Session 5A

What makes a Weed a Major Problem? - Case Studies

Chairman	Mr G W CUSSANS
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Papers	5A-1 to 5A-4

CROP MANAGEMENT AFFECTS THE COMMUNITY DYNAMICS OF WEEDS

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ABSTRACT

In plant ecology the study of stabilized communities makes us understand the factors governing the composition and development of plant communities. With weed communities, however, we are faced with labile habitats and pioneer species interacting with rapidly changing agricultural activities. This paper attempts to establish links between the crop management and the resulting weed flora. It is possible to find some major factors, governing the development of weed communities, but to predict changes in the weed flora on the basis of factual information is difficult, because the multitude of interrelating factors reduces our ability to find cause and effect relationships.

INTRODUCTION

The aim of the study of weed floras is to predict future trends in weed communities with changing land use. But the study of weeds must take several agronomic, soil and climatic factors into account which are all interrelated, or confounded, with each other. These interrelationships make it difficult to unravel the cause and effect relationships. There is a large amount of factual information on weed communities available but there is little understanding of how weed floras are orchestrated by the environment. Crop management is probably one of the most important factors in the development of weed communities but must be viewed as a conglomerate of factors of which the crop species itself may only play a minor part.

This paper attempts to identify some biological and ecological characteristics of crop management and weeds, chosen amongst the infinite, and relate these characteristics to the dynamics of weed communities.

ECOLOGY OF WEEDS

Weed species are pioneers that increase the diversity of agricultural ecosystems by exploiting the environmental potential. These pioneers form the first plant community after a disturbance brought about by harrowing, ploughing, break of season, *etc*. The pioneers pave the way for new colonizing species to gain foothold, species competitively superior to the

pioneers. The presence of weeds on arable land is thus the first step in a succession towards plant communities in equilibrium with the prevailing climate and soil (Holt, 1988). Because weed communities in Northern Europe are mostly annual pioneers they are dynamic communities far from any apparent equilibrium. They can therefore respond rapidly to changes in the environment.

Species which have the same ecological demands are inclined to occupy the same habitats and form a plant community. Weed communities on arable land, like any other plant community, are affected by the environment but the frequent disturbance due to agriculture makes it difficult to define distinct weed communities as in more stable habitats.

Various methods have been employed to describe weed communities (Légère *et al.*, 1993; Post, 1988; Andreasen *et al.*, 1992). The problem with vegetation is that we are working with a multi-variate environment and the number of variates needs to be reduced if we want to understand and predict the floristic composition of weed communities in response to climate, cropping pattern and other environmental conditions (Streibig *et al.*, 1984; 1989).

Although the number of weed species is large, about 200 species, in most crops of Northern Europe the number of predominant weed species are rather low. In barley, 50% of the weed flora was represented by only 5 species and 80% by only 20 species (Andreasen, 1990).

CHANGES IN CROP MANAGEMENT

In the past, rotation of a number of crop types has been an important method of weed control. The chances of any one species becoming predominant were minimized as cultural practices and competitive abilities of crops varied, some weed species were encouraged and others discouraged. The result was a highly diverse weed flora. However, the introduction of the phenoxyalkanoic acid herbicides in the late 1940s, the reduced availability of labor, and the rapid mechanization of agriculture all constituted drastic changes in agricultural management practices. The trend was away from cultural control towards chemical methods of control.

The switch to cereal monoculture has changed not only the proportions of lifeforms of the weed flora but also the distribution of weeds within similar life forms (Haas & Streibig, 1982). This could be linked in the late sixties with other agronomic practices than herbicide use, because these changes were already in progress before the introduction of herbicides. Among the dominant weed species in Finland (species with a frequency of occurrence greater than 65%) only perennial weeds were found in grass leys, whereas the large majority of weed species in winter cereals was annual species (Hansiki & Tiainen, 1988).

In Denmark the percentage of area under cereals has changed only from 59% in 1970 to 56% in 1990, but the proportion between cereal species has changed dramatically. Barley was the predominant crop 20 years ago whereas now wheat has increased its proportion considerably; also winter cereals have increased at the expense of spring cereals. Oats are now virtually nonexistent compared with the area 20 years ago. This shift in cereal growing will affect the weed communities, because the competitive ability of the various cereals differ

(Christensen, 1993).

Any one crop has its own "history" in terms of soil preparation, fertilization, and so on, and each affect the development of a weed flora. The effect of these confounded agronomic factors are difficult to separate and, consequently, conclusions about the causes of changes of the weed flora are often based upon circumstantial evidence.

Parallel with the changes in cereal growing, herbicide use has also changed during the last 30 years. The peak amount of herbicides was in 1980 after 20 years of a steady increase (Fig. 1). The decline in herbicide use after 1980 is mainly due to the introduction of the low-dose sulfonylureas and the Danish Action Plan from 1986 to reduce herbicide use (Haas, 1989; Haas & Streibig, 1993). The herbicide use (Fig. 1) must be considered together with the spraying intensity that denotes the total number of times a unit area is sprayed with recommended herbicide rates within a growing season. Spraying with a recommended rate of 4 g AI of chlorsulfuron per ha in, for example, wheat is biologically just as effective as a recommended dose of 1 kg AI phenoxyalkanoic acid per ha. In both cases the spraying intensity is equal to one. If 2 g chlorsulfuron, 50% of recommended rate, could do the same job under optimum spraying conditions, then the spraying intensity would be 0.5. From a biological point of view the spraying intensity is a relative measure of the environmental impact of herbicides on the agro-ecosystem, irrespective of the quantify used. Since 1984 the spraying intensity has increased somewhat until 1988, even though the amount of herbicides was decreasing (Fig. 1). If the decrease in spraying intensity continues it will affect the floristic composition of the weed flora by favoring species that are able to survive less than recommended rates of common herbicides.

Nitrogen, phosphorous and other important plant nutrients have also shown a dramatic increase since the early fifties and so has farm mechanization. The increased use of fertilizers have made the farmers more independent of soil fertility in that more demanding crops such as wheat could be grown on soil types previously being used for low fertility species, eg. rye and oats.

WEED SURVEYS IN DENMARK

We are fortunate in Denmark to have four weed surveys of arable land. The first one was done at the turn of this century and the second one in the mid-forties; the number of crops and surveyed fields were not large, but the results gave a good indication of the composition of weed communities before the advent of herbicides in the late forties (Haas & Streibig, 1982). The two more recent surveys included more crop types and fields, one was in the sixties and the last one was concluded three years ago (Andreasen, 1990).

A characteristic of the two last surveys is that the samples were taken in fields untreated with herbicides. Consequently, the recorded weed species reflect the effects of the crops without herbicide effects in the year surveyed. Thus, results of the surveys could be looked upon as a reflection of the buffering capacity of the soil seed bank which tends to offset the short term effects of radical environmental changes (Jensen, 1969). The mere number of seeds in the soil, however, may not be correlated with the number of emerged weed species (Hurle, 1988).

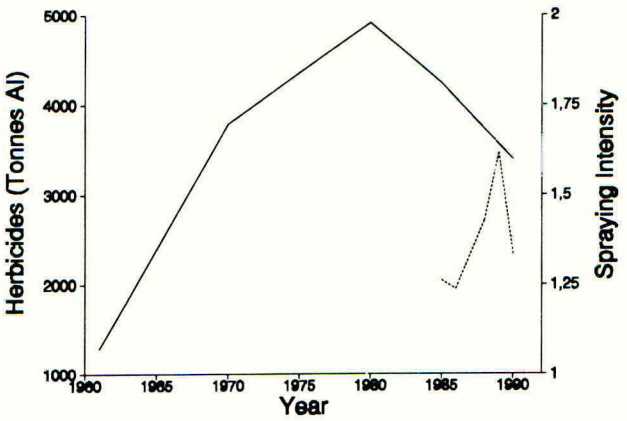

FIGURE 1. Quantity of herbicides used (solid line) and spraying intensity (dotted line) in Denmark. Spraying intensity denotes how many times a unit area is sprayed with recomended rates of herbicides (From Haas & Streibig, 1993).

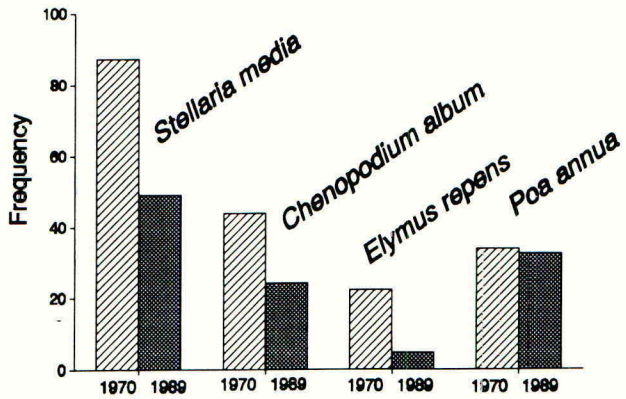

FIGURE 2. Changes in frequency (The probability of finding the species in a field) of some common weed species in spring barley from the 1970 and 1989 surveys. The declines were significant except for *P. annua*.

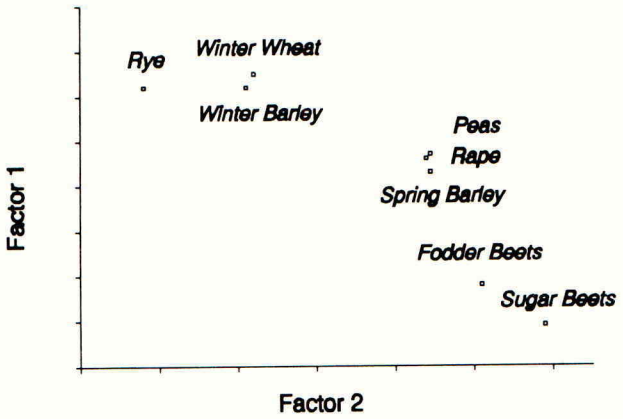

FIGURE 3. Distribution of crops on the two first extracted factors based on weed frequencies within crops from the 1987-1989 survey (Andreasen *et al.*, 1992).

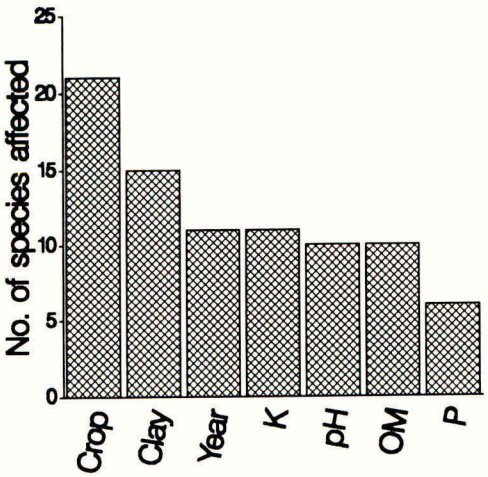

FIGURE 4. Number of the 37 species significantly affected by various ecological factors after being adjusted for the effects of all the other factors the 1987-89 survey. OM = organic matter (From Andreasen *et al.*, 1991).

For the last 20 years the frequencies of some weed species have changed, probably due to the use of herbicides (Fig. 2). In barley, *Stellaria media* and *Chenopodium album* have declined and there has been a dramatic decrease in the frequency of *Elymus repens*. The changes in these species is significant, whereas *Poa annua* has kept its position as one of the most common species.

The analysis of the weed communities of 19 crops in Denmark, based on the survey from the sixties showed clearly that there was a link between the crop types and their associated weed flora. A factor analysis, based on the weed flora of the crops, showed a gradient in the composition of the weed communities and could separate the influence of perennial, winter annual and summer annual crops (Streibig, 1979). A similar analysis was done with the 1987-1989 survey, although perennial crops were not included in this survey, and a similar pattern emerged (Fig. 3). The weed flora of winter and summer annual crops made them fall into two distinct groups. For example, rye which grows on sandy soils and beet, which grows on heavier soils and is a weak competitor early in the growing season, appeared to lie on the periphery of the two main groups.

The distribution of weed species in response to eight crops and seven edaphic factors was evaluated using multiple linear logistic regression with adjustment for over-dispersion (Andreasen *et al.*, 1991). Crop types and sample years were used as classification variables. The regressions showed that crop type is the most important factor governing the distribution of 37 common weeds in the eight crops (Fig. 4) and corresponded well with the factor analysis (Fig. 3). Some detailed studies showed that *Poa annua* was influenced by crop type and organic matter with a significant interaction (Andreasen *et al.*, 1991).

It was surprising that variation between years significantly affected only 11 out of 37 weed species (Fig. 4) although the climatic conditions of the three years were very different. The failure to detect differences in weed frequency among years can be explained by the variability of the data. Differences between years are difficult to detect because the occurrence of species is determined by an array of factors that were not recorded in the survey.

In addition to legislated restrictions on pesticide use in Denmark, there are other constraints on land use and farming that will have an impact on the weed flora. These constraints include: Prohibition of straw burning since 1990: handling of farm manure and fertilizer: compulsory green cover of 65% of arable land during winter. These constraints aim at reducing nitrate leaching and ensuring optimal use of farm manure and commercial fertilizers. These legislative steps will result in an increasing amount of organic matter in Danish arable fields, possibly with positive effects on the water holding capacity of the soil, and thus increase the occurrence of several species, for example, *Poa annua*, *Polygonum lapatifolium* and *Myosotis arvensis* (Andreasen *et al.*, 1991a).

CONCLUSION AND PREDICTION

Among the factors dealt with in this paper, the crop type probably was the most important factor governing the weed communities whilst some soil factors were of importance also. However, the crop type was confounded with soil types.

The use of herbicides has also had an effect; comparison between survey data from the late sixties and the late eighties showed reduction in the frequency of some weed species.

Several legislative constraints on land use, such as reduction of herbicide use, compulsory green cover over arable land during winter, and the new EEC set aside initiative, will change the weed flora.

It is not possible to unambiguously quantify the changes in the weed flora brought about by any one single factor. Although weed communities can shift in response to environmental changes they are buffered by the soil seed bank which in most instances is capable of delivering recruits of weeds to most crop types within a climatic region.

Observations by way of surveys give us a description of the weed flora, but the effect of well-defined non-confounded agronomic factors requires designed long term experiments.

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WHAT MAKES CYPERUS ESCULENTUS (YELLOW NUTSEDGE) AN INVASIVE SPECIES? - A SPATIAL MODEL APPROACH.

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ABSTRACT

A three dimensional spatial model was developed to simulate the demography of Yellow Nutsedge tubers on field level. Beside demographic processes, also spatial redistribution of tubers due to farming operations was simulated. Results showed that farming operations were the main cause of dispersal in the field. Tillage caused a threefold increase in infestation level compared to a no-tillage situation caused by weaker intraspecific competition of the dispersed weed. Growth and control of the weed were affected by intraspecific variation. Intensive and long term control measures were necessary to eradicate the weed from a field.

INTRODUCTION

Cyperus esculentus L. is a troublesome weed in many countries. Recently it was introduced in N.W. Europe where it became a problem in crops like maize, potato and sugar beet (ter Borg & Schippers 1992). It is a pseudo-annual species, mainly propagating and surviving the winter by means of small tubers. These tubers sprout in spring and produce one, occasionally two or three primary shoots; rhizomes develop from the base of the shoots and produce either a secondary shoot or a tuber. Secondary shoots have the same properties as primary shoots. Six generations of shoots can be produced under Dutch climatic conditions during a growing season. Although viable seeds can be produced, seedlings were never recorded in agricultural fields. Therefore the tubers play a pivotal role in the population ecology of *C. esculentus*. Their small size, high winter survival, low sensitivity to herbicides and long viability all contribute to the weediness of the species.

Intraspecific variation of this weed is described by ter Borg *et al.* (1988). They reported morphological as well as ecological variation within the species. One of the varieties, var. *leptostachyus*, was introduced in France in 1947 and is now present in most N.W.European countries including the Netherlands. Var. *macrostachyus* was introduced in the Netherlands in the seventies (ter Borg & Schippers,1992). Both varieties probably originate from the American continent.

Van Groenendael & Habekotté (1988) and Habekotté & van Groenendael (1988) simulated the population dynamics of *C. esculentus* , based on data from the Netherlands. This model calculates the distribution of tubers in several soil layers in time. The model was developed to evaluate control measures and to predict future infestation levels per square meter. Cloutier *et al.* (1988) presented a model, describing the population processes under Canadian field conditions. Both models did not include explicit spatial relations and did not evaluate the effect of intraspecific variation of the weed.

In this paper we want to study the effect of initial tuber distribution, tillage, control measures and intraspecific variation on the long term within-field population dynamics of Yellow Nutsedge. For this purpose a three dimensional spatial model was developed because this allows integration of several processes dependent on horizontal distribution, such as production

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and mortality of tubers, effects of herbicides, as well as dispersal by farm practices. Whenever relevant, results are given separately for two distinct varieties.

THE MODEL

The model simulates the status of the tuber population in a 50 * 50 m2 maize field. This field is divided in square meter grids and eight soil layers of 5 cm are distinguished. All model operations deal with the redistribution, propagation and mortality of tubers in this three dimensional space consisting of 20000 cells, based on data summarized by Habekotté (1988). The structure of the model is based on a series of sequential deterministic equations describing the fate of tubers and plants in each phase of the life cycle in each cell. The processes thus described are: winter survival, redistribution (vertical and horizontal), sprouting, growth and tuber production. The model simulates the situation under continuous maize cultivation. In general the model is comparable to the one presented by Ballaré *et al.* (1987) who simulated the population ecology of *Datura ferox* in Soybean and the model of Auld & Coote who simulated the dispersal of *Avena fatua*. The combination of horizontal and vertical redistribution however is new.

Dispersal of tubers is effected by two distinctly different mechanisms. One is basically due to the plants themselves producing tubers in the network of shoots and rhizomes (Figure 1). The other concerns the effects of farming and cultivation practices, resulting in transport of tubers and plant parts. In the model we consider the tubers as a soil contamination which have the same properties as a soil particle.

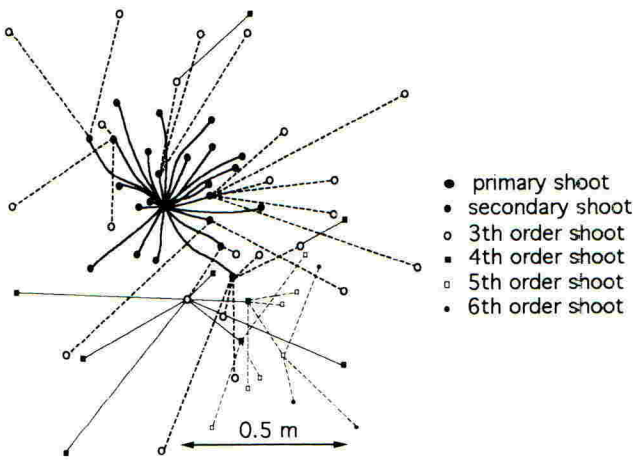

Figure 1. Shoots and the connecting rhizomes of var. *macrostachyus* growing in a sandy soil, without competition.

The model is not completely deterministic. If in a specific cell low numbers of tubers are involved the model treated calculations as a stochastical process. These stochastical calculations are applied when:

$$\mathbf{N}(x,y,z)*\mathbf{P}\leq 5$$

N is the number of tubers in a cell (x,y,z) and **P** is a probability of for instance sprouting, dying or transportation. This ensures more natural dynamics for low population levels.

Modelling the reproduction and mortality of the weed

The two varieties used differ with respect to a number of characteristics, e.g. the number of primary shoots produced by a tuber and winter mortality which, both are higher in var. *macrostachyus* (Table 1). Germination of the tubers is according to data of Stoller and Wax (1973). Germination percentages per soil layer are given in Table 1. They are assumed to be representative for both varieties.

TABLE 1. Tuber germination and winter survival per soil layer. A: spring germination after Stoller & Wax (1973). B: Survival during the 1986/1987 winter season for two varieties (unpublished data L.A.P. Lotz).

	germination in spring	winter survival	
soil layer (cm)		var. *leptostachyus*	var. *macrostachyus*
0-5	1.00	0.55	0.033
5-10	0.87	0.59	0.092
10-15	0.80	0.59	0.20
15-20	0.70	0.54	0.33
20-25	0.60	0.55	0.39
25-30	0.54	0.55	0.45
30-35	0.52	0.55	0.41
35-40	0.52	0.54	0.37
40-45	0.52	0.54	0.37

Parameters for population growth are similar to those used by Habekotté & van Groenendael (1988) and van Groenendael & Habekotté (1988). Three important values can be distinguished:
a) Average number of primary shoots produced by a germinating tuber per year (**P**).
b) Average number of secondary shoots from a primary shoot per year (**S**).
c) Average number of tubers produced by a shoot per year (**T**).
The average number of primary shoots per tuber (**P**) differs between varieties, due to the larger tubers of var. *macrostachyus*. **S** and **T** do not differ (Table 2). The number of tubers in year t+1 (**TU**(t+1)) without density dependent effects, can be calculated as:

$$\mathbf{TU}(t+1) = \mathbf{TU}(t)*\mathbf{P}*\mathbf{S}*\mathbf{T} + \mathbf{TU}(t)$$

If there are many tubers per m2, many primary shoots establish; in such case density dependent effects reduce the formation of secondary and higher order shoots. This results in lower tuber production per germinating tuber. So density effects are operating on the transition of the number of primary

TABLE 2. Parameterisation of population characteristics of two varieties, growing in maize.

	variety	
parameter	var. *leptostachyus*	var. *macrostachyus*
P (primary shoots/tuber)	1.38	1.84
S (total shoots/primary shoot)	3.505	3.505
T (tuber/shoot)	2.37	2.37

shoots (N_p) to the total number of shoots (N_{tot}). Three density levels were distinguished:

$N_{tot} = N_p$	density N_p higher than 794 per m2
$N_{tot} = 30.5 * \sqrt{(N_p - 66.5)}$	between 20 and 794 N_p per m2
$N_{tot} = S * N_p$	for values of N_p under 20.

