.7	103	7.0	152	6.7	101
	3.0	5.7	112	2.7*	51	1.3*	13*	4.0*	57	7.0	139	6.3	144
Metazachlor	1.0	6.0	113	6.3	139	7.0	86	7.0	92	7.0	143	5.7	126
	3.0	6.7	113	5.3*	80	6.7	82	6.3	92	7.0	101	7.0	107
Metsulfuron	0.01	6.0	105	6.7	118	7.0	102	6.0	106	6.0	142	5.7	78
-methyl	0.03	6.7	127	6.7	118	7.0	93	5.3	73	7.0	136	6.0	80
Pendimethalin	2.0	5.7	101	6.7	152	6.7	109	6.7	109	7.0	150	6.0	132
	6.0	6.0	174	7.0	106	7.0	109	7.0	114	6.3	142	5.7	84
Simazine +	0.5+0.5	6.0	85	6.7	120	7.0	102	6.7	140	7.0	126	6.0	120
propyzamide	1.5+1.5	5.7	108	6.3	142	7.0	91	6.3	111	6.3	98	5.3	101
Untreated		6.2		6.6		7.0		6.4		6.3		6.2	
(actual value, g)			(15.4)		(8.4)		(32.4)		(40.5)		(7.1)		(16.6)
SED trt v trt (df 30)		± 0.79		0.62		0.52		0.69		0.67		0.83	
trt v untrt		± 0.64	39.1	0.50	30.7	0.42	16.0	0.56	24.3	0.55	30.2	0.68	48.9

S = 0-7 score of condition 23 Sept 88; 0 = dead, 7 = healthy
W = total shoot fresh wt 8 Feb 89
* = indicates values significantly lower than untreated at P = 0.05
a = generally lower dose indicates recommended dose

TABLE 4 Effect of residual herbicides on biomass applied on 22 March 88 to alder, poplar Fritzi pauley (PF), Rap (PR) and willow Korso (WA) and Bowles Hybrid (WB) (Experiment 4)

Herbicide	Dose (kg a.i. ha^{-1})	Alder S	Alder W	PF S	PF W	PR S	PR W	WA S	WA W	WB S	WB W
		Score of condition and total shoot fresh wt (% of untreated)									
Atrazine	1[a]	5.0	111	2.3*	58	3.3*	62	4.0*	79	4.3*	94
	2	3.3*	41	1.3*	34	3.0*	60	1.3*	9*	2.7*	84
	4	0.3*	0	0*	0*	0.7*	0*	0.7*	0*	0.7*	14
Cyanazine + atrazine	1	5.7	115	4.3*	58	6.3	105	7.0	133	6.3	102
	2	6.3	167	1.7*	35	3.0*	71	3.7*	79	5.0*	119
	4	3.7*	70	0.7*	0	2.0*	49	1.7*	46	4.0*	113
Diuron	1	6.0	146	6.3	163	6.7	112	6.7	130	7.0	112
	2	5.0	32	2.7*	61	5.0	110	4.7	92	4.7*	109
	4	0.7*	16	1.0*	25	0.7*	3*	0.3*	0*	1.3*	17*
Metazachlor	2	4.7	102	4.3*	68	4.0*	102	5.0	118	4.3*	90
	4	4.3*	32	3.7*	67	4.3	140	4.3	97	4.3*	100
	8	3.7*	16	3.7*	84	3.3*	115	3.0*	61	3.3*	62*
Pendimethalin	1	6.0	114	7.0	135	7.5	189	6.7	120	7.0	112
	2	6.3	143	7.0	116	7.0	155	6.7	111	7.0	113
	4	5.7	95	7.0	142	5.7	149	6.3	120	7.0	111
Untreated (actual value, g)		5.8	(62)	6.9	(54)	6.3	(40)	6.3	(31)	6.6	(68)
SED trt v untrt (df 39)		± 0.65	49.0	0.71	35.3	1.13	44.8	1.02	34.6	0.41	16.0

S = 0-7 score of condition 3 June 88; 0 = dead, 7 = healthy
W = total shoot fresh wt 22-23 Aug 88
* = indicates values significantly lower than untreated at P = 0.05
[a] = generally lower dose indicates recommended dose

Experiment 4

Pendimethalin did not damage any of the five biomass species when applied at 2.0 or 4.0 kg ha^{-1} (Table 4). Metazachlor caused leaf necrosis and stunted shoots in spring but the plants recovered later in the season. The only appreciable growth reductions were at 8.0 kg ha^{-1} on willows and 4.0 and 8.0 kg ha^{-1} on alder.