Experimental field data (Habekotté 1988) showed that the average maximum production of tubers in maize cultivation was about 1000 tubers per m^2.Therefore tuber production in the model is limited to 1000 tubers per m^2 per year.

Tubers produced are distributed in the x,y,z directions according to experimental data. They were found to be produced up to c. 0.7 m from the primary shoot under conditions of unrestricted growth. No essential differences were observed between varieties involved with respect to horizontal dispersal. However, the vertical distribution may differ considerably (Figure 2), the more so after winter kill (Table 1).

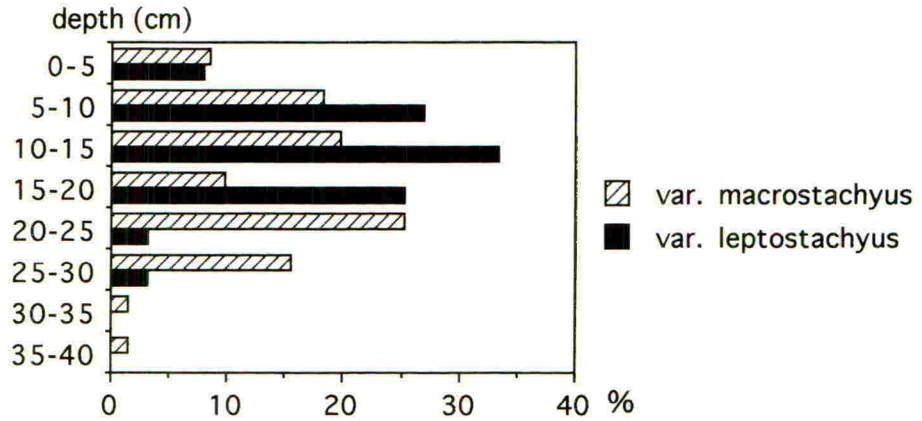

Figure 2. The distribution of new tubers per soil layer of two varieties of *C. esculentus* (original data).

Leaf applied herbicides have only effect on the mortality of shoots. Soil applied herbicides like EPTC and metolachlorine reduce the sprouting of tubers which also reduces shoot formation. Both methods leave the tubers unaffected. The effect of herbicide application in the model results in the reduction of primary shoots which affects the total amount of shoots and new tubers. We assume that herbicide application has no consequences for the dispersal of the weed.

Modelling redistribution of tubers due to farming operations

Several processes determine the redistribution of tubers in the field:

a) Vertical redistribution of the soil due to ploughing and harrowing.
b) Horizontal redistribution of the soil due to soil mixing. Tillage operation causes also horizontal spread.
c) Horizontal redistribution caused by adhering and transportation of soil (and tubers) by farm machinery.

Assuming that tuber behaviour is similar to that of seeds, we used the matrix published by Cousens & Moss (1990) to simulate the vertical redistribution of tubers in the soil. Ploughing was considered to reach a depth of 25 cm only, and tine cultivation a depth of 20 cm.

TABLE 3. Horizontal redistribution probabilities caused by soil mixing processes.

time (year)	grid (y value)								
	-4	-3	-2	-1	0	1	2	3	4
t	.000	.000	.000	.000	1.00	.000	.000	.000	.000
t+1	.000	.004	.054	.242	.397	.242	.054	.004	.000

With respect to horizontal transport in the soil two mechanisms should be distinguished: soil mixing which is a mechanical process caused by tillage and transport processes due to adhesion of soil to machinery. Soil tillage results in both vertical and horizontal redistribution of soil particles. It can be described as a gaussian process (Sibbesen *et al.*, 1985). These authors estimated the variance of all farm operations during one year in sandy soil to be 0.42 m^2 Redistribution probabilities for *C. esculentus* were calculated under the assumption that the tubers are distributed uniformly in the source cell (Table 3). The horizontal spread in the direction of farming operation is assumed to be the same for all soil levels until 25 cm (depth of ploughing).

Soil contaminated with tubers can be attached to farm machinery and can be transported and dropped. This process is very different from soil mixing. Less soil and tubers are involved but the distance of transport can be very high. In contrast to the soil mixing process the soil adhesion process only affects the transport of tubers and soil of the upper 5 cm of soil. Hofmeester (1990) examined the soil adhering to machinery; she found that the amount of soil on a machine is in a steady state, with new soil from the field diluting the soil attached. Two parameters determine this process: the amount of soil attached to a machine and the exchange rate between field soil (upper layer) and soil on the machine. This process can be analytical expressed as a negative exponential relation. Howard *et al.* (1991) used the same relation when analysing the dispersal of *Bromus* species. The machine type in the process of soil adhesion is crucial. Spraying, fertilizing and harvesting are assumed to be clean operations. In the model only a plough, a cultivator and a sowing machine are considered to play a role in this process. Based on data of Hofmeester and Van Dullemen (1989) we estimated the amount of soil attached to these machines to be 2 litre and the exchange rate per square meter as 0.875 liter. With this information we could calculate the effect of there and back tillage, which is common practice in the Netherlands. Figure 3 demonstrates the effect of this mechanism on two lines of contaminated soil.

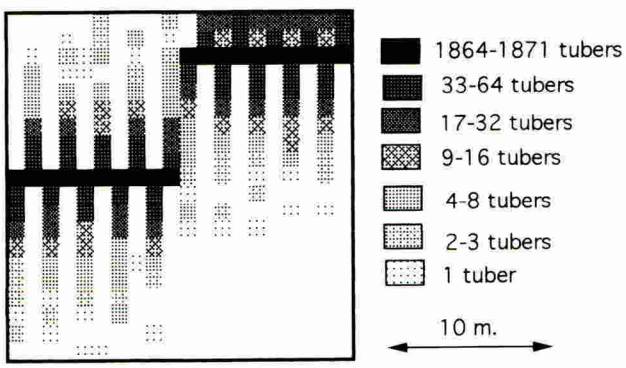

Figure 3. The dispersal of tubers caused by soil adhering to machinery. The pattern is caused by the driving direction. The black lines represent the initial situation of 2000 tubers per cell of 1 m^2 in the upper soil layer.

SIMULATION EXPERIMENTS

A series of simulation experiments was conducted based upon the model described. Several processes are relevant for the population of Yellow Nutsedge:

a) ploughing (vertical redistribution of tubers in five layers)
b) soil mixing (horizontal redistribution of tubers in five layers)
c) deep tine cultivation (vertical redistribution of tubers in four layers)
d) soil adhesion (horizontal redistribution in the first soil layer (twice per year)
e) germination and establishment of primary shoots
f) herbicide application (reduction of primary shoots)
g) development of secondary shoots and tuber formation (natural dispersal in x-y-z direction)
h) winter kill of the tubers in eight soil layers.

The reference is a run with all processes mentioned above except herbicide treatment. It describes the normal growth of *C. esculentus* in the field. All runs concern var. *leptostachyus* except when differences between the varieties are evaluated. The initial situation in the reference run is a local contamination of 10 tubers in the field centre, located in the upper soil layer. All simulations concern this reference run except for processes mentioned explicitly. Tuber distribution in the x-y-z space, infested area (number of cells with at least one tuber) and the total number of tubers in the whole field are evaluated for every run after 10 years.

Initial tuber distribution

The initial distribution of tubers in a field can be an important factor. This was tested by simulating:
a) 10 tubers spread at random in the field
b) 10 tubers situated in one grid-cell in the field centre
c) 10 tubers attached to a farming machine which entering the field from a corner.
The situation after 10 years is given in figure 4. Figure 4a shows the population produced by 10 randomly distributed tubers. Only half of them establish, due to demographic stochasticity. In other words the tuber is ploughed into a deeper layer where it does not germinate, and can be killed during the following winter. Five tubers however survive and the population grows, especially in the direction of tillage. If all 10 tubers are located in the field centre (Fig. 4b), only one population develops, but it is almost certain to do so. However, because of intraspecific competition the total amount of tubers as well as the area covered is reduced. If tubers are imported into the field by machinery, they will be dispersed by the apparatus during the first meters. Because the population is located at the field edge its spread will be limited because of the field boundaries (Fig. 4c).

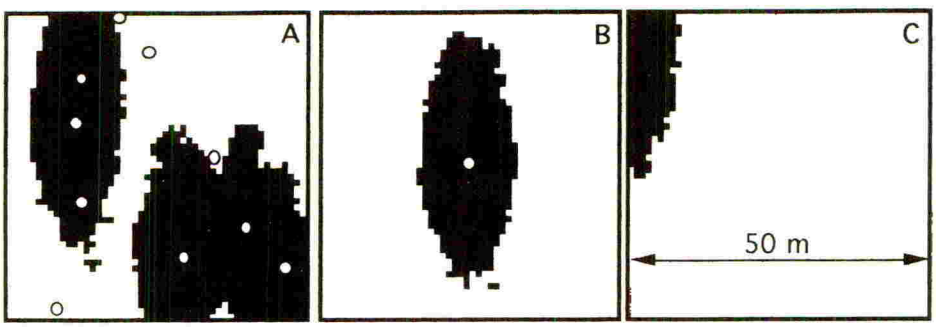

Figure 4. The distribution of *C. esculentus* after 10 years determined by the initial infestation of 10 tubers. a: 10 randomly distributed tubers, b: 10 tubers located in the field centre, c: 10 tubers introduced by farming machinery. White spots and circles indicate initial tuber positions. The black area is contaminated with at least one tuber per m2.

Varietal differences, herbicides and moment of first control

In comparing varieties two questions can be asked: do they differ when growing under standard (reference) conditions, and what is the effect of herbicide treatment? The following simulation experiments were performed:

a) reference run with var. *leptostachyus*
b) var. *leptostachyus* with herbicide control which kills 90% of the shoots; control started in the 4th year.
c) reference run with var. *macrostachyus*
d) var. *macrostachyus*, same as treatment b

Figure 5 shows that var. *leptostachyus* has the highest growth rate, resulting in a c. 10 times larger population after 10 years. The var. *macrostachyus* population appeared to be far more susceptible to herbicide control. It was nearly eradicated after 10 years of control while var. *leptostachyus* is still able to increase under herbicide treatment. The differences can be explained by the differences in winter kill and the deeper tuber formation in the soil which results in lower germination.

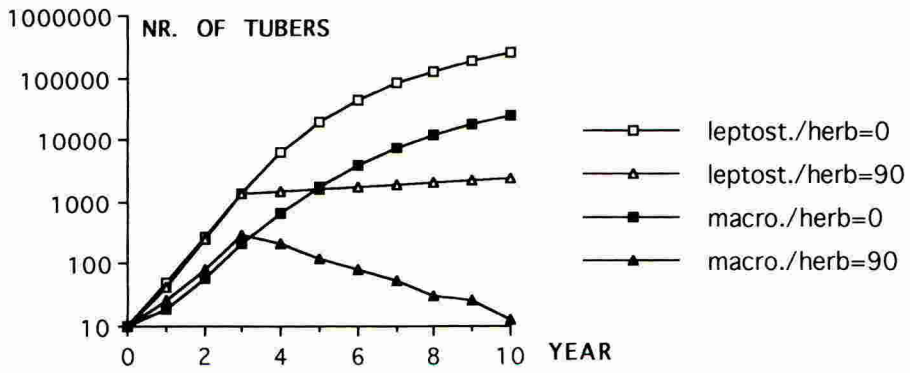

Figure 5. The comparison of two varieties, var. *leptostachyus* and var. *macrostachyus* under normal farming conditions and with a herbicide treatment killing 90% of the sprouts

The effect of herbicide application and the time of discovery / moment of first control

Var. *leptostachyus* is still able to grow under 90% herbicide efficacy (Fig. 5). Application of a more effective herbicide appeared to be necessary. Moreover it might be relevant in which year (after first introduction) the species is discovered and control started. Effects were tested of two levels of effectivity of herbicide treatment (90 and 95 % resp.), and control started after 3, 4 or 5 years after infestation. Figure 6 shows that 95% mortality due to herbicide treatment indeed reduces population size. In the case of early application the population is nearly eradicated in the 20th year. Starting only one year later, this period increases to about 40 years. Note that the number of tubers decreases faster than the size of the infested area, which means that the population density is decreasing. In treatment (H95/Y5) the infested area still increases while the number of tubers in the population is going down. This demonstrates that dispersal mechanisms like soil mixing and soil transport can still be effective, even under low population densities

The effect of horizontal soil moving processes on the spread and distribution of the tubers

To test the effect of the horizontal soil and tuber transport on growth and distribution of the population a sensitivity analysis was carried out described in table 4. The population started with 1000 tubers in the field centre to avoid the effect of too much stochastical variation. The results in Table 4 demonstrate the importance of tillage for growth and distribution of the

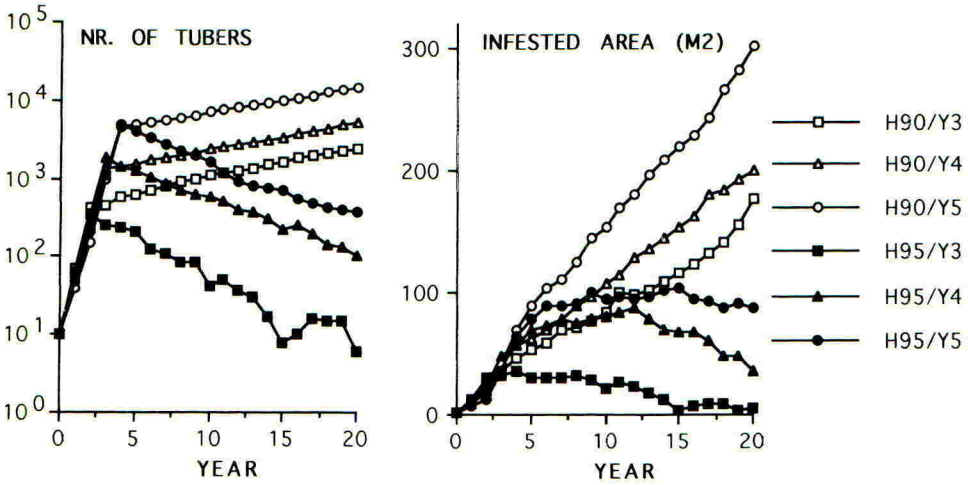

Figure 6. The effect of moment of discovery and start of herbicide treatment (number of years after introduction) and the degree of efficacy of herbicides (90% and 95% effective). a: number of tubers, b: infested area.

population. The soil mixing process appears to be a more important factor than the soil adhesion. This is probably so because the distribution caused by the gaussian mixing process involves all tubers in the upper five soil layers while the soil adhesion process only involves 1.75% of the tubers of the upper soil layer.

TABLE 4. The effect of soil adhering to machinery and soil mixing on the population size after 10 years treatment. The population started with 1000 tubers in the field centre.

treatment		results after 10 years	
adhering	mixing	tuber nr. (*1000)	infested area (m^2)
no	no	135	253
yes	no	339	455
no	yes	385	602
yes	yes	422	693

The effect of exchange rate and soil volume attached to machines on population growth and distribution

The amount of soil attached to a machine depends to a large extent on the type of machine used. Especially potato and sugar beet harvesters transport large amounts of soil, up to 100 litre (Hofmeester 1990). Soil texture and moisture content will affect the process. The rate of tuber transport will also depend on the persistence of the soil at the machine, and on exchange with field soil. Again a sensitivity analysis was carried out (Table 5) under reference conditions (soil mixing included). Table 5 shows that the highest exchange rates gave lower population levels than low and intermediate rates. This is caused by the relatively fast cycle. Many tubers are picked up but most of them are released during the first few meters, where

often tubers are already present. If the exchange rates are low, fewer tubers are transported over a longer distance, but these have a high probability to establish a new subpopulation. At very low rates of exchange the chance of transport becomes too low again.

TABLE 5. The effect of soil transport by machinery and the exchange rate between field and machine on tuber numbers (*1000) and infested area (in brackets) after 10 years.

soil adhering to machinery (l)	exchange rate (l/m^2)			
	0.0	0.2	0.875	2.0
2.0	385 (602)	542 (795)	422 (693)	399 (633)
10.0	385 (602)	649 (939)	631 (855)	611 (821)

DISCUSSION AND CONCLUSIONS

The population growth in a field is found to be limited by the dispersal rate of Yellow Nutsedge. This reduction is caused by intraspecific competition. An increment of the dispersal rate reduces competition which leads to an increment in the population growth rate. Factors limiting dispersal have the reverse effect, which happens for instance when a population meets the field boundary. Therefore also the potential spread of a (sub)population determines its future development and its danger.

Three mechanisms of dispersal of *C. esculentus* are distinguished: dispersal due to plant growth and the production of rhizomes, spread due to soil mixing and spread caused by soil adhering to machinery. All of them are important factors under field conditions. Natural spread is limited to less than one meter per year; hence the species requires a century to reach the other end of a field of just 100 meter. Yet in field conditions the natural spread is still important because it is responsible for the dispersal in the non-tillage direction. Soil mixing concerns tubers in the upper five soil layers, but the distances of transport are relatively small. However the increment caused by the soil mixing causes a rise in tuber production up to three times the natural growth in ten years. Soil adhering on machinery is less effective compared with soil mixing. This is because only a small fraction of the tubers in the upper soil layer is involved in the process. The distance of transportation, however, can be long, therefore the effect on the population growth is still remarkable. Sensitivity analysis of this process shows that soil adhesion is even more important if the exchange rate drops to small amounts of soil. This is because in this case less tubers are transported over a longer distance and can start new subpopulations without competition with the old population. This makes the process of soil adhering the most unpredictable of the three dispersal mechanisms and the most dangerous one. Hofmeester (1990) found that the soil left on machinery after a rough cleaning and a short drive of about 1 km varies from 2 up to 280 kg. This indicates that the soil adhesion process can also be responsible for dispersal between fields.

Control of var. *macrostachyus* seems to be less problematic than that of var. *leptostachyus*. This difference is caused by the lower sensitivity to winterkill of var. *leptostachyus*. This can explain the wide distribution of var. *leptostachyus* in north west Europe.

The moment of the first discovery of the weed is extremely important if the time required for eradication is considered. One year delay in starting the control can double the time needed for complete eradication of *Cyperus* on a field (see Figure 6). This and the long timespans involved in complete eradication indicate that it is nearly impossible to weed out Yellow Nutsedge. These conclusions are supported by results of Rotteveel and Naber (1993) who reported on long term eradication experiments.

In conclusion, model results indicate that *C. esculentus* is an invasive species because: it has a high population growth rate in the field which is supported by soil tillage; the weed is difficult to eradicate because it is difficult to affect the tubers directly by herbicides or weeding, and because its dispersal is increased by soil cultivation. Further dispersal between fields can be caused by contaminated soil adhered to farming machinery.

ACKNOWLEDGEMENTS

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THE ABUNDANCE OF BROME GRASSES IN ARABLE AGRICULTURE - COMPARATIVE POPULATION STUDIES OF FOUR SPECIES

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ABSTRACT

In 1970, *Bromus commutatus*, *B. interruptus*, *B. mollis* and *B. sterilis* were indigenous to arable habitats in the UK. *B. interruptus* has subsequently become extinct and *B. sterilis* is a serious weed of autumn sown cereals. This paper presents an analysis of the reasons for the present relative abundance of these grasses today, focusing on dispersal and germination behaviour. In arable habitats, population renewal occurs annually from seed shed by the previous generation. In a wheat crop, natural seed dissemination rates varied amongst the four species being slowest in *B. interruptus*, the other species being comparable. *B. interruptus* exhibited precocious germination in response to water supply and readily established seedling populations under water regimes and soil conditions prohibitive to the other species. It is argued that crop harvesting promotes seed dissemination of all four species but the absence of seed dormancy mechanisms in *B. interruptus* renders seedling populations vulnerable to autumn cultivation. Conversely the other species show a protracted germination span allowing establishment to occur after autumn crop sowing. Depth of cultivation selected interspecifically with substantially reduced emergence from seed buried below 100 mm in all species and from 50 mm in *B. mollis*.