All species except Fritzi pauley (PF) were unaffected by cyanazine + atrazine at 1 kg ha^{-1}, but higher doses caused damage, particularly to poplars and Korso (WA). Diuron applied at 4 kg ha^{-1} damaged all species, though on Rap (PR) and the willows the damage was short-term; no damage resulted from the 1 kg ha^{-1} dose. All doses of atrazine were damaging to all species except the lowest dose on alder.

DISCUSSION

The type of evaluation used in these experiments enables us to rank herbicides in order of tolerance, and this information can be used in planning subsequent field trials with mixtures. Currently, the only recommended herbicide for use in transplant lines in forestry is simazine, but for ornamental trees and shrubs other herbicides such as cyanazine + atrazine, diphenamid, diuron, napropamide, propyzamide and simazine have recommendations (Ivens, 1989), and their safety on many species has been confirmed in these experiments.

In the four experiments on the eleven species the results indicated that diphenamid, metamitron, napropamide, oxadiazon and pendimethalin are worth testing in field trials. These herbicides appear the safest of those tested, when applied to 'dormant' newly-planted trees and cuttings. However, there could be a risk of soil splash damage from oxadiazon as observed in the biomass experiment at early growth stages, although this was outgrown. Damage by oxadiazon on cherry and ash was also reported by Bentley and Greenfield (1987) but was attributed to spray contacting emerging foliage. Soil splash damage from oxadiazon has been observed on blackcurrants (Clay & Lawrie, 1987).

Cyanazine + atrazine, lenacil, lenacil + diphenamid and simazine + propyzamide were shown to be relatively safe on the forestry species. Earlier work on oak (Turner & Clipsham, 1984) and other species (Bentley & Greenfield, 1987) also showed that simazine + propyzamide mixture was safe. Propyzamide appeared relatively safe at the lower rates on most species.

Isoxaben, oryzalin, metazachlor and metsulfuron-methyl were generally well tolerated at lower doses by forest and biomass species; beech and oak were least damaged by these herbicides. The significance of short-term damage caused by metazachlor on several species requires further investigation. Metsulfuron-methyl caused growth reduction on cherry and sycamore, which could have been through foliar uptake since these species had started growth when sprayed. In conifers, at least, there is no evidence of damage from metsulfuron-methyl uptake by roots (Clay & Lawrie, unpublished data).

In general terbuthylazine, terbuthylazine + atrazine and atrazine were more damaging than simazine. This is unlikely to be due to contact with foliage except possibly cherry and sycamore. Williamson and Tabbush (1988) found that the mixture was particularly damaging to birch and cherry when applied pre-flush. Imazapyr may be useful as a pre-planting treatment, but there is a need to establish safe time intervals between spraying and planting because of possible root uptake.

These results confirm that some residual herbicides are potentially safe for use on newly-planted broad-leaved trees; mixtures of some of these herbicides could widen the spectrum of weed species controlled. This may also permit reduction of doses of individual components to reduce cost and possible phytotoxicity to the crop e.g. simazine + propyzamide. Further field work is needed to establish safe and effective programmes for the control of weeds amongst the different tree and biomass species.

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RESPONSE OF ESTABLISHED WILLOW STOOLS AT DIFFERENT GROWTH STAGES TO FOLIAR HERBICIDE APPLICATIONS

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ABSTRACT

To investigate the crop tolerance of established willow biomass stools to herbicides with potential for use against weeds of willow beds, six year-old stools were oversprayed with a range of herbicides at up to seven growth stages. Dormant stools were unaffected by paraquat, clopyralid, amitrole or glyphosate at recommended, or amitrole or glyphosate at double, dose rates. Stools with shoots up to 12 cm long were largely unaffected by paraquat or clopyralid: glyphosate was damaging. Amitrole, at recommended rates, damaged both 12 cm and 20 cm shoots. Use of double rate amitrole may be possible at early growth stages for very intractable weed problems.