INTRODUCTION

Three decades ago, four brome grasses were recorded in the UK as ruderal species of field margins and weeds of arable land (Hubbard, 1984). By the mid 1970's, *Bromus interruptus* (Hack.) Druce (interrupted brome) was considered extinct in the UK (Lucas and Synge, 1978) and *B. sterilis* L. (barren brome) had become noted as a more important grass weed of arable agriculture (Froud-Williams and Chancellor, 1982; Gray, 1981). Neither *B. commutatus* (meadow brome) or *B. mollis* (soft brome) have been viewed as serious grass weeds although the former is increasing in abundance in arable land. All four of these grasses display annual life cycles in arable habitats but periodic defoliation may result in limited perenniality.

This paper reports autecological comparisons of the population ecology of these bromes in an analysis of the underlying reasons for their relative abundance. The four grasses are considered to be discrete species (Smith, 1972) despite noticeable hybridisation within the genus. All species reproduce prolifically by seed with germination in the autumn. A principal reason advanced for the spread of *B. sterilis* is that this species is pre-adapted (Mortimer, 1990) to the niche created by the increased use of winter sown cereals and minimum tillage techniques, its competitive ability (Cousens *et al.*, 1988) and the absence of effective pre- and post-emergence graminicides.

MATERIALS AND METHODS

The seed (caryopses and surrounding lemma and palea) used in all experiments were obtained from plants grown as isolated populations in the field at Liverpool University Botanic Gardens, Ness, England. Source seed of *B. interruptus* was obtained from a germ

plasm bank (Dr. P. M. Smith) whilst sources of other species were obtained from natural populations.

Seed production in the field

As part of a larger field trial into the population dynamics of each species, single plants of all four bromes were raised in 2 m^2 plots in the field. Two experimental treatments were factorially applied ; a) crop density and b) soil compaction at sowing. Plants were raised in monoculture, or winter wheat (*Triticum aestivum* cv Avalon) sown at either 188 kg ha^{-1} (standard sowing density) or at 376 kg ha^{-1} (double density). Wheat was sown by seed drill in October 1987 and brome seed hand broadcast immediately afterwards, plots being thinned to a single brome plant after seedling establishment. After sowing, half of the plots were compacted by wheeling with a tractor at 3 km hr^{-1}, applying a tyre surface pressure of approximately 5000 kg m^{-2}, when stationary. Twenty kg N ha^{-1} supplied as 9:24:24 N:P:K fertiliser were incorporated into the crop seed bed at sowing. Subsequent additions of N were applied to wheat plots as main and top dressings, a total of 140 kg N ha^{-1} being given to wheat at the standard density and 280 kg N ha^{-1} at the double density sowing. Plots were handweeded throughout the course of the experiment and counts of the number of seeds per plant made at harvest in August 1988. Full experimental details are given in Howard (1991).

Rate of Seed Dissemination

Each species was grown as individual plants at the centre of 0.25 m^2 field plots either alone or in winter wheat. Plants were sown in early October 1988 and subsequently thinned to one per plant, wheat being sown simultaneously at 188 kg ha^{-1}. A completely randomised design was used enabling 10 replicate plants of each species to be taken at each of 16 destructive harvests. Nitrogen fertiliser was applied at sowing (20 kg N ha^{-1}) and in February 1989, at 70 kg N ha^{-1}. Once the first species (*B. sterilis*) displayed seed loss from the panicle (noted from daily observations in June), plants were harvested over a 16 week period from 30 June 1989 until 2 November 1989. At each harvest the number of seeds remaining per plant was counted. Linear regression analysis of logarithmic number of seeds in the panicle against time was used to calculate a measure of the dissemination rate and analysis of covariance used to statistically compare rates of dissemination.

Seedling emergence in the field

Freshly collected seed samples of all four brome species were sown in 0.5 x 0.5 m^2 field plots on 7 September 1989. Plots contained either a winter wheat stubble cut one week previously (stubble treatment) or bare soil rotovated to a depth of 100 mm (cultivation treatment) on the day preceding sowing. Within each plot, 100 seeds of each species were evenly sown into the central 0.1 x 0.1 m area. In the stubble treatment, seeds were sown directly onto the soil surface, whereas on the cultivated soil treatment they were covered by raking to a depth of approximately 5 mm with soil. A total of 128 plots were arranged in four replicate blocks, on a split plot design, main-plots being stubble/cultivation treatments and sub-plots the harvest/species combinations. Four destructive harvests were made at fortnightly intervals from sowing. At each harvest, all *Bromus* seeds and seedlings at the soil surface were removed and counted within the entire area of each plot. During the course of the experiment, the mean minimum temperature, at grass level, was 6.8 ± 0.46 °C and the average weekly rainfall was 10.8 ± 2.5 mm $week^{-1}$.

Seedling emergence from depth

Twenty seeds of each species were sown at depths of 10, 50, 100 and 150 mm in 250 mm diameter pots containing compost. Three replicates of each species/depth combination were sown and arranged in a complete randomised block design. The pots were placed in a polythene tunnel house on 24 February 1989 and regularly watered. The number of seedlings

that emerged were counted at weekly intervals for 8 weeks. After the final monitoring, pots were excavated and examined for the remains of seedlings that had not emerged.

Germination in response to water availability in laboratory conditions

Four replicate sets of twenty seeds of each species were sown on the surface of 50g of acid washed sand in separate 90 mm Petri dishes. Watering regimes consisting of three supply volumes, 1, 2 or 5 ml distilled water were applied either every day or on a weekly basis. Water supplied at 5 ml per day provided a continuous 1mm film of water above the sand surface. The experiment was conducted in darkness at 20 ^{o}C. The number of seeds that had germinated were recorded daily, germination being considered to have occurred when the coleoptile had reached 5 mm in length. At each census, seedlings were removed. Seed viability (by tetrazolium chloride testing) was > 89% in all stocks and data presented are corrected for inviability.

Germination in response to water availability and substrate in *B. interruptus* and *B. sterilis*

Fifty seeds of *B. interruptus* (viability 82.6%) and *B. sterilis* (viability 98.2 %) were sown into 355 x 215 mm trays containing either a) acid washed silica sand, or b) John Innes No. 1 peat based compost or c) a mixture (50/50 by volume) of sand and compost. Trays were filled to a depth of 80 mm and substrates were air dried for 7 days at the start. Seeds were sown either in the surface layer or at 50 mm depth of each substrate in a factorial arrangement with four watering regimes; 50, 100, 200 or 500 ml of distilled water given on alternate days. Treatment combinations were replicated twice in a complete randomised block design. Seedling emergence was then monitored over a 14 day period from the start of watering in an unheated glasshouse in March 1991.

RESULTS

Seed production in the field

Seed production in the study season was highest in monoculture plots, *B. mollis* being the most fecund (> 2000 seeds per plant), Figure 1. In this treatment there was no statistically significant effect of soil compaction and mean seed production of the other species was in rank order *B. commutatus* ≈ *B. sterilis* > *B. interruptus*. This ranking was not preserved in the presence of wheat (*B. commutatus* ≈ *B. sterilis* > *B. mollis* > *B. interruptus*), seed production being lowered as expected by the companion crop. At double density wheat, seed production was suppressed further and differences amongst species were minor but plants in compacted plots outyielded those not receiving this treatment.

Rate of Seed Dissemination

Disarticulation of seed from panicles was evident first in *B. sterilis* in late June 1989 with initial seed shed in the other species occurring over the next 14 days. Figure 2 shows that seeds were lost from mature panicles at a constant rate and log-linear regression analysis accounted for at least 70% of observed variation with time. Covariance analysis indicated significant differences amongst species ($F=2.17$, df 3,124, $P<0.01$), between treatments, and a species x treatment interaction ($F=87.02$, df 3,124, $P<0.001$). Dissemination by *B. sterilis* was similar in monoculture and in wheat, 31.5 days being required for 50 % seed loss from panicles. Similarly *B. interruptus* was insensitive to the presence of the crop but took longer (an additional 9 days) to disseminate 50% of seeds per plant. Both *B. commutatus* and *B. mollis* lost seed from the panicle at a faster rate in winter wheat than in monoculture. This was particularly conspicuous in *B. commutatus* where the dissemination rate was doubled in wheat (31 days for the loss of 50% of seed).

FIGURE 1. Seed production (mean ± sem) of spaced brome plants in monoculture □; in winter wheat, standard density ▨; in winter wheat, high density ■. - C, uncompacted soil; + C, compacted soil.

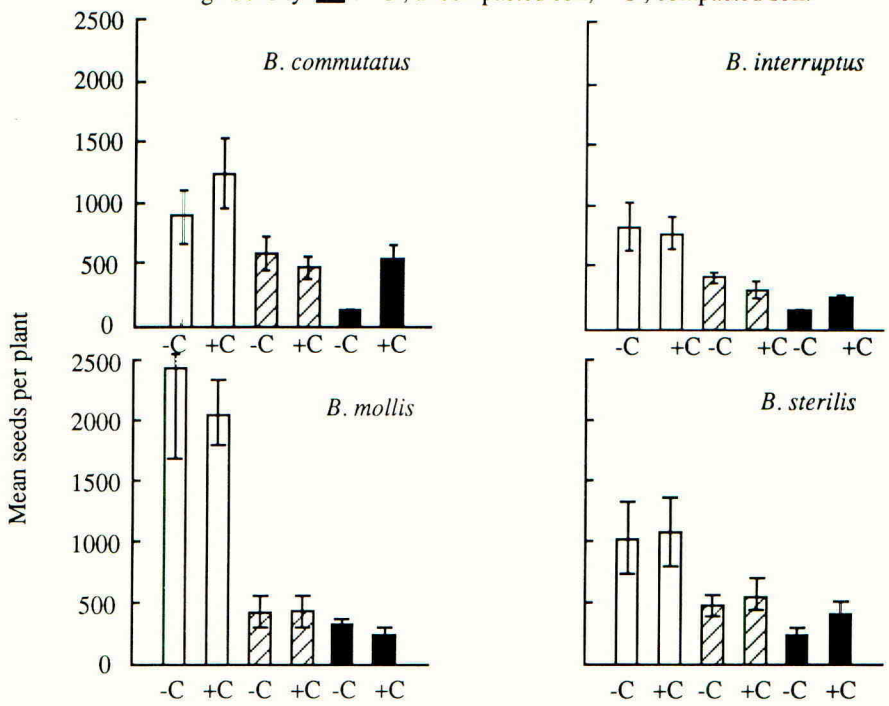
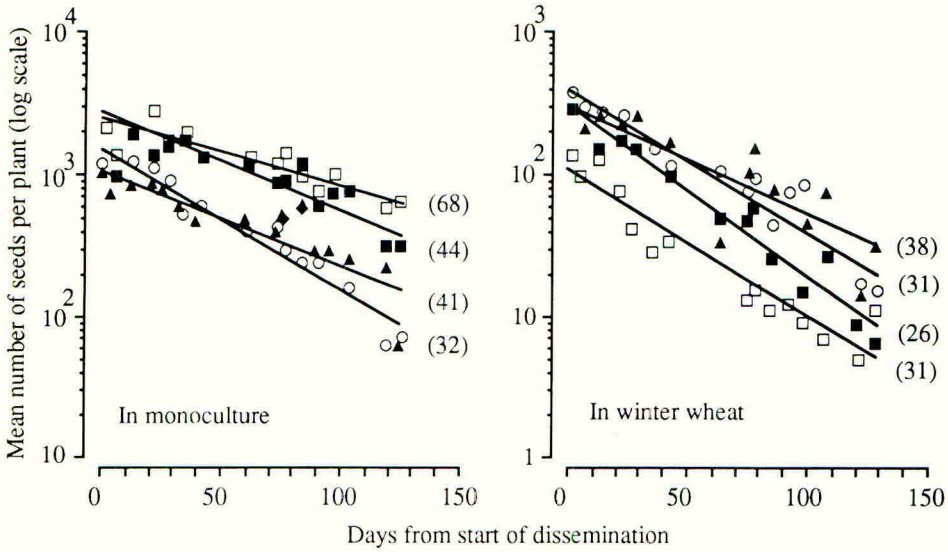


FIGURE 2. Seed loss from plants of brome grasses grown in monoculture and in wheat. *B. commutatus*, □; *B. interruptus*, ▲; *B. mollis*, ■; *B. sterilis*, ○. The time in days to disseminate 50% of seed is shown in parentheses.



Seedling emergence in the field

There were significant differences amongst species in patterns of seedling emergence ($F=7.57$, df 3,87, $P<0.001$) between the stubble and cultivation treatments. *B. sterilis* showed the greatest initial seedling establishment in cultivated soil, 57% in the first 10 days as opposed to less than 50% for the other species (Figure 3). These differences diminished over time and 90% of sown viable seed had established seedlings by late autumn. Recovery of ungerminated seed and subsequent analysis (Howard *et al.*, 1993) suggested that small proportions (<5%) seed of all species may have been induced into dormancy. In the stubble treatment 14 days after sowing, there were three-fold more seedlings of *B. interruptus* than of the other three species and peak seedling emergence in *B. interruptus* was recorded 40 days after sowing. Cumulative increases in seedling number were evident in the other species in stubbles over October and November, these being fastest in *B. commutatus* and *B. mollis*.

Seedling emergence from depth

Planting depth had a pronounced effect on seedling emergence ($F=59.51$, df 3,30 $P<0.001$), all species showing a marked decline in the numbers of seedlings that emerged with increased depth (Figure 4). At the shallowest planting depth of 10 mm, all bromes displayed high levels of emergence (> 91 %). In contrast and with the notable exception of *B. interruptus*, emergence from 100 mm planting depth was greatly reduced, species displaying less than 5 % emergence.

No emergence was recorded for any of the species at the deepest planting depth (150 mm). On termination of the experiment the pots for this treatment were excavated and it was found that for all four species, seedlings had extensive coleoptiles but consistently failed to emerge from this depth.

Germination in response to water availability in laboratory conditions

The four species showed differences in germination capacity after 27 days ($F=8.79$, df 3,69 $P<0.001$) in relation to water availability, Figure 5. *B. interruptus* displayed the highest overall percentage germination, 96%, compared to the other three species which ranged between 80-84 %. Both the rate and the volume of water supply were highly significant in their effect ($F=23.05$, df 1,69 $P<0.001$ and $F=25.98$, df 2,69 $P<0.001$ respectively) on the final numbers of seeds that germinated. *B. interruptus* showed no differences in final numbers germinated and only very slight differences in the speed of germination with reduction in water availability. *B. mollis* showed the largest reduction in germination at the 1 ml week $^{-1}$ water supply and, as with *B. commutatus*, the germination rates increased with increasing volume of water received per week. This pattern was not as evident in *B. sterilis*, the initial rate of germination in the 2 ml week $^{-1}$ treatment was comparable to the other treatments (except 1 ml week $^{-1}$) but declined after the first week.

Germination in response to water availability and substrate in *B. interruptus* and *B. sterilis*

Watering regime, depth of planting and substrate all had significant effects on the pattern of germination of *B. interruptus* and *B. sterilis* species ($P< 0.001$ in all cases). At both planting depths there was an interaction between watering regime and substrate ($F=4.08$ df 6,47, $P< 0.02$) after 7 days. For the seeds planted in the surface layer, germination under the 50 ml watering regime was negligible whilst under the 100 ml watering regime, *B. interruptus* in compost showed earlier and higher germination than the other species-substrate combinations (Figure 6). Under the higher watering regimes, the speed of germination was similar for both species on all three substrates. For the seeds planted at 50 mm depth *B. interruptus* in compost showed considerable germination even under the lowest (50 ml) watering regime. Under the 100 ml watering regime a low proportion of both species germinated rapidly in compost with a similar flush of germination on sand six days later.

FIGURE 3. Seedling emergence (mean ± sem) in the field in four brome grasses in cultivated soil (———) and in wheat stubbles (— — -). Data are corrected for initial seed inviability.

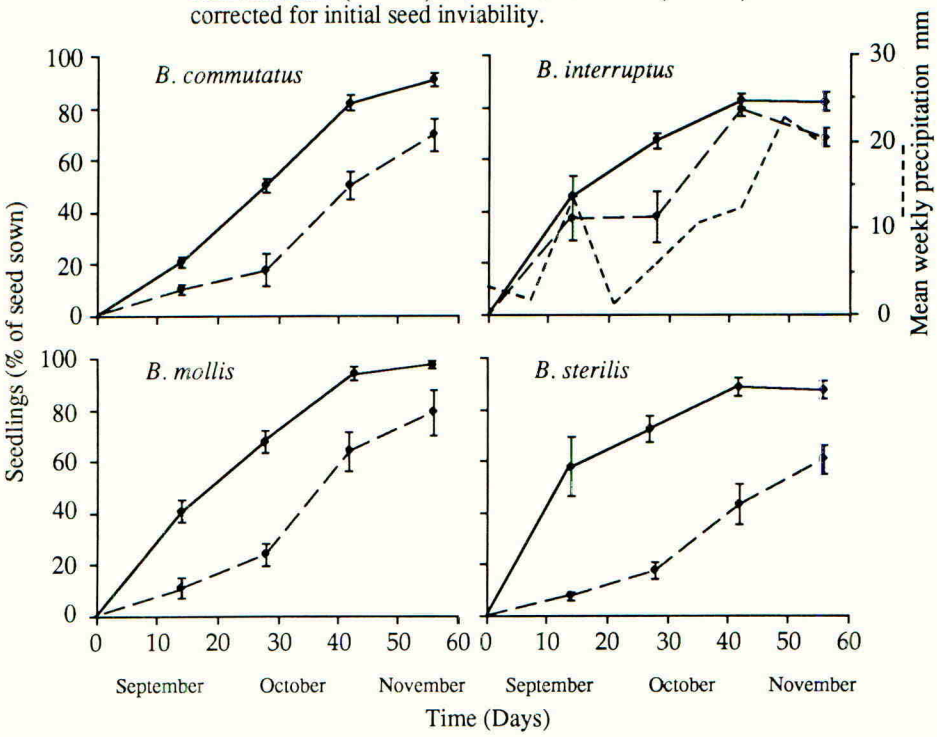
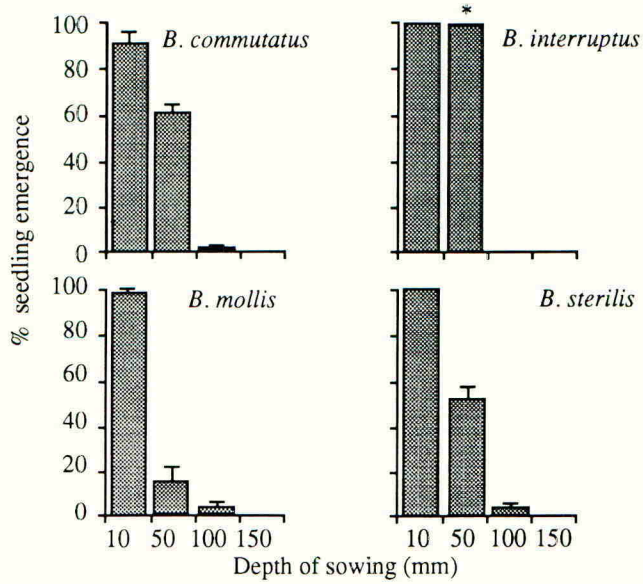
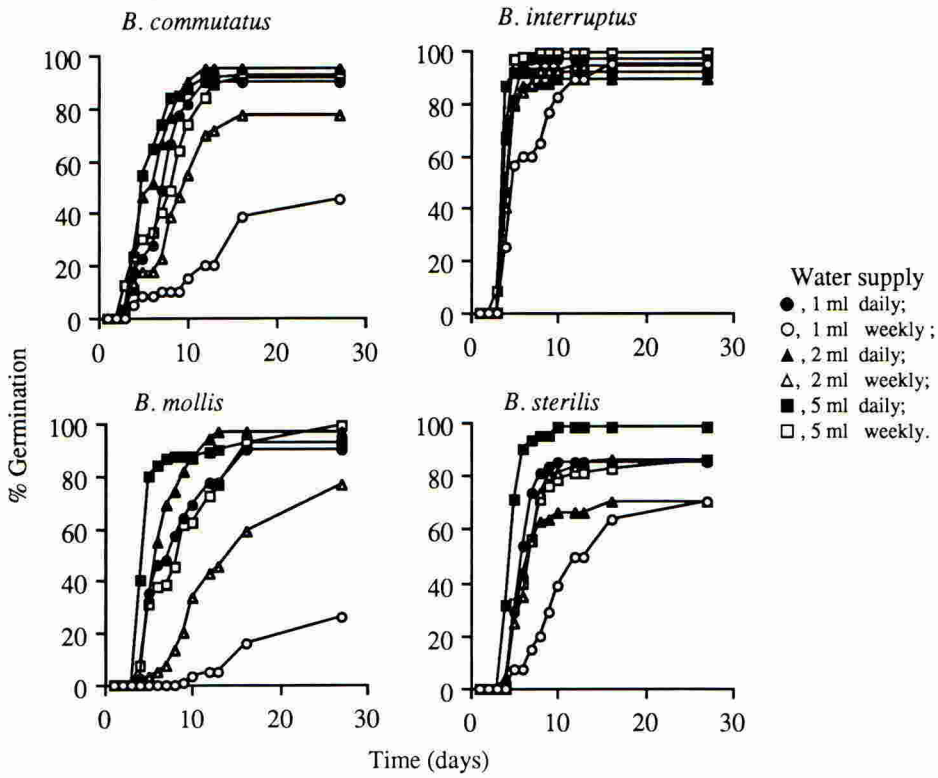


FIGURE 4. Seedling emergence (mean ± sem) in brome grasses in relation to sowing depth. (*, data taken from Howard *et al.*, 1991).