INTRODUCTION

Short rotation coppice biomass (SRCB) is attracting more interest now as an alternative crop for the production of energy, pulp, or fibre-board. Work at Long Ashton has demonstrated both the value of effective weed control for this crop and the difficulty of achieving it (Stott _et al_., 1989).

Weed control in established SRCB plantations requires herbicides, applied between the coppicing cycles, ideally when weeds are susceptible and stools are resistant. Past experience indicates that foliar-acting herbicides are required, but none of the candidate herbicides is completely selective. There is a need, therefore, to investigate the crop tolerance to foliar-acting herbicides known to be effective on the commonest weeds of SRCB (Clay _et al_., 1989).

MATERIALS AND METHODS

The experiment was done on six year-old stools of _Salix burjatica_ (syn. Aquatica Gigantea) Korso planted at 0.7 m between and 0.35 m within the rows, with a previous history of two annual cuts followed by one triennial cut and one annual cut. Plots consisted of two rows of five stools each (0.7 m x 3.5 m) in four completely randomised blocks.

Single applications of each herbicide treatment were applied by an Oxford Precision Sprayer using a single deflector nozzle at 1 bar pressure and a rate of 500 l ha^{-1}, to a 0.7 m swathe. Dose rates, dates and growth stages (mean length of the three longest shoots) at application are shown in Table 1. All treatments were oversprayed except the final amitrole (see Discussion).

At the end of the growing season, shoot fresh weights and numbers per stool were recorded. An additional count of the number of shoots per stool that exceeded the mean length in a block for unsprayed control plots was included as an indication of quality. No attempt was made to assess the effects of herbicides on weeds; few developed on any treated plots.

Because of the large range of treatment means for all analyses, data were transformed to give sufficient homogeneity. The transformations used were:-

LOG_e - Total weight and mean shoot weight.
Square root - Number of shoots per stool.
Angular transformation ($\sin^{-1}(\sqrt{\%})$) - % shoots exceeding control mean length.
Back transformed means are included in Tables in parentheses.
Analysis of variance was done on the transformed data and treatment interactions derived by Student's T-test.

Some treatments killed all the stools sprayed, leaving no measurable shoots. Others killed some stools or shoots, but at a later stage so that measurable, although dead, shoots remained. In each case, there were insufficient data for analysis. Where all stools were killed, tables are marked *; where some stools were killed, means are included in the tables (marked $^{+}$), but not included in statistical analyses. These means cannot be used for the statistical testing of differences between treatments.

RESULTS

Table 1 shows the percentage of dead stools combining both of the above categories. Stools were not killed by any treatments applied at the dormant stage, nor by paraquat or clopyralid at any growth stage. Glyphosate increasingly caused mortality from bud break onwards, killing most or all stools when applied to 6 cm shoots (recommended rate) or 3 cm shoots (double rate). Recommended rate amitrole did not cause stool deaths until applied to 12 cm shoots, killed all stools when applied to 20 cm shoots, but none when applied to 30 cm shoots. This pattern was repeated for the double rate from the 6 cm shoot stage.

TABLE 1. Effect of treatments on stool mortality: (percent). Nov. 1988.

Spray date Shoot length* Herbicide	Dose (kg a.i. ha^{-1})	5/4 0	13/1 1	19/4 3	25/4 6	5/5 12	10/5 20	17/5 30
Amitrole	4.0	0	0	0	0	30	100	0
Amitrole	8.0	0	0	0	15	75	100	0
Glyphosate	2.25	0	15	20	80	100		
Glyphosate	4.5	0	5	85	80	100		
Paraquat	1.0	0	0	0	0	0		
Clopyralid	0.2	0	0	0	0	0		
Untreated control		0						

*(mean: 3 longest stool shoots: cm)

TABLE 2. Mean fresh weight (g) of shoots per stool (LOG_e transformed). Nov. 1988. Back transformed data in parentheses.