Differences in the speed of germination amongst substrates for both species were apparent under the 200 ml watering regime, germination being fastest in the compost.

FIGURE 5. Germination responses of four brome grasses to water supply in laboratory conditions.

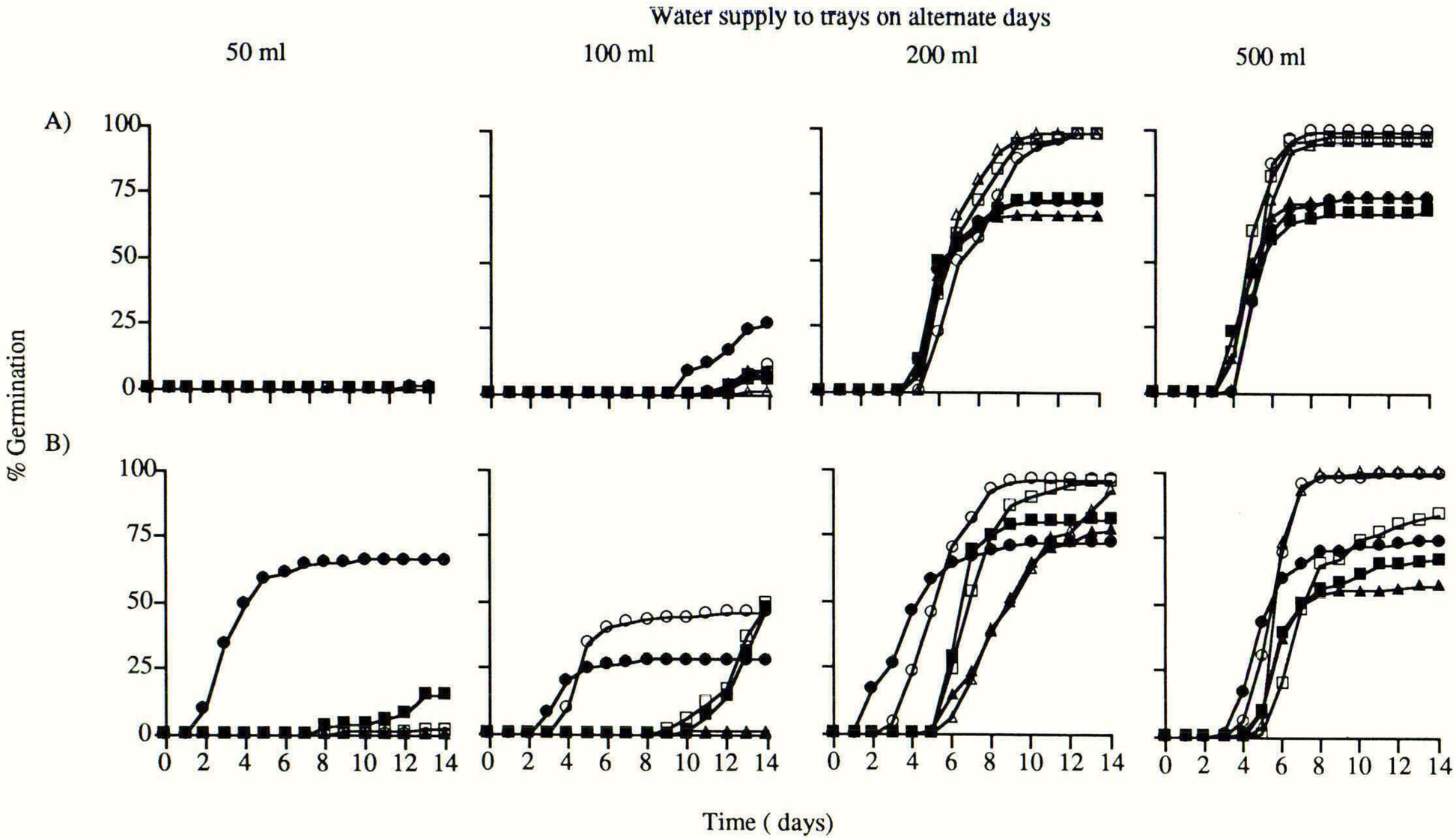


DISCUSSION

The results presented above indicate significant interspecific differences amongst these brome grasses that have major ecological implications to autumn germinating species with annual life histories in arable ecosystems. *B. sterilis*, and to a lesser extent *B. commutatus*, are important weeds of autumn sown cereal crops and seed production by individual plants of both weeds was comparable in winter wheat. *B. interruptus* and *B. mollis* were less fecund. The fate of these seeds and the size of the seedling infestation in the subsequent autumn will depend principally upon the magnitude of losses experienced since seed banks of all four species are transient (sensu Thompson & Grime, 1979) in most arable habitats (but see Peters *et al.*, 1993).

A significant source of weed seed loss occurs during crop combining (Howard *et al.*, 1991) and the timing of crop harvest relative to the start of dissemination and dissemination rate will have a major influence on the size of the seed population rained on to the soil. A 14 day difference in the initiation of seed dissemination was observed amongst species, from simultaneous sowings the previous autumn, with seed rain starting with *B. sterilis*. Dissemination rates of *B. sterilis*, *B. commutatus* and *B. mollis* were similar, circa one month being required for 50% seed shed, and were faster than in *B. interruptus*. In consequence the greatest proportion of seed likely to be exposed in the panicle to machinery at crop harvest will be of *B. interruptus* and the least, of *B. sterilis*. Howard *et al.*, (1991) reported that in combine harvesting the probability of a seed of *B. sterilis* being removed with the cereal grain

FIGURE 6. The germination of *B. interruptus* (closed symbols) and *B. sterilis* (open symbols) in response to substrate, water supply and depth of sowing. A) surface sown seed; B) seed sown at 50 mm depth. Substrates sand, triangle; compost circle; sand/compost, square.



(as opposed to dispersal to the ground) was 0.34 in contrast to 0.15 for *B. interruptus*. Thus combine harvesting has the effect of disseminating a greater proportion of *B. interruptus* to the ground surface in comparison to *B. sterilis*, the absolute amount depending upon the number of seeds retained in the panicle, which in turn is dependent on weed maturity date relative to crop harvest. Calculation suggests that where crop harvest is 5 to 7 weeks after the start of seed shed ca 87% and 77% of *B. interruptus* and *B. sterilis* seed respectively will have been dispersed to the ground. No data is published on the risk of seed loss by combine harvesting in *B. commutatus* and *B. mollis* which have similar shape and size to those of *B. interruptus*. Resultant population sizes of these species in wheat are likely to fall within the range suggested above due to similarity in maturity date and rate of dissemination.

The fate of seed post dispersal will depend upon factors determining predation, burial and germination. Freshly harvested dry seed samples of all species stored for one month in the dark displayed high germination capacity and no innate dormancy, enforced dormancy being broken by adequate water supply (Figure 5). It is noticeable that time to 50% germination was strongly correlated with water supply (volume and frequency) in all species except *B. interruptus*. Observations in the field also indicate that water supply influenced seed germination of *B. interruptus* ; emergence of seedlings from seed sown into stubbles was halted in late September when natural precipitation was low (days 14 -21, Figure 3). The somatic polymorphism in response to water supply in *B. commutatus*, *B. mollis* and *B. sterilis* provides a mechanism for delaying germination after seed dispersal and staggering seedling emergence. Conversely in *B. interruptus*, seedling populations will rapidly emerge after short duration rainfall on substrates with low water holding capacity.

The chance of germination and seedling establishment in all species was enhanced by shallow (10 mm) burial. However cultivations that bury seeds below 50 mm are likely to have a differential effect on species establishment. Whilst very few seedlings emerged from depths > 100 mm, seed burial at 50 mm reduced establishment in *B. mollis* more than in *B. commutatus* and *B. sterilis*.

The comparisons above suggest that summer climate and cultivation practices are key determinants of interspecific selection of seedling brome populations on arable land. The precocious germination behaviour of *B. interruptus* in response to water will result in early development of seedling populations that then will suffer high (possibly complete) mortality during land preparation for autumn crop sowing. In contrast a fraction of seed of the other three species are more likely to remain in enforced dormancy up to and during land preparation. Cultivations that bury seeds > 50 mm within the soil profile will tend to select against *B. mollis* in favour of *B. commutatus* and *B. sterilis*. These arguments take no account of the induction of dormancy by photo-inhibition that has been demonstrated in *B. sterilis* (Pollard, 1982, Hilton, 1987). Photo-inhibition may occur in all species but the response of *B. interruptus* in the glasshouse (Figure 5) would suggest this is least likely in this species.

Seed dispersal of species grown in monoculture occurred at a significantly lower rate in all bromes except *B. sterilis*. This may reflect delayed maturity of seed in the infructescence as plants were considerably larger and more fecund (Figure 1). In open undisturbed habitats that occur in field margins and headlands, dispersal will occur over a prolonged time span resulting in a seed rain from early July through to October with a consequent prolonged episodic germination span.

These studies suggest that the extinction of *B. interruptus* from arable habitats may have occurred because of a narrowly defined establishment niche arising from lack of innate and induced seed dormancy and rapidity of germination at low moisture levels. Temporally therefore, the niche is prescribed by summer water supply soon after dispersal and subsequent absence of autumn soil disturbance causing seedling death. Soil temperature is unlikely to be a constraining factor as all species have a wide temperature tolerance for germination (Howard, 1991). In the other bromes, enforced and induced seed dormancy ensures a wider window in

autumn for seedling emergence, the establishment niche being spatially constrained by depth of seed burial. It is possible that the much greater sensitivity of *B. mollis* to depth of burial (Figure 4) is an important factor limiting its spread in arable agriculture in the UK. *B. commutatus* and *B. sterilis* on the other hand are pre-adapted to establish in habitats arising from shallow cultivations in autumn.

The relative competitive abilities of all four species remain to be investigated but all species appear to be competitive with wheat even at high density (Figure 1). Comparative studies on the competitive ability of *B. interruptus* and *B. sterilis* in the glasshouse have suggested that in wheat *B. sterilis* is a stronger competitor than *B. interruptus* when species were sown simultaneously (Howard *et al.*, 1989) and in the absence of wheat, *B. sterilis* outcompetes *B. interruptus* (Howard 1991). Prior to its extinction, *B. interruptus* may have co-existed with the other species in natural populations in patches of field margins and headlands subject to no tillage due to the competitive advantage accrued from rapid germination in the summer and early autumn.

ACKNOWLEDGEMENTS

The work reported here was conducted whilst C.L.H. was in receipt of a N.E.R.C. studentship and latterly a grant from the Frank Horne Memorial Fund. Thanks are also due to Dr. P.M. Smith who provided the original supply of *B. interruptus* seed.

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An Ecological Comparison of Weed and Non-Weed forms of *Arrhenatherum elatius* (L.) Beauv. ex J & C Presl

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ABSTRACT

Arrhenatherum elatius is a useful model species for the study of the adaptive features of perennial arable weeds. Within the species both weed and non-weed forms occur, and are commonly found growing in close proximity. The paper reviews work carried out at Imperial College over a number of years. The results of a number of studies into the comparative ecology of these weed and non-weed growth forms are presented, and key adaptive features of the weed form which result in its success are identified. The comparative features of the growth forms are assessed using the scheme of functional ecology proposed by Grime (1977). The specific adaptive features observed are interpreted in terms of the weed form's overall strategy, and this strategy is contrasted with other common weed species.

INTRODUCTION

Arrhenatherum elatius is a perennial grass, almost ubiquitous within the U.K. (Pfitzenmeyer,1962). The species is highly polymorphic, and exists as a number of sexually compatible growth forms (sometimes raised to the rank of subspecies or variety). The primary morphological characteristic which differentiates growth forms is a swelling of one or more basal internodes. In the growth form which occurs as a troublesome weed of arable land, known as Onion Couch, this swelling is at its most extreme, with a chain of 4 to 7 swollen basal internodes of typically 7 to 12mm in diameter being present. In semi-natural habitats which occur in close proximity to arable land the most common form is non-bulbous and this form is often referred to as Tall Oatgrass. A complication is that a less widespread form of the species, which is not considered in this paper, occurs in semi-natural habitats, especially in the West, in which the basal internodes are intermediately swollen (See Fig. 1). Despite the intermediate morphology there is evidence that this form does not represent a functional intermediate between non-bulbous non-weed forms and bulbous weed forms of the species(Cussans *et al*, 1992). Figure 1 shows a provisional distribution map of the bulbous forms of the species and it

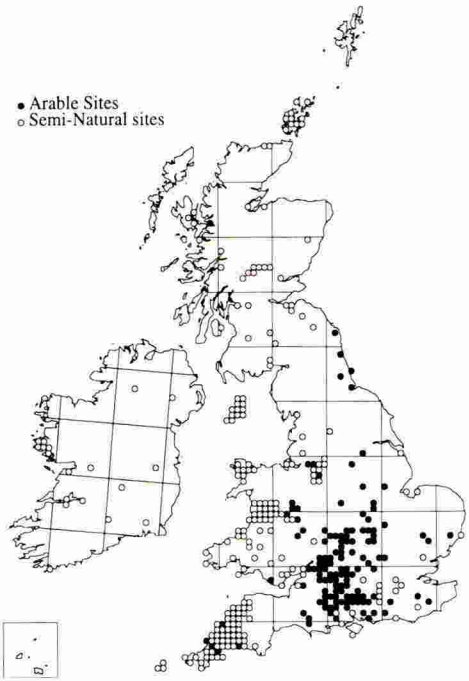

FIGURE 1 - A provisional map of the distribution of *Arrhenatherum elatius* subsp. *bulbosum* at a resolution of 10km. Produced by DMAP (Morton, 1993)

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is apparent that the weed form, Onion Couch, is most prevalent in Central Southern England, where it represents a significant weed problem. The distribution of the non-bulbous (non-weed) Tall Oatgrass form of the species is virtually ubiquitous and is therefore not shown.

The existence within this species of two closely related growth forms which are successful in very contrasting habitats, but in close proximity to one another, means that study of the comparative ecology of the growth forms of the species can provide interesting data about the key adaptive features of a perennial weed which set it apart from similar non-weedy populations.

In this paper, the comparisons of weed and non-weed forms of *A.elatius* are discussed with reference to Grime's C - S and R categories. Grime (1977) has proposed a model for the study of the adaptive ecology of plants from semi-natural habitats. This model has been shown to be successful for a number of semi-natural situations and has been applied to experimental studies (Campbell *et al*, 1991). The model is based on the proposal that within semi-natural vegetation there are three primary adaptational strategies, the first is adaptation for highly competitive habitats (C), the second is Stress tolerance (S), and the third is a disturbance tolerant or 'Ruderal' (R) adaptation. Populations can thus be described according to the degree to which they possess each of these primary strategies.

METHODOLOGY

i) Competition.

The relative performance of the different forms of *Arrhenatherum elatius* and Wheat under competitive conditions was assessed using de Wit (1960) replacement series experiments. Plants of Tall Oatgrass, Onion Couch and Wheat were grown, at differing relative densities under high nutrient and optimum moisture regimes. The full methodologies are described in Khan (1987). The different plant types were grown in all possible pairs. In this way an indication of the relative competitive abilities of the plant types under optimum conditions was obtained. The results are expressed as the relative crowding coefficient (RCC) of type A on type B and an estimate of this coefficient is provided by fitting a function to the data from the mixture experiments (Fig. 2). A full explanation is given in de Wit (1960) but essentially the higher the RCC value obtained, the more competitive plant type A is against plant type B. In all cases in this paper, where a pair of competing species is mentioned, the first is type A and the second type B.

ii) The effect of Stress.

a) Moisture. The effect of varying moisture supply is demonstrated here by the effect of three levels of moisture treatment on the relative competitivity of Onion Couch and Tall Oatgrass populations grown in pots. The results are expressed as the RCC of type A (is this case Onion Couch) on type B (Tall Oatgrass) (See Fig. 3). A full account of the methodology is provided in Cussans (In prep.).

b) Nutrient Supply. The relative effect of nutrient availability on Onion Couch and Tall Oatgrass was illustrated by the effect of nutrient treatments on the competitivity of the plants. In addition to the direct assessment of the competitivity of Onion Couch and Tall Oatgrass, a comparison with Wheat is shown (Fig. 4). A full account of the methodology is provided in Khan (1987).

iii) The effect of Disturbance.

a) Defoliation. An experiment was carried out in which monthly harvests of monocultures of Tall Oatgrass and Onion Couch were taken from the same plots between April and October. The weight of cuttings and the cumulative effect of the

monthly cutting regime was then compared with uncut control plots. The results are shown in Fig. 5; A) shows the dry weight of successive cuttings and B) the cumulative effect of the cutting regime at the end of the experiment in October. In Fig. 5A the ranges of least significant differences have been superimposed ($P<0.05$).

b) Root disturbance. Plants established in field plots for a year were carefully removed from the field and subjected to three pot treatments. 'Control' pots were established where minimal disturbance was exerted; whole plants, together with a surrounding root ball, were transplanted into 25l pots. A 'drought' treatment intended to simulate arable field conditions was established where plants were roughly dissected and left on the surface of large soil trays without receiving any water other than natural rainfall. In a third, 'high moisture', treatment plants were roughly dissected in the same way and placed on the surface of comparable soil trays, but received daily additions of moisture to prevent total drying out of the plant material without causing water-logging or damping-off. A full description of the experimental methodology is given in Cussans (In prep.). Results from this experiment are presented in Fig. 6 as number of shoots produced (expressed as a percentage of the control plot value). Where bars are not labelled with identical letters (A to E) the treatments were significantly different ($P<0.05$).

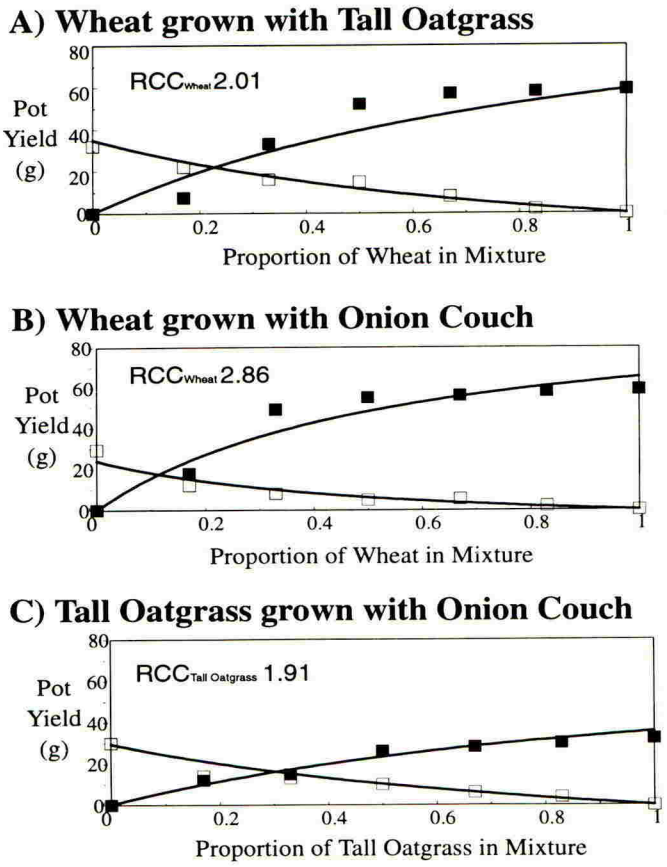


FIGURE 2 - The results of three de Wit replacement series experiments (Khan, 1987).

RESULTS

i) Competition.

Figure 2 shows the results of the de Wit replacement series experiments (Khan, 1987) under optimum, highly competitive, conditions. Using the estimates of RCC obtained the three plant types can be ranked according to their competitive ability under such conditions; Wheat > Tall Oatgrass > Onion Couch. These results are in agreement with more recent experimental findings in which the RCC of (different) Tall Oatgrass populations grown with Onion Couch populations was estimated as 1.416 (Cussans, 1992)

ii) The effect of Stress.

a) Moisture. Figure 3 shows estimates of the RCC obtained for Tall Oatgrass grown with Onion Couch under three differing levels of moisture regime. As the level of moisture supply decreases, the RCC values obtained become closer to one, suggesting that the competitive advantage which Tall Oatgrass has under optimal conditions is reduced with decreasing moisture supply.

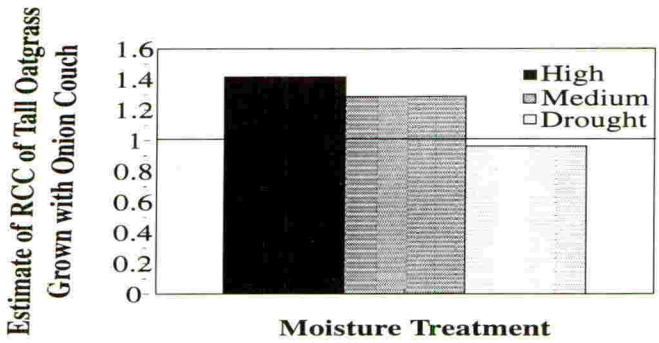


FIGURE 3 - Results from an experiment demonstrating the modifying effect of three moisture treatments on the relative competitive abilities of Tall Oatgrass and Onion Couch (Cussans *et al*, 1992).

b) Nutrients. Figure 4 shows estimates for the RCC of Wheat grown with Tall Oatgrass, Wheat grown with Onion Couch and Tall Oatgrass grown with Onion Couch under high and low nutrient supply treatments (Khan, 1987). The estimates of RCC obtained suggest that the relative competitive abilities of both Onion Couch and Wheat decrease under low nutrient conditions and the relative competitive ability of Tall Oatgrass increases. Using the results illustrated in Fig. 4, the plant types can be ranked in terms of their success in low nutrient, high stress (S), conditions; Tall Oatgrass > Onion Couch > Wheat. Separate experimentation has confirmed the sensitivity of Onion Couch to low nutrient conditions but comparable data are not available (Cussans, in prep).

iii) The effect of Disturbance.

a) Defoliation. The results of an experiment (Khan, 1987) on the effect of successive cuttings on monocultures of Tall Oatgrass and Onion Couch are shown in Fig. 5. Figure 5A shows the dry weight yields of the successive harvests. It is apparent that there is a significant regrowth after the first defoliation in April (seen in the dry weight of the May harvest), most markedly in Onion Couch. Regrowth

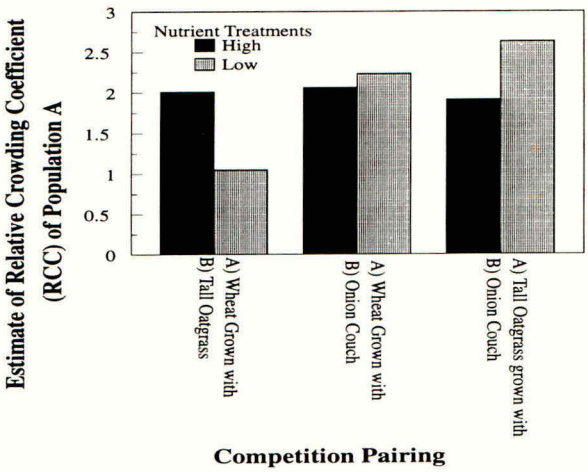
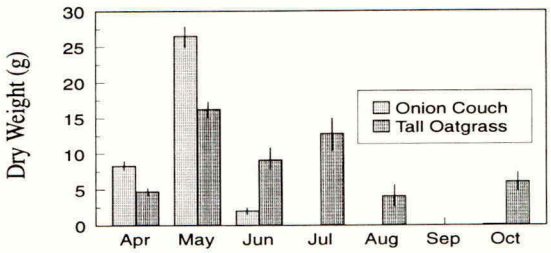


FIGURE 4 - The modifying effect of nutrient treatment on the relative competitive abilities of Tall Oatgrass, Onion Couch and Wheat. (Data from Khan (1987))

A) Dry Weight of Clippings at several sucessive cuttings.



B) Final yield (October) observed in cut compared to control plots.

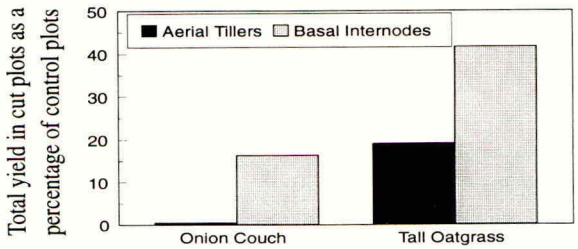


FIGURE 5 - Results from an experiment on the effect of successive cutting on the performance of Tall Oatgrass and Onion Couch monocultures. (Data from Khan (1987)).

after subsequent defoliations rapidly declines, more so in Onion Couch than in Tall Oatgrass. These results indicate that Onion Couch performs well under conditions of single aerial disturbance events, but is greatly affected by a continuous defoliation regime. Tall Oatgrass appears much less effected by high levels of aerial disturbance. This effect is confirmed in Fig. 5B where data on the cumulative yield at the end of the experiment in cut, compared to uncut plots, are shown. The cutting regime has reduced the yield of both Onion Couch and Tall Oatgrass, but the reduction is much more marked in Onion Couch.

b) Root Disturbance. Figure 6 shows the results of an experiment into the effect of disturbance on the regrowth of ramets of Onion Couch and Tall Oatgrass, and the ameliorating effect of moisture supply on disturbance. The experiment attempts to address the problem inherent in assessing root disturbance in arable cultivation, in that any root disturbance which occurs is often confounded with a period of extreme water stress. In the 'high moisture' treatment, plants received the same level of physical disturbance, but were not subjected to the same degree of water stress as the 'drought' treatment. In both disturbed treatments Tall Oatgrass is more affected than Onion Couch compared to the Control plots. Where root disturbance was not associated with extreme water stress both Onion Couch and Tall Oatgrass were less affected compared to the control plots; in fact regrowth from Onion Couch clumps was significantly increased.

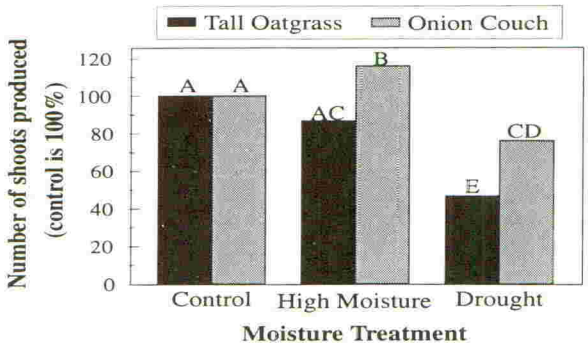


FIGURE 6 - The effect of disturbance and subsequent moisture treatment on the emergence of new shoots from Tall Oatgrass and Onion Couch. (Cussans , in prep.).

DISCUSSION AND CONCLUSIONS

The first point about these results is that it is clearly inadequate to partition the adaptive attributes of weed and non-weed populations simply to Competition, Stress and Disturbance. A further sub-division is needed taking into account the very specific nature of the arable habitat in which trends in Stress factors and Disturbance factors are variable and confounded, for example, nutrient stress is kept low by external additions, whilst moisture stress is introduced and maintained during periods of disturbance (cultivation). The model suggested by Grime provides a useful and rigorous framework for firstly organising the study of the attributes of weed species in relation to Stress, Disturbance and Competition adaptation, and then for considering how these attributes represent a strategy. A degree of care must be exercised, however, where a comparative study between populations growing in arable and semi-natural habitats is undertaken.

TABLE 1 - A summary of the adaptive attributes of weed and non-weed forms of *Arrhenatherum elatius*. The indication of 'LOW' and 'HIGH' in this table are intended as relative between Onion Couch and Tall Oatgrass, and are not intended as indicating high or low levels relative to other species.

	(WEED) Onion Couch	(NON-WEED) Tall Oatgrass
(C) Competitivity		
Competitive Ability under optimal conditions	LOW	HIGH
(S) Tolerance of Stress		
Moisture Status	HIGH	LOW
Nutrient Supply	LOW	HIGH
(R) Tolerance of Disturbance		
Aerial (Defoliation)	LOW	HIGH
Root (Cultivation)	HIGH	LOW

In addition to the attributes displayed by established perennial populations of the weed, its reproductive features must be considered. Vegetative reproduction is mainly by perennation from the shoot bases, but the plants do not spread significantly in this way; rhizomes are produced but are short and not creeping. Although *A.elatius* produces inflorescences with large numbers of seed, sexual reproduction appears in practice to be limited. Weed populations do not breed true, with a proportion of the offspring being non-bulbous (non-weed). Of those offspring which do resemble the weed parent in being bulbous it has not been established what proportion represents potential weed plants, since it has been demonstrated that bulbosity is not an absolute indication of weediness (Cussans, 1992). The relative importance of sexual and vegetative regeneration in this species is the subject of further study by the co-workers at Rothamsted.

The set of attributes presented in Table 1 when taken together with the reproductive situation outlined above represents the strategy of Onion Couch as a weed. The strategy appears to be based on extreme persistence (S and R), but without being highly invasive or competitive (C). The extremely patchy distribution pattern of Onion Couch reflects its strategy; there are very localised regions, both within fields and within the country as a whole, where Onion Couch is found at very high levels, whereas in other regions it is non-existent. The patchy distribution of Onion Couch appears to be driven more by its biology than by environmental factors such as soil type and rainfall.

Comparison with other successful weeds suggests that there is a 'trade-off' between the persistence of a weed (S and R) and its invasiveness and competitiveness (C), with trends in increasing resistance to root disturbance and tolerance of desiccation being associated with trends in decreasing competitiveness and reproductive spread. The obvious comparison is with Common Couch (*Elymus repens*) which is similar to Onion Couch in that it is not highly competitive with the crop (Cussans, 1970), but differs in that it is more invasive, both vegetatively and, to an extent, by seed (Williams, 1971). Whilst it is difficult to find results from comparable experimentation, Common Couch is considered slightly less persistent than Onion Couch.

At the other end of the spectrum are annual weeds such as *Bromus sterilis* which is relatively highly competitive (Cousens *et al*, 1988) and extremely invasive(C), but whose adult plants display no persistence (S and R) and has seed of limited life-span. Similarly to Onion Couch, the strategy of such a weed is reflected in the nature of its success and distribution; highly competitive and invasive plants will tend to be more widely and evenly distributed (for example

Bromus sterilis (Cussans *et al*, In Press)), with any apparent patchiness in its distribution being on a much larger scale.

ACKNOWLEDGEMENTS

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Session 5B

The Molecular Basis of Herbicide Action and Resistance in Weeds

Chairman and Session Organiser	Professor A H COBB
Papers	5B-1 to 5B-5

MOLECULAR MODELLING OF HERBICIDE INTERACTIONS WITH THE D1 PROTEIN OF PHOTOSYSTEM II

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ABSTRACT

Molecular modelling studies, combining molecular graphics and computational chemistry are used to investigate the principle intermolecular interactions governing herbicide binding to the D1 protein of photosystem II. Using a model structure for the D1 protein based on homology modelling with the experimentally derived structures for the L proteins of *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*, various herbicides of the urea and triazine class are docked into the herbicide binding site of the D1 protein. Based on the calculated intermolecular energy of interaction between the herbicide and its binding site and the correspondence with the inhibition behaviour of mutant species, favoured orientations for various types of herbicides within the binding site are proposed.

INTRODUCTION

The commercially important herbicides of the urea and triazine class function biochemically by displacing a plastoquinone, usually termed Q_B, from its binding site in the D1 protein of the photosystem II (PSII) reaction centre (RC) (Draber *et al.*, 1991). As the quinone is an essential electron carrier for photosynthesis, its displacement results in plant death.

The availability of high resolution crystal structures for the photosynthetic RCs of the purple bacteria *Rps. viridis* (Deisenhofer *et al.*, 1985) and *Rb. sphaeroides* (Allen *et al.*, 1987), along with the realisation that the L and M subunits show functional and partial sequence homology with the D1 and D2 subunits of photosystem II (PSII) (Trebst, 1986; Michel and Deisenhofer, 1988) have had a considerable impetus for molecular modelling with a view to the rational design of new herbicides for the Q_B binding site. The Q_B and herbicide binding domains of the L and M subunits of the RCs possess a number of conserved residues and molecular models of this region show significant structural similarities (Trebst, 1987; Ruffle *et al.*, 1992) . In the absence of high resolution crystal structures of PSII, analysis of the parameters which govern the binding of the secondary quinone and herbicides such as the triazines with the L subunit of bacterial RCs can help in the construction of interactive models of analogous ligands with the D1 protein (Draber *et al.*, 1991). However, because the phenylurea type herbicides such as dichlorophenyldimethylurea (DCMU) do not bind to wild type bacterial RCs, indirect methods have had to be employed to predict the binding environment of these herbicides with PSII. These predictions have been based mainly upon mutations of the Q_B binding domain which affect the interaction with DCMU. To date, various modelling studies have produced a number of potential orientations of the phenylureas within the binding pocket (Shigematsu *et al.*, 1989a; Bowyer *et al.*, 1990; Egner et al., 1993).

The characterisation of the herbicide–resistant mutants from *Rps. viridis* has revealed that one of the mutants, T4 (Tyr L222 to Phe) is sensitive to the urea type inhibitors (Sinning *et al.*, 1989a) in common with the PSII RC. In addition, the semiquinone–iron EPR signal of Q_B^- in *Rps. viridis* T4 mutants is similar to that reported for PSII (Sinning *et al.*, 1989b). These similarities provide us with a potential model in T4 for the construction of interactive structures of DCMU with PSII.

METHODS

The model of the D1 protein was from the PSII RC of *Pisum sativum* (coordinates supplied by J.H.A. Nugent, Department of Biology, University College London). The Q_B binding domain was represented by Leu 210 to Val 280 (Fig. 1) and all other residues were deleted from the model for simplification. All hydrogen atoms, polar and non–polar were included. The models of the herbicides were constructed using the *Insight II* molecular graphics modelling program (Version 2.1.0, Biosym Technologies, San Diego, CA) and minimised accordingly. Atom partial charges and potentials for both protein and herbicide models were assigned according to the parameters defined within the Consistent Valence Force Field (CVFF) used by the *Discover* molecular mechanics program (Version 2.8.0, Biosym Technologies, San Diego, CA). The herbicides were orientated within the Q_B binding site until a minimum intermolecular energy was achieved. Energy minimisation of the combined structures involved constraining the herbicide heavy atoms and the backbone atoms of the peptide whilst allowing the amino acid side chains to relax to relieve further unfavourable interactions between the protein and the herbicide. This was performed using steepest descents and conjugate gradients algorithms successively until the average first derivative was less than 0.005 kcal mol $^{-1}$ angstrom^{-1}. The cancellation of the nonbonded interactions between atoms after a specified cutoff distance was not carried out during minimisation in order to achieve a more accurate final structure. A dielectric constant of one was employed throughout the study. The same minimisation procedure was adopted after replacing amino acid residues in line with reported mutations. Intermolecular energies were calculated using the docking module of the *Insight II* modelling program.

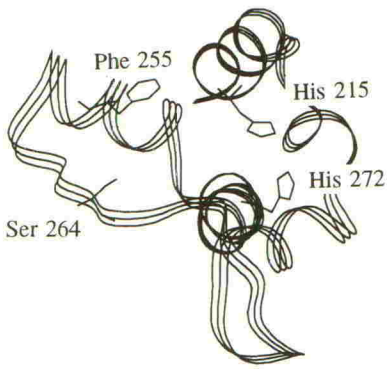

Fig.1. The Q_B binding site of the D1 protein.

RESULTS AND DISCUSSION

Egner (Egner *et al.*, 1992) has stated, when investigating the binding of triazine herbicides to bacterial reaction centres, that parameters within a molecular mechanics/dynamics program may have to be adjusted to achieve a correlation with biological and calculated data. Only when this correlation has been found can the impact of different substituents be compared and new compounds designed. Our study demonstrates that such adjustments are not required when examining triazine, phenylurea and plastoquinone binding to PSII, there being an apparent correlation between biological and calculated data using the standard forcefield parameters. It is important to point out that modelling the interaction energy only in terms of the electrostatic and van der Waals terms is a severe approximation. It does, for example, ignore completely polarisation effects. Calculation of intermolecular interactions depends critically on the parameterisation method. In essence, by taking a certain combination of values for the electrostatic and van der Waals parameters one can model the total interaction energy. The combination of electrostatic and van der Waals parameters supplied in the *Discover* program have been shown to be suitable for this purpose. Modification solely of the electrostatic component is therefore likely to lead to a breakdown of the approximations inherent in equations used to calculate the total intermolecular energy.

Plastoquinone binding to the D1 protein (Fig. 2) is characterised by a hydrogen bond between each quinone carbonyl group and the His 215 and Ser 264 residues. This resembles binding of the quinone in the *Rb. sphaeroides* binding site which is hydrogen bonded to the equivalent residues His L190 and Ser L223 (Allen *et al.*, 1988), rather than in *Rps. viridis* which has three hydrogen bonds to His L190, Ser L223 and Gly L225 (Michel *et al.*, 1986). The binding interaction with the D1 protein is also stabilised by ring stacking of the quinone head group and the phenyl side chain of Phe 255. The unsaturated isoprenoid tail forms extensive hydrophobic interactions with amino acid residues in the immediate vicinity of the binding site (Phe 255, Ala 263, Asn 266 and Phe 274) and with residues through the protein as it orientates out to the surface (Ile 259, Phe 260, Gln 261 and Trp 278).

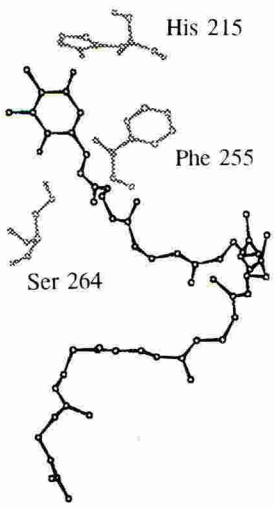

Fig. 2 The plastoquinone quinone binding orientation in the D1 receptor site

The mechanism by which triazines bind to the bacterial RC of *Rps. viridis* (Michel *et al.*, 1986) has been studied by X-ray crystallography which indicates that terbutryn binds to the L subunit of the reaction centre in the Q_B binding pocket through two hydrogen bonds; one between the side chain oxygen of Ser L223 and the ethylamino NH and the other between the peptide NH of Ile L224 and N3 of the *s*-triazine ring system. Employing our model of the Q_B binding site from PSII, we propose a hydrogen bond between the ethylamino NH and the side

chain oxygen of Ser 264, the equivalent residue to Ser L223 in the bacterial L subunit, in agreement with other models (Shigematsu *et al.*, 1989a; Bowyer *et al.*, 1990). However, we speculate that the second hydrogen bond involves the *t*–butylamino NH and the peptide carboxy group of Phe 265 as opposed to being between the triazine ring and the peptide NH of the same residue. The methylthio group of terbutryn is in proximity to Met 214, the residue labelled by azido[^{14}C]atrazine (Kleier *et al.*, 1987), and the *t*–butyl group is enclosed in a hydrophobic pocket consisting of the Phe 211, Tyr 262, Phe 265 and Phe 274 residues (Fig. 3).

The differences in size and hydrophobicity of the substituents on the basic triazine ring system may cause different interactions with the protein (Ewald *et al.*, 1989). Our model of atrazine bound to the Q_B site of PSII shows a binding site very similar to that of terbutryn (Fig. 4). In both cases, the same hydrogen bonds with the protein matrix are formed and the chloro substituent is close to Met 214. Further confirmation of this binding site is provided by the modelling of the interaction of optically active isomers of atrazine analogues. The (S) isomer of N^2–α–methylbenzyl–N^4–ethyl–6–chloro–2,4–diamino–*s*–triazine is reported to have a greater binding affinity for the Q_B binding site of PSII than the (R) isomer (Gardner and Sanborn, 1987) and the intermolecular energies of the modelled structures reflect this difference, being –42.4 kcal mol^{-1} and –3.0 kcal mol^{-1} respectively. We propose that the hydrophobic pocket consisting of the residues Phe 211, Tyr 262, Phe 265 and Phe 274 is the stereoselective region of the binding site (Mackay and O'Malley, 1993a).