Spray date		5/4	13/1	19/4	25/4	5/5	10/5	17/5
Shoot length*		0	1	3	6	12	20	30
Herbicide	Dose (kg a.i. ha^{-1})							
Amitrole	4.0	5.62 (276)	5.40 (221)	4.94 (140)	4.99 (146)	4.70^{+} (109)	*	5.20 (182)
Amitrole	8.0	5.52 (250)	4.49 (89)	4.72 (112)	4.68 (107)	5.56^{+} (259)	*	4.38 (80)
Glyphosate	2.25	5.38 (217)	4.02 (56)	4.23^{+} (69)	4.26^{+} (71)	*		
Glyphosate	4.5	5.24 (189)	3.56 (35)	4.26^{+} (71)	4.22^{+} (68)	*		
Paraquat	1.0	5.63 (278)	5.37 (215)	5.46 (235)	5.14 (171)	4.98 (145)		
Clopyralid	0.2	5.59 (268)	5.15 (172)	5.41 (223)	5.39 (218)	5.04 (154)		
Untreated control		5.40 (220)						
SED		0.269 (treatments); 0.233 (control v. treatments) 76 df						

*(mean: 3 longest stool shoots: cm)

In all tables, * = all stools killed; + = too many stools killed for meaningful analysis.

TABLE 3. Mean number of shoots per stool (square root transformed). Nov. 1988. Back transformed data in parentheses.

Spray date		5/4	13/1	19/4	25/4	5/5	10/5	17/5
Shoot length*		0	1	3	6	12	20	30
Herbicide	Dose (kg a.i. ha^{-1})							
Amitrole	4.0	4.52 (20.4)	4.38 (19.1)	3.22 (10.4)	3.99 (16.0)	3.90^{+} (15.2)	*	3.75 (14.0)
Amitrole	8.0	4.20 (17.6)	3.29 (10.8)	3.65 (13.4)	3.34 (11.2)	5.05^{+} (25.5)	*	3.04 (9.2)
Glyphosate	2.25	4.22 (17.8)	3.26 (10.6)	3.44^{+} (11.8)	3.87^{+} (15.0)	*		
Glyphosate	4.5	4.16 (17.3)	3.34 (11.2)	4.12^{+} (17.0)	3.90^{+} (15.2)	*		
Paraquat	1.0	4.41 (19.5)	3.95 (15.6)	4.36 (19.0)	3.56 (12.7)	3.84 (14.7)		
Clopyralid	0.2	4.55 (20.7)	4.38 (19.2)	4.51 (20.3)	4.24 (18.0)	3.82 (14.6)		
Untreated control		4.38 (19.2)						
SED		0.333 (treatments) 0.289 (control v. treatments) 76 df						

*(mean: 3 longest stool shoots: cm)

The mean weight of shoots per stool (Table 2) harvested from treated plots was unaffected by any herbicide treatment sprayed on dormant stools, or by paraquat or clopyralid sprayed at any growth stage. Stools which survived recommended rate application of amitrole grew on to give yields not significantly different from the control. Double rate amitrole significantly reduced yield compared with control, paraquat and clopyralid treatments when sprayed at any stage after dormancy; glyphosate at either dose had the same effect, and double rate glyphosate reduced yield more than did the double rate amitrole.

The response to treatments expressed as the mean number of shoots per stool (Table 3) was very similar to that shown by mean weight per stool. Additionally, recommended rate amitrole applied to 3 cm or 30 cm shoots, and paraquat applied to 6 cm shoots significantly reduced shoot numbers compared with both the untreated control and the clopyralid sprayed at the same time.

Mean shoot weight, although responding similarly, differed significantly from the untreated control only where double rate amitrole was applied to 1 cm and 3 cm shoots, and where either rate of glyphosate was applied to 1 cm shoots (Table 4).

TABLE 4. Mean shoot weight (g) (LOG_e transformed). Nov. 1988.

Spray date		5/4	13/1	19/4	25/4	5/5	10/5	17/5
Shoot length*		0	1	3	6	12	20	30
Herbicide	Dose (kg a.i. ha^{-1})							
Amitrole	4.0	2.63	2.47	2.62	2.25	2.05^{+}	*	2.60
Amitrole	8.0	2.68	2.16	2.18	2.43	2.33^{+}	*	2.28
Glyphosate	2.25	2.52	1.69	1.78^{+}	1.57^{+}	*		
Glyphosate	4.5	2.42	1.28	1.43^{+}	1.51^{+}	*		
Paraquat	1.0	2.67	2.64	2.53	2.63	2.33		
Clopyralid	0.2	2.58	2.24	2.44	2.53	2.39		
Untreated control	2.47							
SED		0.165 (treatments)						
		0.143 (control v. treatments) 76 df						

For brevity, the back transformed data, which were derived from data in Tables 2 and 3, are not included.