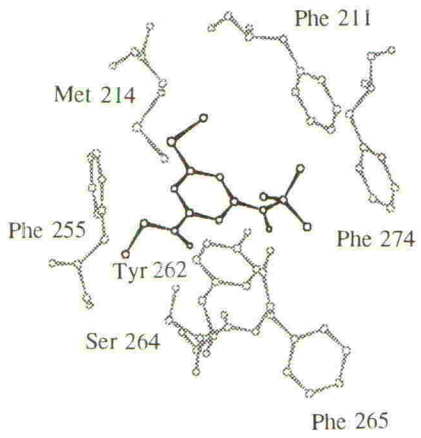

Fig.3 Terbutryn binding orientation in the D1 receptor site

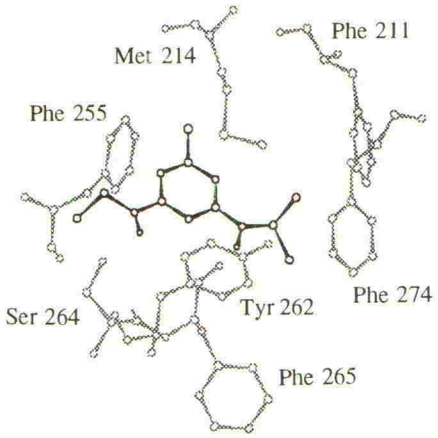

Fig. 4 Atrazine binding orientation in the D1 receptor site

Previously, modelling discussions concerning DCMU have focused upon the formation of hydrogen bonds with the top of the Q_B binding pocket of PSII, involving the Ser 264, Phe 265 or Ser 268 residues (Shigematsu *et al.*, 1989a; Bowyer *et al.*, 1990). We were able to demonstrate that the adoption of the *cis*–amide conformation by the herbicide was necessary for the formation of a

hydrogen bond between the phenylamino NH of DCMU and the side chain oxygen of Ser 264, to maximise van der Waals dispersion forces between the phenyl ring and the side chain of Phe 255 and to minimise repulsive forces between the dimethylamino group and the protein matrix producing an intermolecular energy with the protein of −17.0 kcal mol^{-1} (Table 1, Fig. 5). Whilst the *trans*−amide isomer is considered to be the more stable conformation (Creuzet *et al.*, 1989), *in vivo* adoption of the *cis*−amide form cannot be discounted.

TABLE 1. Calculated Nonbonded Interactive Energies Between DCMU and the Q_B Site of *P. sativum* Containing Mutated Amino Acid Residues

MUTATION	INTERMOLECULAR ENERGY (kcal mol^{-1})	
	(1)	(2)
Wild type	−19.7	−17.0
Val 219 to Ile	+274.3	−18.4
Ala 251 to Val	+173.0	+6000.0
Phe 255 to Tyr	−15.6	−13.0
Ser 264 to Gly	−24.5	−16.5
Ser 264 to Ala*	−24.0	−17.0
Ser 264 to Thr*	−24.5	−14.4

* *Calculated intermolecular energy without conformational rearrangement of the protein structure.*
Column (1): DCMU hydrogen bonding with His 215
Column (2): DCMU hydrogen bonding with Ser 264

X−ray crystallographic analysis of the reaction centres from T4 with bound DCMU revealed that the phenyl ring of the DCMU stacks with the Phe L216 residue, and the favoured orientation of the urea moiety is with the carbonyl group directed towards the His L190 residue forming a hydrogen bond (Sinning *et al.*, 1990; Sinning, 1992). Based upon this data, we have shown that a favourable orientation can also be achieved for the interaction with PSII (Mackay and O'Malley, 1993b). Studies investigating the effect of herbicides on the EPR spectra of Fe (II) (Diner and Petrouleas, 1987) in PSII suggest that DCMU may have one binding site similar to that of *o*−phenanthrolene which probably hydrogen bonds to His 215 in an analogous manner to it forming a hydrogen bond with His L190 in the bacterial reaction centre (Michel *et al.*, 1986). The studies do not discount the possibility of additional binding sites for DCMU within the Q_B binding pocket which do not affect the iron environment. In our model, the phenyl ring of DCMU is in proximity to Phe 255 (equivalent to Phe L216 in the bacterial RC), and a hydrogen bond is formed between the carbonyl group of the urea and His 215 (Fig. 6). We have shown that DCMU can adopt the more stable planar *trans*−amide form when binding with the protein and that the carbonyl group is the more likely hydrogen bonding function (Creuzet *et al.*, 1989). The intermolecular energy with the protein for this orientation was −19.7 kcal mol^{-1} which is comparable to the binding energy when stabilised by a hydrogen bond with Ser 264 (Table 1). Other workers have suggested that water molecules act as bridges to connect the DCMU hydrogen bonding functions and the His 215 and

Ser 264 together (Egner *et al.*, 1993).

Particularly supportive of our model where DCMU binds to His 215, are the effects on the intermolecular energy between the herbicide and the protein when specific residues are replaced in line with reported site directed mutagenesis(Table 1). The mutation of Phe 255 to Tyr reportedly does not induce DCMU resistance (Erickson *et al.*, 1985) and this change has very little effect upon the intermolecular energy of the DCMU with the D1 protein model.

Replacement of Ala 251 with Val in our model, in line with the mutation which induces DCMU resistance (Johanningmeier *et al.*, 1987), produces a significant increase in binding energy as a result of steric interaction between the larger Val side chain and DCMU (Table 1).

Gingrich and coworkers (Gingrich *et al.*, 1983) have suggested that the mutation Val 219 to Ile may alter the binding of DCMU directly by being near to His 215. Increasing the side chain to the more bulky Ile leads to steric hindrance of DCMU binding to His 215 below as both side chains are predicted to be facing in the same direction (Trebst, 1986). In this conformation, such a residue replacement significantly worsens the binding interaction (Table 1).

Mutations affecting Ser 264 produce DCMU resistance to differing degrees. The Ser to Thr (Shigematsu *et al.*, 1989a/b) and Ser to Ala (Erickson *et al.*, 1984) mutations induce resistance to DCMU whereas Ser to Gly (Pfister and Arntzen, 1979) does not. Replacement of Ser 264 with Gly in our model results in no change in the binding energy (table 1). In the absence of hydrogen bonding between Ser 264 and DCMU, little change in binding, and thus activity would be expected. Mutations of Ser 264 to Ala or Thr may produce conformational changes to the Q_B binding pocket (Erikson *et al.*, 1984; Shigematsu *et al.*, 1989b) which are absent in the Ser to Gly mutation and lower the relative affinity of DCMU with respect to the natural quinone for the protein. Without such changes in our model, the Ser to Ala or Thr mutations have little effect on binding energies. It is difficult to predict the structural effect of such a conformational change on the rest of the protein

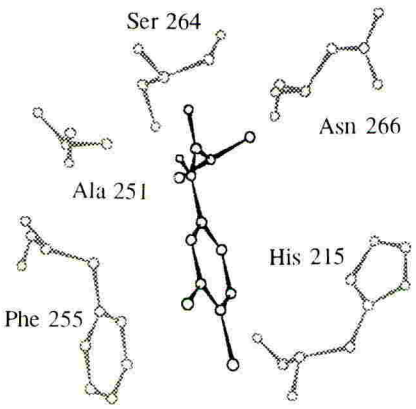

Fig. 5 DCMU binding orientation when hydrogen bonded to Ser 264

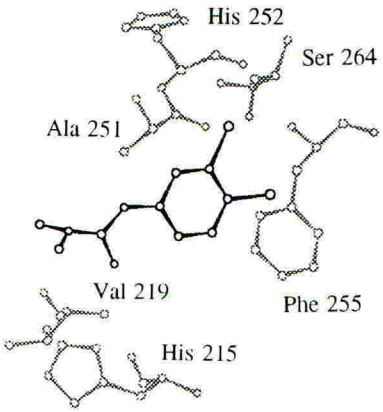

Fig. 6 DCMU binding orientation when hydrogen bonded to His 215

without recourse to crystallographic data and therefore we have not attempted to model such an interaction.

When DCMU is hydrogen bonded to Ser 264 at the top of the binding site, changes in the intermolecular energy with the receptor as a consequence of mutations to the Phe 255 and Ala 251 residues give the expected results (Table 1), however a discrepancy between experimental and theoretical results occurred for the Val 219 to Ile mutation. We can report no significant change in the binding interaction between DCMU and the mutated protein, a change at the bottom of the pocket, as demonstrated by Bowyer (Bowyer *et al.*, 1990). Little change to the binding energy occurs when Ser 264 is replaced by Gly or Ala, even though the electrostatic interaction with the phenylamino NH of DCMU is lost.

ACKNOWLEDGEMENTS

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Target site-based resistance to herbicides inhibiting Acetyl-CoA carboxylase.

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ABSTRACT

Aryloxyphenoxypropanoate and cyclohexanedione herbicides inhibit the plastid enzyme ACCase in susceptible species. Resistance in four weed species is currently documented as due to the selection of individuals expressing herbicide resistant ACCase. There are different forms of resistant ACCase, although these remain to be characterised at the molecular level. Overproduction of ACCase is a possible mechanism, but has not yet been found in natural populations. When due to herbicide resistant ACCase, resistance is inherited as a single, nuclear, partially-dominant gene. Unlike most previous cases with other herbicide groups, resistance to ACCase inhibitors can appear after a period of selection as short as three years. A possible explanation for this is the absence of a fitness penalty imposed by resistant ACCase allowing the presence in wild population of resistant genes at frequencies higher than the normally expected mutation rate.

INTRODUCTION

The excellent postemergence aryloxyphenoxypropanoate (APP) and cyclohexanedione (CHD) classes of herbicide are widely used because they can effectively control a variety of annual and perennial grasses in most dicotyledonous and some cereal crops. However, their efficacy is a double edged sword, since resistance to these herbicides is developing rapidly, and may threaten their commercial viability.

The primary target site of these herbicides is the plastid enzyme, acetyl-CoA carboxylase (ACCase). ACCase is thought to be a key regulatory enzyme in lipid biosynthesis and its inhibition results in severe growth disruption (Gronwald, 1991). While selectivity in some cereals is achieved by a high rate of metabolic inactivation (Devine *et al.*, these proceedings), selectivity between dicotyledonous and monocotyledonous plants is due to differential herbicide susceptibility at the enzyme level (Gronwald, 1991). Resistance to ACCase inhibiting herbicides in formerly susceptible weeds was first reported in *Lolium rigidum* in Australia in the early 80's (Heap and Knight, 1982) and has since increased dramatically. To date, there are at least eight weed species which have evolved resistance to CHD and APP herbicides (Holt *et al.*, 1993; M. D. Devine, pers. comm.). In Australia, there are currently about 2200 farms on which resistance to these herbicides has developed in *L. rigidum* (unpublished results). Resistance can be conferred by several

mechanisms but in this paper, we will discuss the cases that involve modification of the target enzyme ACCase.

CHARACTERISATION OF ACCASE

ACCase is a biotinylated, plastidic enzyme encoded in the nuclear genome. ACCase has been purified from some grass species and consists of two subunits of approximately 220 kD for a native molecular weight of 500 kD (Egli *et al.*, 1993). ACCase is found in all plant organs but is particularly active in young developing tissues like root tip and leaf meristems (Gronwald, 1991). ACCase inhibition results in lipid deprivation which is of minor consequences in mature tissues like leaves, but is lethal in actively growing tissue where the demand for lipids is particularly high.

Another form of ACCase has recently been identified in maize with a molecular mass of 212 kD and which is localised in the cytoplasm (Egli *et al.*, 1993). This cytoplasmic ACCase is poorly recognised by antibodies raised against the plastid form of ACCase and is far less susceptible to inhibition by haloxyfop and sethoxydim. This strongly suggests that there are two different isoforms of ACCase in plants. The exact role of this second form of ACCase is not yet known but may not be crucial to herbicidal effects as it is minimally affected by APP and CHD herbicides in susceptible species (Egli *et al.*, 1993).

DEVELOPMENT OF RESISTANCE

Modification of ACCase target site

Evolution of resistance due to modification of ACCase has been documented in the weed species *Lolium rigidum* and *Avena sterilis* in Australia (Tardif *et al.*, 1993; Maneechote *et al.*, 1993), *L. multiflorum* (Gronwald *et al.*, 1992) and *Sorghum halepense* in the USA (M. D. Devine, pers. comm.), and *Setaria viridis* in Canada (Marles *et al.*, 1993). Furthermore, resistance has also been artificially selected in maize cell culture lines (Parker *et al.*, 1990a). In all these cases ACCase modification provides a high level of resistance and the level of resistance at the enzyme level is correlated with resistance at the whole plant level.

In most cases, resistance is found not only to the herbicide that selected for resistance, but there is target-site cross-resistance to other APP and CHD herbicides. However, the relative levels of target-site cross-resistance to APP and CHD herbicides varies and it is possible to identify different forms of ACCase within and between species based on the inhibition profiles. For example, both *L. rigidum* biotypes SLR 3 and WLR 96 have a form of ACCase that is highly resistant to APP and only moderately resistant to CHD herbicides, while biotype SLR 31 (a subset of the population) has a modified ACCase that is highly resistant to both groups (Table 1). In a resistant *L. multiflorum* biotype,

resistance is very high to diclofop, moderate to other APPs and low to CHD herbicides (Gronwald *et al.*, 1991). It is likely that there are different mutations endowing resistant ACCase, corresponding to different alleles of the same gene. Indeed, evidence exists from mutant corn cell lines that different alleles of the same locus are coding for ACCases with variable target site cross-resistance patterns (Marshall *et al.*, 1991).

Although resistance is generally higher to the herbicide providing the selection pressure, there are noteworthy exceptions. *L. rigidum* biotype SLR 3 has a form of ACCase that is resistant to sethoxydim following selection with this herbicide. However, at both the whole plant and ACCase level, this biotype is far more resistant to herbicides of the APP group, despite such herbicides never being used on this population (Table 1). Taking into account this variation among types of ACCase, it is virtually impossible to predict what type of resistant allele has been selected without biochemical and/or whole plant screening.

Table 1. Selection pressure and level of enzyme resistance in three biotypes of *Lolium rigidum*.

Biotype	Selection pressure	APP	CHD	Reference
SLR 3	Sethoxydim	High	Moderate	Tardif *et al.*, 1993
WLR 96	Diclofop	High	Moderate	Holtum and Powles, (unpublished)
SLR 31 (subset)	Diclofop Sethoxydim	High	High	Tardif and Powles, 1993

There are indications that possession of an insensitive form of ACCase does not reduce fitness of the plant. It seems that alteration of the herbicide binding site does not result in reduced ACCase catalytic activity in *L. rigidum*, *L. multiflorum*, *A. sterilis*, *S. viridis*, and maize cell lines (Parker *et al.*, 1990a; Gronwald *et al.*, 1992; Maneechote *et al.*, 1993, Tardif *et al.*, 1993). At the whole plant level, fitness of biotypes of *L. rigidum* (Matthews and Powles, unpublished) and *A. sterilis* (Mansooji, Holtum and Powles, unpublished) with resistant ACCase are not inferior to that of susceptible biotypes.

ACCase overproduction

It is possible for an organism to develop resistance to a pesticide by increasing the amount of the target enzyme present in the cells (Klee *et al.*, 1987). Hence, weed biotypes expressing increased amount of ACCase might be able to withstand higher

doses of APP and CHD herbicides than normal types. This resistance mechanism has been demonstrated only *in vitro* in some non-regenerable maize tissue culture lines artificially selected on sethoxydim (Parker *et al.*, 1990b). Growth of the most resistant line was 90 and 58 times more resistant to haloxyfop and sethoxydim, respectively, than that of the susceptible type and this corresponded to a 2.6-fold increase in the specific activity of ACCase in the cells. This increased activity was also correlated with a 4.7-fold increase in the amount of a biotinylated enzyme of 220 kD, suggesting that higher ACCase activity was due to enzyme overproduction. Whether this was due to gene duplication or to an alteration of post-transcriptional regulation has not been determined.

Overproduction of ACCase as a possible resistance mechanism has been excluded in resistant biotypes of *L. rigidum* (Matthews *et al.*, 1990; Tardif *et al.*, 1993), *L. multiflorum* (Gronwald *et al.*, 1992), *A. sterilis* (Maneechote *et al.*, 1993), *S. viridis* (Marles *et al.*, 1993) and *A. fatua* (Devine *et al.*, 1993). In all these cases, the specific activity of ACCase was found to be similar in resistant and susceptible biotypes. That this mechanism of resistance has not so far appeared in weed species may indicate that enzyme overproduction could be associated with a higher metabolic cost and would incur a fitness disadvantage.

GENETICS

Inheritance

The inheritance of APP and CHD herbicides resistance in *L. rigidum* biotype WLR 96 has been investigated using haloxyfop as the test herbicide. Segregation of resistance to susceptibility in F_2 plants from reciprocal crosses between resistant and susceptible parents fitted a 3 to 1 ratio, indicating control by a single, semi-dominant, nuclear gene (Table 2).

Table 2. Segregation of resistance and susceptibility to haloxyfop in F_2 populations from reciprocal crosses between resistant *L. rigidum* biotype WLR 96 and a susceptible biotype.

Haloxyfop (g ai ha^{-1})	Resistant	Susceptible	χ^2 (3:1)	Probability (df = 1)
	(Number of plants)			
26	441	159	0.72	0.369
208	478	153	0.19	0.662

Single gene inheritance is expected for an enzyme such as ACCase which is encoded in the nuclear genome. Such inheritance of resistance has also been found in resistant *A. sterilis* and *L.*

multiflorum as well as in artificially selected resistant maize cell lines (Parker *et al.*, 1990a, Betts *et al.*, 1991; Barr *et al.*, 1992). Inheritance of resistance due to ACCase overproduction has not been determined since the maize tissue lines used were non-regenerable (Parker *et al.*, 1990b).

Population genetics

In a biotype of *L. rigidum* (WLR 96), the resistant ACCase trait is semi-dominant because there are differences in the LD_{50} or GR_{50} of the resistant homozygous and the heterozygous. However, this is apparent only when very high rates of herbicides are used (Figure 1). At agriculturally relevant rates (100 to 200 g ai ha^{-1}), both homozygous resistant and heterozygous individuals are unaffected. This may well contribute to the increase in resistance as the heterozygous individuals are as successful as the homozygous resistant individuals.

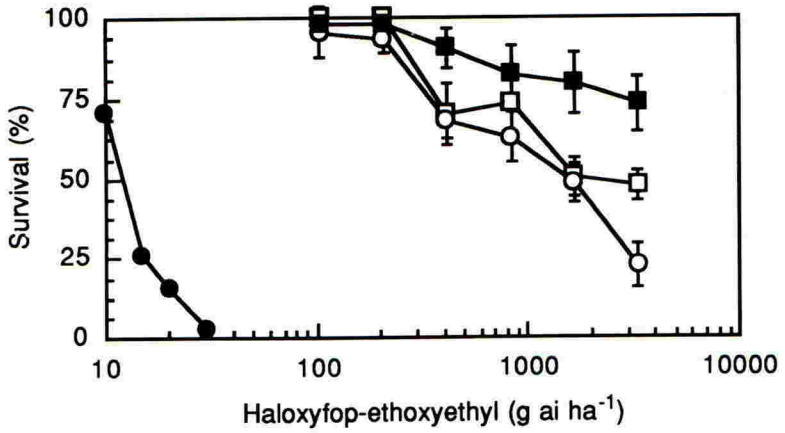

Figure 1. Survival of reciprocal F_1 families (male R (□) and female R (○)) from a cross between an APP and CHD herbicide resistant (■) and susceptible (●) *L. rigidum* biotypes.

The initial frequency of resistance genes in a population can be a critical factor for the development of resistance. As yet, there have been few studies conducted to determine the initial frequency for resistant ACCase. However, there are indications that, in some species, initial gene frequency may be high. *L. rigidum* biotype SLR 3 was treated for only three consecutive seasons with sethoxydim before resistance was observed (Tardif *et al.*, 1993). This population is now comprised entirely of individuals expressing a resistant ACCase. Similarly, it was found that selection of a susceptible *L. rigidum* population with diclofop led to resistance in only three generations (Matthews and Powles, 1992). In one case the resistant individuals all expressed resistant ACCase (Matthews and Powles, unpublished results). Although, alternative explanations are possible, these

results strongly suggest a high frequency of ACCase-based resistance in *L. rigidum*.

The frequency of resistance genes in susceptible populations is often estimated around 10^{-6} to 10^{-11}, which corresponds to the mutation rate (Gressel and Segel, 1983; Maxwell *et al.*, 1990). This is due to the equilibium between the rate of mutation and the depletion of new mutants because of lower fitness. Compared to the generally long time required to select for herbicide resistance in general and triazine resistance in particular (>10 years), this estimate of initial gene frequency appears too low to explain ACCase based resistance evolving as rapidly with only three years of selection (Tardif *et al.*, 1993). That herbicide resistant ACCase does not impose a fitness penalty may explain its higher frequency. A mutation conferring resistance could have appeared in a population long before herbicide use. Then, the resistant gene frequency could have only slowly increased for reasons unrelated to herbicide use (eg. slight advantage under some environmental conditions). However, upon the commencement of herbicide selection, this would make a difference between resistance appearing in 10 years or much more rapidly.

An explanation for the possible high frequency of resistant ACCase alleles in *Lolium* spp. could lie in their phylogeny. *Lolium* spp. form a taxonomic complex with the *Festuca* spp. and the two genera have common ancestry and similar enzyme polymorphism (Jauhar, 1993). It has been documented that red fescue (*Festuca rubra*) and the blue fescues (*F. ovina* var. *glauca* and *F. amethystina*) are resistant to the APP and CHD herbicides due to herbicide resistant ACCases (Gronwald, 1991; Catanzaro *et al.*, 1992) while another member of that genus, *F. arundinacea*, is susceptible. Taking into account the close genetic distance between the *Festuca* and *Lolium* spp., it is possible that there is a continuum in the frequency of resistant forms of ACCase present in the different species of these two genera. For example, resistant alleles are very abundant in *F. rubra*, *F. ovina* and *F. amethystina*, and rare in other species like *F. arundinacea*. Ryegrass species *L. rigidum* and *L. multiflorum* could fit somewhere in between these two extremes, with some biotypes having a higher frequency of resistant ACCase alleles.

MOLECULAR BIOLOGY

Many research teams are currently attempting to clone the gene coding for the chloroplastic form of ACCase in plants. A cDNA expression library has been screened with antibody raised against a 225 kD biotinylated protein from maize leaf (Ashton *et al.*, 1993). The positive clones showed the best alignment with the 3′ end of cDNAs encoding rat and chicken ACCases. A region comprising 4.5 kb of the maize cDNA ACCase has 48% homology with chicken cDNA nucleotide sequence and the deduced amino acid sequences are 37% similar. Greater similarity was found in smaller regions of the sequence: 56% identity over 400 amino acids and 70% over 80 amino acids. Further work is currently underway on the cDNA clones from maize. It is possible that a range of nucleotide substitutions leading to different amino acid

changes in the herbicide binding site exist. This would explain the different alleles of resistant ACCase that are observed. However, the types of mutation will be revealed only when ACCase genes from resistant plants are cloned and sequenced. Knowing the mutations which can result in herbicide resistant ACCase while maintaining enzyme functionality will help determine the likelihood of further weed species developing ACCase inhibitor resistance. Molecular tools can eventually be developed for the screening of weed populations providing information that could be included in the design of management programs.

Given the widespread use of APP and CHD herbicides and the presence of different resistant ACCase mutants in otherwise susceptible weeds, as well as non target-site resistance mechanisms, we expect many more cases of resistance to appear. An exciting area of research over the next few years will be the molecular elucidation of both the resistant ACCase and the non target-site resistance mechanisms which are evident in weed species.

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ALTERNATIVE MECHANISMS OF RESISTANCE TO ACETYL-COA CARBOXYLASE INHIBITORS IN GRASS WEEDS

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ABSTRACT

Resistance to aryloxyphenoxypropanoate and cyclohexanedione herbicides can be conferred by at least two mechanisms. One mechanism involves a mutation in the herbicide target site, acetyl-CoA carboxylase (ACCase), that renders it less sensitive to inhibition by herbicides. In some resistant weed biotypes the ACCase is sensitive to herbicides, suggesting a second mechanism distinct from the target site alteration. In these weed biotypes, resistance has been correlated with an altered effect of the herbicides on the plasma membrane electrogenic potential (Em). Evidence from electrophysiological experiments indicates that the Em is permanently depolarized in susceptible tissue, but can recover in resistant tissue. It is not clear how this observation is related to herbicide resistance. The recovery of Em in resistant biotypes may indicate that the herbicide is rapidly compartmentalized away from the target site in resistant tissue. The results of preliminary experiments examining herbicide uptake into isolated plasma membrane vesicles or protoplasts indicate a 15 to 30% reduction in uptake into resistant compared to susceptible tissue. The physiological basis for this uptake difference and its significance in conferring herbicide resistance are not yet known. Further compartmental analysis will determine the possible roles of the cell wall, vacuole or chloroplast envelope in conferring resistance in these biotypes. Various alternative herbicide resistance mechanisms, and approaches to examine them, are discussed.

INTRODUCTION

Resistance to aryloxyphenoxypropanoate (APP) and cyclohexanedione (CD) herbicides in grass weed species has become widespread in recent years, with at least nine species worldwide showing evidence of resistance development (Devine and Shimabukuro, in press). In some of the resistant weed biotypes resistance has been associated with altered sensitivity of the herbicide target enzyme, acetyl-CoA carboxylase (ACCase). ACCase-based herbicide resistance in *Lolium rigidum*, *Setaria viridis* and other grass weeds is discussed in detail in a companion paper (Tardif and Powles, 1993).

In other weed species, however, no differences have been found in ACCase sensitivity between resistant and susceptible biotypes. In resistant *Avena fatua* (Devine *et al.*, 1992; 1993) and in some resistant *L. rigidum* biotypes (Holtum *et al.*, 1991) the ACCase is sensitive to APP and CD herbicides in *in vitro* assays. In addition, there is no evidence for substantial differences in herbicide uptake, translocation, or metabolism between the resistant and susceptible biotypes of these species; the minor increases in herbicide detoxification in resistant biotypes compared to susceptible biotypes of *L. rigidum* (Holtum *et al.*, 1991) and *S. viridis* (Marles *et al.*, 1993) are unlikely to be sufficient to confer the high level of resistance observed at the whole-plant level. Consequently, it is proposed that a second resistance mechanism, not based on an alteration

in ACCase sensitivity or in herbicide metabolism, is present in some resistant weed biotypes.

Diclofop and diclofop-methyl disrupt the proton gradient across the plasma membrane and tonoplast of several plant species (Lucas *et al.*, 1984; Ratterman and Balke, 1989; Wright and Shimabukuro, 1987). Although the relevance of this effect to the phytotoxicity of diclofop has been questioned (e.g., DiTomaso *et al.*, 1991), it has provided one of the few clues to the mechanism of herbicide resistance in the non-ACCase-based resistant biotypes. In this paper we review the evidence for a membrane-based mechanism of resistance to APP and CD herbicides, and present some recent results of our investigations into the nature of resistance in weed biotypes that have a sensitive ACCase.

EVIDENCE FOR A DIFFERENTIAL EFFECT OF DICLOFOP ON THE PLASMA MEMBRANE OF RESISTANT AND SUSCEPTIBLE BIOTYPES

As indicated previously, diclofop (or diclofop-methyl) depolarizes the plasma membrane electrogenic potential in various plant species. The effect is rapid, and can usually be measured within several minutes of exposing the tissue to a bathing solution of diclofop. In typical susceptible tissue the effect is permanent (at least within the time-course of these experiments, up to 1 hr), and when the diclofop solution is removed and the tissue flushed with herbicide-free solution, the Em remains depolarized.

In resistant *A. fatua* and *L. rigidum* biotypes with a sensitive ACCase the response is somewhat different. The plasma membrane Em is depolarized in much the same way as in susceptible tissue, but recovers fully when the herbicide solution is replaced with fresh, herbicide-free solution (Häusler *et al.*, 1991; Shimabukuro and Hoffer, 1992; Devine *et al.*, 1993; Holtum *et al.*, submitted). Typical data from *A. fatua* R and S coleoptile tissue are shown in Figure 1. Similar results have been obtained with diclofop in R and S *L. rigidum*

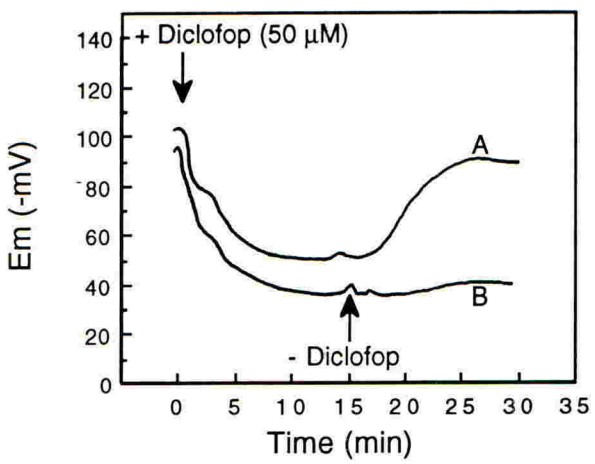

Figure 1. Effect of diclofop (50 µM) on the plasma membrane Em of resistant (A) and susceptible (B) *A. fatua* coleoptile tissue. The coleoptiles were peeled and held in a flow-through cuvette filled with a bathing solution, then flushed with diclofop solution at time zero. The diclofop solution was replaced with herbicide-free solution after 15 min. Em was measured by inserting a microelectrode into a single cell. (From Devine *et al.*, 1993.)

root tissue (Shimabukuro and Hoffer, 1992) and with a variety of APP and CD herbicides in *L. rigidum* coleoptile tissue (Häusler *et al.*, 1991).

To further examine the effect of diclofop on Em in the R and S *A. fatua* biotypes, we have measured its effect on H+-ATPase activity and on proton pumping in purified plasma membrane vesicles. The vesicles were prepared by two-phase partitioning (Larsson *et al.*, 1987; Gallet *et al.*, 1989). Vesicle purity was checked by chlorophyll content (for chloroplast contamination), cytochrome C oxidase activity (mitochondrial contamination) and ATPase senstivity to various inhibitors (vanadate, nitrate, azide, molybdate). Diclofop (100 µM) had no effect on plasma membrane H+-ATPase activity (Figure 2), but did reduce proton flux across the membrane in both R and S vesicles (Figure 3D). However, when the vesicles were pretreated with diclofop and then assayed in the absence of diclofop, proton flux was restored to its full (control) value in both biotypes (Figure 3B and C). In this system, therefore, there was no difference in the response to diclofop between the R and S biotypes. This suggests that cell wall or other sub-cellular components may be required for the differential effect of diclofop on the Em of resistant and susceptible membranes.

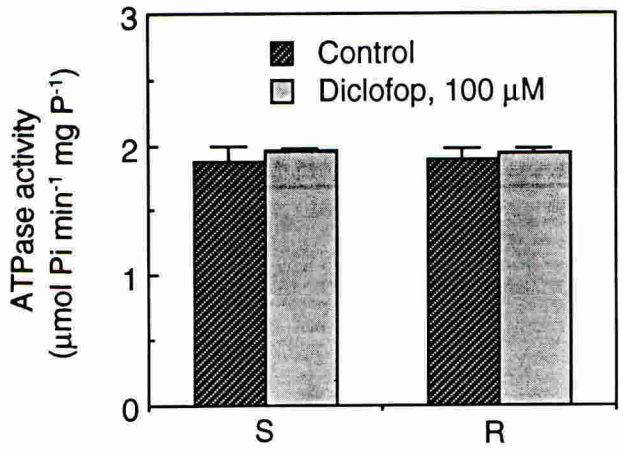

Figure 2. Effect of diclofop (100 µM) on plasma membrane H^+-ATPase activity in purified plasma membrane vesicles. H+-ATPase activity was measured by release of phosphate after addition of 3 mM ATP at 37 °C for 30 min. Results are the mean of six experiments ± standard error.

The relevance of membrane depolarization to the phytotoxicity of APP and CD herbicides has been the subject of much recent debate. Most available evidence suggests that membrane depolarization is not phytotoxic, and may not be directly linked to the phtotoxicity caused by ACCase inhibitors. Other evidence (reviewed by Devine and Shimabukuro, in press) indicates that the effect on Em cannot be ignored in accounting for the overall action of ACCase inhibitors. However, until the precise mechanism of membrane depolarization and its relationship to phytotoxicity have been determined, its relevance remains unclear.

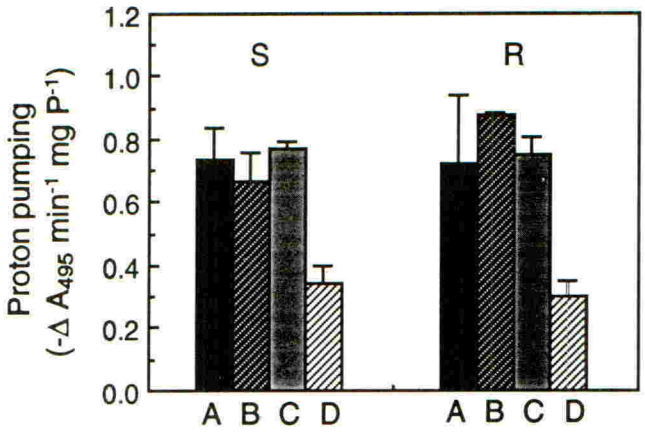

Figure 3. Effect of diclofop (100 μM) on proton flux in purified inside-out plasma membrane vesicles. Proton flux was determined by measuring the change in absorbance of acridine orange (a pH sensitive indicator) at 495 nm. A, control; B, C, vesicles pretreated with 100 μM diclofop for 15 or 30 min, respectively, then assayed in the absence of diclofop; D, vesicles pretreated with diclofop for 15 min and assayed with diclofop in the incubation medium. Results are the mean of six experiments ± standard error.

HERBICIDE COMPARTMENTATION IN RESISTANT AND SUSCEPTIBLE WEED BIOTYPES

If ACCase in the resistant varieties is truly sensitive to the herbicides, survival of these plants when treated with ACCase inhibitors must be due to one of the following: (a) the herbicide fails to reach the target site in sufficient quantities to have a lethal effect; (b) inhibition of ACCase (and hence fatty acid biosynthesis) is not lethal to the plant, at least in the short term; or (c) these plants have an alternate pathway for synthesizing malonyl-CoA, the product formed by ACCase. The fact that most grasses are killed when exposed to ACCase inhibitors argues strongly against possibility (b). While alternative malonyl-CoA synthesis mechanisms may exist (c), possibly via other biotinylated carboxylating enzymes, there is no evidence that these can be a major source of malonyl-CoA in higher plants. Therefore, (a) is a plausible explanation for herbicide resistance.

A compartmentation mechanism would result in less herbicide reaching the target site in the chloroplast. This could be a result of decreased foliar penetration, a reduction in the passage of the herbicide through the internal tissue, or in enhanced transport of the herbicide into a sink such as the vacuole. Cuticular penetration of various ACCase inhibitors has been shown to be equal in resistant and susceptible weed biotypes (Holtum *et al.*, 1991; Devine *et al.*, 1992, 1993). Therefore, compartmentation, if it occurs, must operate at the cell wall or one of the semi-permeable membranes that the herbicides pass through before reaching ACCase in the plastids. To date, we have examined the uptake of diclofop-methyl into purified plasma membrane vesicles and isolated protoplasts of resistant and susceptible *A. fatua* biotypes. In both systems, uptake was greater in the

susceptible than in the resistant biotype (Figure 4 and Table 1). However, the difference was not great, ranging from 15 to 30% less uptake in the resistant than in the susceptible protoplasts or vesicles. Similar results have been obtained with tralkoxydim in isolated protoplasts. It is not clear whether the relatively small difference in herbicide uptake between the resistant and susceptible biotypes could confer the high level of resistance seen at the whole-plant level.

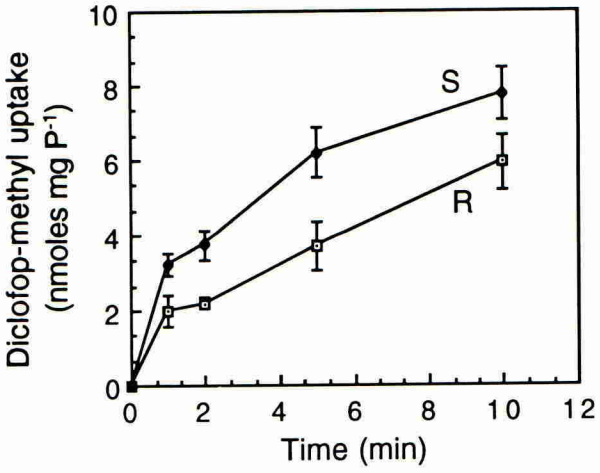

Figure 4. Uptake of ^{14}C-diclofop-methyl in purified plasma membrane vesicles from resistant (R) and susceptible (S) *A. fatua* biotypes. Uptake was measured at pH 5.5. Initial diclofop-methyl concentration was 2.0 µM. Each value is the mean of 12 observations.

Table 1. Uptake of ^{14}C-diclofop-methyl in isolated protoplasts from resistant (R) and susceptible (S) *A. fatua* biotypes. The data are means of two experiments each at two starting concentrations of diclofop-methyl. Uptake was measured at pH 5.6.

	Uptake of diclofop-methyl (pmol ($5x10^5$ protoplasts^{-1}))			
	2.6 µM		6.3 µM	
Time (sec)	R	S	R	S
10	485	570	965	1370
30	525	680	915	1335
60	540	650	1105	1425

Other possible exclusion/compartmentation sites include the cell wall, vacuole and chloroplast envelope. Herbicide uptake or retention by these barriers has not yet been examined. Since resistance in this *A. fatua* biotype is conferred by a single, semi-dominant gene (Murray, B. and Morrison, I.N., unpublished results), one single mechanism should

account for the observed resistance. Whether one mutation could alter herbicide transport across more than one membrane remains to be determined.

Alterations in herbicide uptake into protoplasts or plasma membrane vesicles could be due to changes in either the lipid or protein components of the membrane. However, detailed analysis has shown no difference in the lipid components of plasma membranes form resistant and susceptible *A. fatua* biotypes (Renault, S., Giblin, M., MacKenzie, S. and Devine, M., unpublished results). The membranes of sub-cellular organelles have not yet been examined. Differences in the protein profiles of purified membrane fractions of resistant and susceptible biotypes could provide preliminary evidence for a compartmentation mechanism located in that membrane. No differences in the plasma membrane protein profiles of resistant and susceptible *A. fatua* have been observed. However, preliminary evidence indicates a difference between the resistant and susceptible biotypes in the expression of a 45 kDa protein in the microsomal membrane fraction (Phelps, S.M. and Devine, M.D., unpublished results). This protein is present in untreated preparations from the resistant and susceptible biotypes and in treated preparations from the resistant biotype, but absent in herbicide-treated, susceptible preparations. Identification and characterization of this protein may shed some light on the resistance mechanism in *A. fatua*.

One remaining possibility is that the herbicide is prevented from reaching the target site by more rapid detoxification in the resistant biotypes. Herbicide detoxification has been examined in several weed biotypes resistant to ACCase inhibitors, but the results do not support enhanced herbicide metabolism as an important resistance mechanism. As stated previously, the slight increases in the rate of conversion of diclofop-methyl to polar (non-herbicidal) metabolites in *L. rigidum* (Holtum *et al.*, 1991) and of fenoxaprop-ethyl in *S. viridis* (Marles *et al.*, 1993) are very unlikely to confer the high level of resistance seen in these biotypes.

OTHER RELATED RESEARCH

The possibility that resistance in these weed biotypes is based on an altered ACCase cannot be ruled out until the ACCase from the resistant and susceptible biotypes has been characterized in detail. Recent evidence from maize indicates the existence of two distinct forms of ACCase (either isozymes or post-translationally modified forms); the major form of ACCase was sensitive to haloxyfop and sethoxydim, whereas the minor form was considerably less sensitive (Egli *et al.*, 1993). It is possible that there are two (or more) forms of ACCase in the resistant weed biotypes, only one of which is herbicide-resistant. If the resistant form was present in very small amounts, or was expressed or became functional only when required (i.e., when the susceptible ACCase was inhibited), it may not be apparent in *in vitro* assays of crude enzyme preparations. We are currently examining the possibility that expression of a second, herbicide-resistant form of ACCase confers resistance in the resistant biotype when the major form of ACCase is inhibited.

CONCLUSIONS

The evidence available at present indicates that at least two separate resistance mechanisms may be capable of protecting plants against APP and CD herbicides. Resistance based on reduced sensitivity of the target enzyme, ACCase, has been demonstrated in several species (Marles *et al.*, 1993; Tardif *et al.*, 1993; Tardif and Powles, 1993). However, the apparent sensitivity of ACCase in other resistant biotypes

(Devine *et al.* 1992; 1993; Holtum *et al.*, 1991) suggests that there is a second mechanism in some weed biotypes. The most likely explanation for resistance in these biotypes is that the herbicide is prevented from reaching the chloroplast in sufficient concentrations to inhibit ACCase. Preliminary evidence indicates that reduced uptake of herbicides into cells of the resistant biotype may be a component of the resistance mechanism.

With the exception of the multiple-resistant *L. rigidum* biotypes, most of the resistant in the plastid. It is intriguing, therefore, that there may be a resistance mechanism based on compartmentation that can discriminate between ACCase inhibitors and other herbicides. Many different resistant *A. fatua* populations have been identified, and these differ considerably in their cross-resistance among APP and CD herbicides (Heap *et al.*, 1993). This suggests that there may be one general resistance mechanism based on compartmentation or exclusion within which a range of more subtle changes allows for variable patterns of resistance to ACCase inhibitors.