*(mean: 3 longest stool shoots: cm)

Table 5 shows the percentage of stool shoots exceeding the mean length of each block's untreated control. By reducing shoot numbers or, possibly, weed competition, all treatments gave values greater than 50% (the control set value). For recommended rate amitrole, spraying shoots of 1 cm and 30 cm gave the highest values, which were significantly greater than the dormant or 12 cm shoots. At the double amitrole rate, spraying 6 cm shoots gave the highest value, significantly more than the dormant, 12 cm or 30 cm shoots: double rate amitrole gave significantly lower values than normal rate when applied to 1 cm and, particularly, 30 cm shoots.

TABLE 5. Percent stool shoots exceeding the control mean length (angular transformation). Nov. 1988. Original data in parentheses.

Spray date		5/4	13/1	19/4	25/4	5/5	10/5	17/5
Shoot length*		0	1	3	6	12	20	30
Herbicide	Dose (kg a.i. ha^{-1})							
Amitrole	4.0	61.4	70.9	68.6	65.8	58.5+	*	71.1
		(77.1)	(89.2)	(86.7)	(83.2)	(72.6)		(89.5)
Amitrole	8.0	57.4	60.8	61.9	68.4	57.7+	*	54.0
		(71.0)	(76.2)	(77.8)	(86.5)	(71.5)		(65.4)
Glyphosate	2.25	63.6	58.4	62.4+	62.8+	*		
		(80.2)	(72.5)	(78.6)	(79.0)			
Glyphosate	4.5	58.0	54.9	58.2+	56.9+	*		
		(71.9)	(66.9)	(72.3)	(70.2)			
Paraquat	1.0	64.2	69.2	72.1	71.7	66.9		
		(81.0)	(87.4)	(90.6)	(90.2)	(84.6)		
Clopyralid	0.2	64.0	63.6	68.8	63.1	66.2		
		(80.8)	(80.3)	(86.9)	(79.5)	(83.7)		
Untreated control not included in analysis (constant 50% original: 45° transformed)								
SED		4.53	69 df					

*(mean: 3 longest stool shoots: cm)

Analysis of the lengths of the three longest shoots per stool at the time of spraying showed significant difference only as time progressed: there were no differences between stools sprayed at the same time. Thus, herbicide response was not affected by differences in shoot length at any given spray date.

DISCUSSION

The results for the latest two amitrole treatments, where spraying 20 cm shoots killed all stools but spraying 30 cm shoots left the stools largely unaffected, may have been due to a necessary adaptation of the spraying method. To have oversprayed 30 cm shoots would have risked spray reaching the adjacent treatments. Thus, these were treated in a modified way by spraying shoots between the rows of stools, at the correct nozzle height above the ground and not the whole crop, so that only about 60% of the length of each shoot was sprayed.

Treatments which kill appreciable numbers of stools are obviously unacceptable. The risk of killing a few stools to control otherwise intractable, and perhaps localised, weeds may be worthwhile: double rate amitrole applied to 6 cm shoots or either rate of glyphosate applied to 1 cm shoots are examples of such treatments.

Numbers of shoots per stool, though markedly affected by treatments, are of practical significance only where annual harvests are considered, when all may contribute to yield. However, in the three-to-five year cutting cycles envisaged as optimal for biomass production, many of the initial shoots are shaded out, leaving only two to ten productive shoots, dependent on spacing, at harvest (Stott et al., 1981). At the spacing used in this experiment, the lower figure applies. Thus, a reduction in shoot numbers may be advantageous, provided the surviving shoots grow well. No treatment reduced shoot numbers below these critical levels, and reference to mean shoot weight and percentage shoots exceeding the control mean length (Tables 4 & 5) shows that only double rate amitrole, at some growth stages, and either rate of glyphosate, post-dormancy, significantly reduced mean shoot weight, whilst all other treatments effectively increased shoot length. Notably, recommended rate amitrole applied to 3 cm shoots, or double rate to 6 cm shoots, whilst decreasing total weight or number of shoots per stool, had no effect on mean shoot weight.

Although it may be dangerous to base so critical an operation as herbicide application on experiments on only one cultivar in one season, these results support previous experience that dormant stools can be safely oversprayed with a wide range of herbicides. This safe period may be extended by delaying cutting to produce stools which, whilst not strictly dormant, at least have no expanding buds.

At later stages, paraquat or clopyralid may be used safely to control susceptible weeds (i.e. grasses, many annuals and thistle). More intractable weeds may be controlled, although at some risk, by early applications of glyphosate or later by amitrole at increased strength, particularly if sprayed between, rather than over, the crop rows.

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LONG-TERM STUDY OF HEXAZINONE EFFICACY IN PINE PLANTATIONS

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ABSTRACT

A trial was established in a young scots pine (*Pinus silvestris*) plantation in 1975. Treatment with hexazinone at rates of 1.8, 2.7 and 3.6 kg ha^{-1} was made in June 1976. No phytotoxicity on pine was observed at any rate used. Compared to an untreated hand-weeded control, deeper green colour of needles and increased radial growth was observed in the year following planting. By 1980 hexazinone at 1.8 kg ha^{-1} increased stem diameter by 46% and by more than 80% at 2.7 and 3.6 kg ha^{-1}. Hexazinone at 1.8, 2.7 and 3.6 kg ha^{-1} increased tree height by 15, 27 and 30% respectively. Measurements in 1982 showed smaller differences in growth, but trees treated with hexazinone were still taller than the untreated ones.

INTRODUCTION

Afforestation is considered one of the most important branches of forestry. New technologies and new methods of labour use are required, including the application of the most up-to-date chemicals to enhance productivity and lower costs.

MATERIALS AND METHODS

The experimental plots were at Chotoviny, in the Southern Bohemian State Forests. The area is flat and the forest contains a mixture of spruce and oak trees. The dominant weeds were *Calamagrostis arundinacea* and *Calamagrostis epigejos*, *Carex brizoides*, *Juncus conglomeratus*, *Avenella flexuosa* and *Agrostis tenuis*.

Scots pine seedlings were planted in 1975. The experiment had four plots each of 10 x 40 m with 2 m wide isolation zones. A CP3 knapsack sprayer with a Polijet red nozzle was used to spray hexazinone as a water soluble powder containing 90% a.i., on 10th June 1976. The weather was sunny.

The treatments were as follows:-

1. No chemical treatment, hand weeding only.
2. Hexazinone 1.8 kg a.i. in 300 l water ha^{-1}.
3. Hexazinone 2.7 kg a.i. in 300 l water ha^{-1}.
4. Hexazinone 3.6 kg a.i. in 300 l water ha^{-1}.

RESULTS

Application of the herbicide at an early stage of seedling development was beneficial. A large difference in height and radial growth was found between the untreated and all the treated plots, four years after planting (Table I). 1.8 kg ha^{-1} hexazinone increased stem diameter by 47% and at 2.7 and 3.6 kg ha^{-1} it increased stem diameter by 80%. Seedlings also grew significantly taller averaging 15% higher after treatment with either 2.7 or 3.6 kg ha^{-1} hexazinone.

Six years after planting differences in growth (relative to the untreated control) were smaller than after four years. However, the treated plots contained seedlings which were still substantially taller and which had larger stem diameters than those in the untreated plots.

Table 1. - The effect of hexazinone on height and stem diameter of scots pine seedlings planted in 1975, four and six years after spraying.

Year	1980			1982		
Hexazinone kg a.i. ha^{-1}	No. of trees	Av. height (m)	Av. d* (cm)	No. of trees	Av. height (m)	Av. d* (cm)
0	234	1.50	0.98	220	2.38	2.56
1.8	321	1.73	1.44	315	2.41	2.61
2.7	241	1.90	1.79	240	2.49	2.88
3.6	341	1.95	1.76	331	2.77	3.10

* d = breast height stem diameter

DISCUSSION

The tests results have shown quite clearly that the removal of competition by weeds at the early stages of development of scots pine seedlings improves growth. Seedlings planted in the plots treated with hexazinone have a greater access to moisture, soil nutrients and sunlight. Moreover, hexazinone has another benefit; the dead weeds turned into a protective mulch around the seedlings that helped to keep moisture in the soil. The degrading organic matter may also have played an important role in enhancing the effectiveness of treatment.

These tests clearly showed that 1.80 kg ha^{-1} hexazinone provided adequate protection for early stages of scots pine seedlings development. Although the higher doses resulted in greater growth, there is no point in over burdening the ecological system of the forest. Furthermore, the increased effect of the higher dose declined after several years.

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