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HERBICIDE RESISTANCE AND CYTOCHROME P-450

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ABSTRACT

Weed resistance to field doses of herbicides is becoming an increasing problem worldwide, with over 100 resistant weed biotypes identified thus far. Some species have developed cross-resistance to a number of herbicides with different modes of action, notably the grasses *Alopecurus myosuroides* in Britain and *Lolium rigidum* in Australia. Metabolism of herbicides to less phytotoxic derivatives is one of the mechanisms employed by plants to overcome sensitivity. Cytochrome P-450 monooxygenase enzymes have been shown to catalyse the initial oxidation of a number of herbicides, which ultimately leads to their detoxification, in several crop and weed species. In wheat, this enzyme has been shown to oxidise phenylurea and sulfonylurea herbicides, and thus to be fundamental to the selectivity of these herbicide classes. We are interested in studying the role of Cyt P-450 enzymes in herbicide metabolism in crops and weeds. Initial attempts to purify Cyt P-450 enzymes for detailed biochemical characterisation have met with little success. We describe work on the purification of Cyt P-450 from wheat, and characterisation of the enzyme. In addition, work on molecular cloning of plant Cyt P-450, in particular isoforms from *A. myosuroides* and *L. rigidum*, using a more general, PCR-based approach, are described.

INTRODUCTION

The cytochrome P-450 superfamily encodes numerous enzyme isoforms mediating a wide range of reactions in animals, microorganisms and plants. The involvement of cyt P-450 in the oxidative metabolism of xenobiotics in animals is well-documented (Ortiz de Montellano, 1986). It has been suggested that this metabolic capacity might have evolved in response to the ingestion of plant-derived defence metabolites (Gonzalez & Nebert, 1990). In plants, these enzymes are known to be involved in a number of biosynthetic pathways, including those leading to terpenoids, steroids, alkaloids, fatty acids, lignins and phenolics (Durst, 1991). Plants have also evolved detoxification pathways for xenobotics which in a number of cases have been shown to involve cyt P-450 (Donaldson & Luster, 1991).

The involvement of plant cyt P-450 in the detoxification of herbicides has been established for maize and wheat, where the initial oxidative attack on chlorotoluron, primisulfuron, chlorsulfuron and diclofop has been unambiguously attributed to cyt P-450 (McFadden *et al*, 1989; Fonne-Pfister *et al*, 1990; Mougin *et al*, 1990). This inherent metabolic capability is the basis for the selectivity of these herbicides in cereal crops, where weeds are unable to carry out sufficient detoxification of the applied compound. However, the severe selection pressure imposed by repeated applications of herbicides with similar modes of action has resulted in the appearance of such metabolic capacity in a number of previously sensitive weed species (Khan *et al*, 1985; Gonneau *et al*, 1988; Le Baron & McFarland, 1990). In particular, the grasses *Alopecurus myosuroides* (blackgrass) in the U.K. and *Lolium rigidum* (annual ryegrass) in Australia have evolved herbicide cross-resistant biotypes. A number of different mechanisms are likely to be involved overall, depending on the individual biotype. However, it is clear that

accelerated, cyt P-450 mediated, metabolism of chlorotoluron in blackgrass (Kemp & Caseley, 1987; Kemp & Caseley, 1991) and chlorsulfuron and chlorotoluron in certain ryegrass biotypes (Christopher *et al*, 1991; Burnet *et al*, 1993;) contributes to their tolerance to these herbicides.

A deeper biochemical understanding of the enzymes responsible for herbicide detoxification is needed in order to effectively combat resistance, and avoid this phenomenon with newly-developed herbicides. This understanding requires detailed study of the cyt P-450 isoforms responsible, either in a purified form or, following molecular cloning, heterologously expressed in a host with naturally low levels of endogenous cyt P-450 isoforms.

PURIFICATION OF PLANT CYT P-450

The purification of plant cyt P-450 has proved more problematic than that of the bacterial or mammalian isoforms. Principally, this has been due to the relatively low amounts of cyt P-450 in plant tissues. For example, phenobarbital-induced rat liver microsomes may contain about 2.45 nmol of spectroscopically-detectable cyt P-450 mg^{-1} of microsomal protein, whereas one of the best plant sources, ripe avocado fruit mesocarp, contains only 0.3 nmol mg^{-1} (O'Keefe & Leto, 1989). These levels may be increased by treatment with inducers (e.g. wounding and aging of Jerusalem artichoke tuber tissue, which increases the amount of detectable cyt P-450 by 110%; Reichhart *et al*, 1980; Durst, 1991). However, the levels rarely approach those obtainable from animal or bacterial sources. In addition, plant tissues often contain phenolics and pigments which render spectroscopic detection of these enzymes difficult. Despite these difficulties, the number of examples of plant cyt P-450 purified to homogeneity has increased dramatically over the past few years. TABLE 1 lists those plant isoforms whose purification to homogeneity has been reported thus far. In general, attempts to purify plant cyt P-450 have been successful when relatively large amounts of non-pigmented tissue (fruit, tubers or cultured cells) were available.

TABLE 1. Purified plant cyt P-450 isoforms.

Species	Tissue	Activity	Reference
Tulipa gesneriana	Bulb	AOS	Higashi *et al*, 1985 Lau *et al*, 1993
Persea americana	Fruit mesocarp	unknown	O'Keefe & Leto, 1989
Glycine max	Cell culture (Ind.)	D6aH	Kochs & Grisebach, 1989
Catharanthus roseus	Cell culture	G10H	Meijer *et al*, 1990
Helianthus tuberosus	Tuber slices (Ind.)	C4H	Gabriac *et al*, 1991
Linum usitatissimum	Seed	AOS	Song & Brash, 1992
Berberis stolonifera	Cell cultures	BTIQox	Stadler & Zenk, 1993
Vigna radiata	Etiolated seedlings	C4H	Mizutani *et al*, 1993
Phaseolus vulgaris	Cell culture (Ind.)	C4H	Rodgers *et al*, 1993

AOS, allene oxide synthase; D6aH, 3,9-dihydroxypterocarpan 6α-hydroxylase; G10H, geraniol 10-hydroxylase; C4H, cinnamate 4-hydroxylase; BTIQox, benzyltetrahydroisoquinoline alkaloid oxidase; Ind., induced by wounding, aging or elicitor challenge.

Purification of wheat cyt P-450

We have attempted the purification of cyt P-450 from blackgrass, rigid ryegrass and etiolated wheat seedlings. Levels of spectroscopically-detectable cyt P-450 in microsomes prepared from the weed grasses were extremely low, and this precluded further attempts at purification. However, appreciable levels (0.2 nmol mg^{-1} protein) of cyt P-450 were present in

microsomes prepared from four-day old wheat coleoptiles. The total amount of cyt P-450 in these preparations could be elevated by treatment of seedlings with the inducers napthalic anhydride, phenobarbital, and aminopyrine. However, no NADPH-dependent metabolism of ^{14}C-labelled chlorotoluron could be detected with microsomal preparations from induced or uninduced tissue. Partial purification of cyt P-450 from uninduced wheat coleoptiles was achieved by successive chromatography of detergent-solubilised membranes on Immobilised Artificial Membrane (IAM) (Pidgeon *et al*, 1991) and MonoQ ion-exchange columns. This procedure separated two fractions of cyt P-450. The first to elute from MonoQ, constituting approximately 50% of recoverable cyt P-450, was shown to catalyse allene oxide synthase (AOS) activity. We were unable to identify catalytic activity of the isoform(s) present in the second fraction. Attempts to further purify these fractions were unsuccessful. However, it is clear from this work that by far the most prominent individual cyt P-450 isoform in this tissue is an AOS, in agreement with previous results (Lau *et al*, 1993). This isoform is not reducible by NADPH:cyt P-450 reductase, and is therefore unlikely to be involved in NADPH-dependent herbicide metabolism.

It would appear, therefore, that in graminaceous species of agronomic importance (cereal crops and weed grasses) the purification of individual isoforms of cyt P-450 which oxidise herbicides will prove difficult.

MOLECULAR BIOLOGY

The low level of cyt P-450 in most plant tissues has meant that only preliminary characterisation of those isoforms involved in herbicide metabolism has been possible to date. Molecular cloning of these isoforms would enable their expression in a heterologous host (eg., yeast) for more detailed biochemical study. However, the conventional approach to cloning requires the availability of purified protein for the generation of antibody or oligonucleotide probes, which as discussed earlier, is likely to prove highly problematic in many cases.

Recently, cinnamate 4-hydroxylase cDNA sequences have been cloned from Jerusalem artichoke and mung bean using probes developed after purification of the corresponding polypeptide (Teutsch *et al*, 1993; Mizutani *et al*, 1993b). The first report of cloning of a plant cyt P-450, however, did not result from such an approach. Rather, differential colony hybridization using probes derived from mRNA's of ripe and unripe avocado fruit was used to identify ripening-related clones (Bozak *et al*, 1990). This procedure led to the identification of cDNA's encoding a ripening-related cyt P-450 (CYP71A1), with N-terminal predicted amino acid sequence identical to the previously-purified avocado cyt P-450 (O'Keefe & Leto, 1989). Differential hybridization of induced/non-induced cDNA's from cell cultures of *Catharanthus roseus* also led to the isolation of two cyt P-450 cDNA's, of as yet unknown function (Vetter *et al*, 1992). Meijer *et al* (1993) have described an efficient strategy for the isolation of cyt P-450 cDNA's using the polymerase chain reaction (PCR). This approach led to the cloning of 16 different cyt P-450 cDNA sequences from a cDNA library of *Catharanthus roseus*. We have also successfully applied a PCR strategy to the molecular cloning of a cyt P-450, geraniol 10-hydroxylase, from catmint (*Nepeta racemosa*) (Hallahan & Forde, 1991). Thus, molecular approaches exist which effectively bypass the need to purify protein, and which are therefore particularly suited to the isolation of low-abundance plant cyt P-450 sequences.

Molecular cloning of cyt P-450 from weed grasses using a PCR strategy

The true physiological role of the ripening-related cyt P-450 from avocado (CYP71A1) remains unidentified. However, we believe it is likely to be involved in terpenoid metabolism (Hallahan *et al*, 1992). This enzyme was first characterised as carrying out general xenobiotic metabolism (demethylation of *p*-chloro-N-methyl aniline; Dohn & Krieger, 1984), and we therefore chose it as a suitable starting point for molecular cloning of plant cyt P-450 species from the resistant weed grasses. Oligonucleotide PCR primers were constructed based on conserved sequence domains in CYP71A1, and used to amplify cDNA's from annual ryegrass.

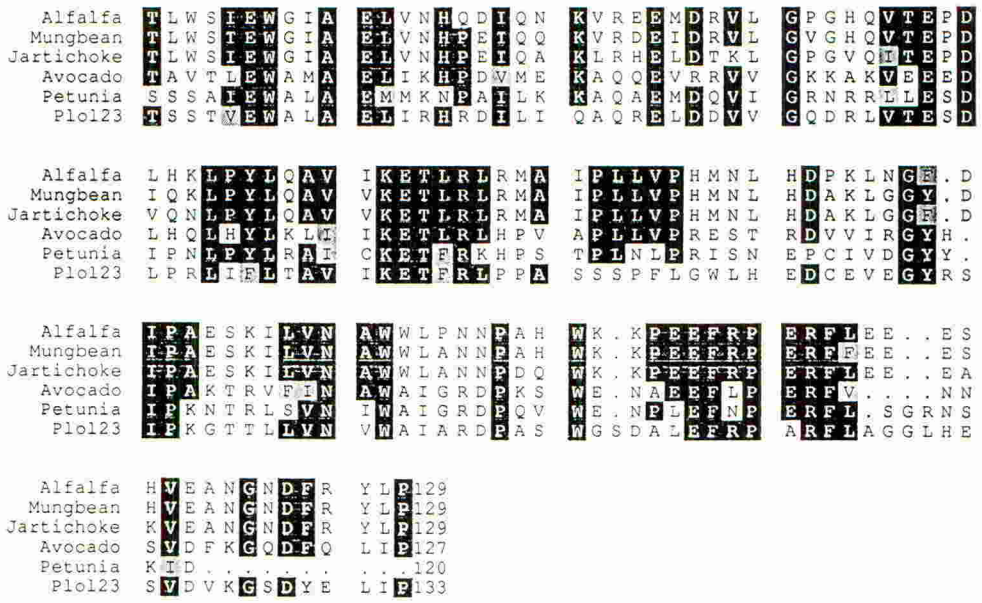

FIGURE 1 Amino acid sequence deduced from the PCR product plol23 aligned with other known plant cyt P-450 amino acid sequences; Alfalfa (L11046); Mung bean (L07634); Jerusalem artichoke (Z17369); Avocado (M32885); Petunia (X71130). Alignment obtained using the program PILEUP, with gap weight set to 3.0 and gap length weight at 0.1.

The PCR amplified a cDNA of the expected size (approximately 0.42 kb). This product was cloned into the vector pUC9, and sequenced using the dideoxynucleotide method. The deduced amino acid sequence of this clone (pLol23) is presented in FIGURE 1, aligned with several other plant cyt P-450 sequences using the PILEUP programme. FIGURE 1 shows that the amino acid sequence deduced from plol23 exhibits significant homology with all the other cyt P-450 sequences. The highest degree of homology is seen with the sequences from petunia and avocado; at the amino acid level, plol23 exhibited 48% identity with both of these cytochromes.

Such "shotgun" approaches to cloning plant cyt P-450 will initially yield cDNA fragments without information as to the function of the corresponding enzyme. However, these fragments will be used as probes of Northern blots, with mRNA derived from different plant parts with or without herbicide or inducer treatment. The information derived from such experiments should indicate whether a particular cyt P-450 gene might be involved in a response to herbicides. The fragment of interest will then be used to isolate full-length cDNA from expression libraries. Expression of full-length cDNA's in a heterologous host should then enable detailed biochemical characterisation of the cloned isoform.

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SURVEY AND GENE FLOW IN ACETOLACTATE SYNTHASE RESISTANT KOCHIA AND RUSSIAN THISTLE

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ABSTRACT

Weeds resistant to the ALS (acetolactate synthase) inhibitor herbicides are a problem in western North America. Two of the resistant weeds with the most important economic impact are the tumbleweeds, Russian thistle (*Salsola iberica* Senn. & Pau.) and kochia [*Kochia scoparia* (L.) Schrad]. Surveys to determine the range and proportion of ALS resistant Russian thistle and kochia were conducted from 1991 to 1993. Russian thistle was surveyed in Washington State and kochia was surveyed in Colorado, Idaho, and Montana. Resistant Russian thistle was found on 70% of the survey sectors. In 1991, the percentage of samples with resistant kochia by state was: Colorado 9%, Idaho 49% and Montana 75%. In 1992, percent of samples with resistant kochia by state was: Colorado 46%, Idaho 55% and Montana 45%. Pollen movement was measured between resistant and susceptible kochia plants in field experiments. Crosses were found at the limits of the experiment, 30 m. Maximum outcrossing was 13% in 1991 and 4% in 1992. Russian thistle plant movement and weight loss were measured. Plants moved 4 km in 1991 and 3.5 km in 1992.

INTRODUCTION

Sulfonylurea resistant prickly lettuce and kochia were identified in 1987 and sulfonylurea resistant Russian thistle was identified in 1989 (Thill *et al.*, 1991). By 1993, 14 species resistant to ALS inhibitors had been identified (Saari *et al.*, 1993). Two strategies were undertaken to study ALS inhibitor resistant weeds. One approach was to survey weed populations for resistant weeds and the other was to determine the contribution of gene flow, through pollen and seed movement, to the spread of resistance.

In order to understand the range and proportion of resistant weeds, surveys were conducted over wide geographic areas (Stallings *et al.*, 1992; Mallory-Smith *et al.*, 1993; Wright *et al.*, 1993). Russian thistle was surveyed in Washington State where it was first identified. Three states were chosen to provide baseline information about the occurrence of resistant kochia. Chlorsulfuron had been widely used in Montana, moderately used in Colorado, and sparingly used in Idaho. Therefore, it was expected that the frequency in which resistant plants were found in each state would reflect the pattern of use and the initial survey could be used to study the development of resistant weed populations over long periods of time.

Models, to predict herbicide resistance dynamics which include abiotic and biotic factors, have been have been developed (Gressel and Segel, 1982; Maxwell *et al.*, 1990). To effectively manipulate the abiotic factors, such as herbicide effectiveness and use, there must be a greater understanding of the biotic factors involved with the evolution of herbicide resistance. Gene flow is one of the biotic factors addressed by the Maxwell model (1990). Gene flow processes, both pollen and seed movement, directly alter the frequencies of resistant and susceptible alleles in a population. These authors suggest that it may be possible to manipulate gene flow from susceptible plants to manage resistance. Before management strategies can be applied to prevent or delay resistance, gene flow must be evaluated. Kochia and Russian thistle are open-, wind-pollinated tumbleweeds. Therefore, both pollen and seed movement could be expected to play a critical role in the spread of resistance.

The objectives of this research were 1) to determine the frequency and range of resistant Russian thistle in Washington and kochia in Colorado, Idaho and Montana, and 2) to measure gene flow of the resistance gene via pollen and seed movement.

MATERIALS AND METHODS

Surveys

Similar survey systems, with some modifications, were used for Russian thistle and kochia. The areas sampled were divided into sectors. Sectors were 373 km^2 for the Russian thistle survey. For the kochia survey, sectors were 92 km^2 in Colorado, 156 km^2 in Idaho, and 164 km^2 in Montana. There was one sample site per sector. Each sample site was near the center of a sector to enable uniform sampling. Sample sites were located adjacent to primary and secondary roadways to facilitate access and timely collection. Sites included cropped fields, road sides, railroad right-of-way, fence lines, industrial areas, conservation reserve land and rangeland. Selection of the specific site-location and type within a sector was random.

Seeds were collected from 30 randomly selected plants per sector and stored in individual packets. A composite sample was made for each sector and tested for resistance by spraying with chlorsulfuron. Russian thistle was treated with two post-emergence applications, 5 d apart, of 52.5 g AI ha^{-1} plus a nonionic surfactant at 0.25% v/v. Treatments were applied with a CO_2 pressurized, greenhouse chain-driven sprayer calibrated to deliver 140 L ha^{-1} at 275 kPa. Kochia was treated with 78.1 g ha^{-1} pre-emergence and 14 d later with 17.5 g ha^{-1} plus a nonionic surfactant at 0.25% v/v post-emergence. The composite samples were rated as resistant, susceptible or mixed. The mixed composite samples were then retested by individual plants to determine the percentage of resistant plants per sector.

Gene flow

Pollen Movement

Pollen movement was studied under field conditions in 1991 and 1992. Kochia was planted into a spring barley field using a Nelder plot design. The area chosen for the study had no native kochia present. The study consisted of four Nelder plots with a minimum of 31 m between plots. Each 61 m diameter plot had 16 rays spaced 22.5° apart and contained 211 kochia plants. There were 12 susceptible and 1 resistant plant on each ray. The R plant was 1.5 m from the center of the plot. Three susceptible plants were placed in the center of the plot. The susceptible plants were spaced at 3.0, 4.8, 6.1, 7.6, 9.1, 12.2, 15.2, 18.3, 21.3, 24.4, 27.4, and 30.5 m from the center of the plot. Wind direction was measured every 5 s, averaged, and recorded at 3 h intervals. Temperature and precipitation were recorded daily. Seed was collected at maturity from each susceptible plant, counted, planted and sprayed with a pre-emergence application of 78.8 g AI ha^{-1} chlorsulfuron to test for resistant F_1. Surviving kochia plants were sprayed 2 weeks after planting with 17.5 g ha^{-1} chlorsulfuron plus a nonionic surfactant at 0.25% v/v to ensure that there were no escapes of susceptible plants. All surviving resistant plants were the result of pollen moving from a resistant plant to a susceptible plant.

Seed Movement

Field studies were conducted in 1991 and 1992 to measure how far and how fast Russian thistle plants would move and how much seed would be lost over time and distance. Twenty-four plants were placed on wheat stubble, 24 on fallow and 24 were secured to the ground to prevent tumbling. Each plant was coded so the movement of individual plants over time and distance could be determined. On each sampling date, four pre-selected plants from each site were collected, measured and seeds counted. At every collection date, the location of all plants was recorded with a global positioning receiver. A weather station was centrally located to the three sites to measure wind direction and rate.

RESULTS

Surveys

Russian thistle samples were collected in 86 of 149 sectors. Of the 86 sectors, 50 were cropland, 31 were roadside, 11 were rangeland and 8 were industrial. Resistant Russian thistle was found in 60 (70%) of the sectors that contained Russian thistle. Homogeneous and heterogenous resistant populations were found within the sectors with resistance. Twenty-four sites contained only resistant plants and 36 sites had both susceptible and resistant plants. Resistance in the heterogeneous populations ranged from 1 to 29 plants of the 30 plants sampled.

Kochia was found in all of the 300 sectors sampled in Colorado and the 343 sectors sampled in Montana. In Idaho, kochia was found in 70% of the 422 sectors sampled. In 1991, resistant kochia was found in 9% of the sectors in Colorado, 49% of the sectors in Idaho and 75% of the sectors in Montana. In 1992, resistant kochia was found in 46% of the sectors in Colorado, 55% of the sectors in Idaho, and 43% of the sectors in Montana. As with Russian thistle, homogenous and heterogeneous resistant kochia populations were identified.

Gene flow

Pollen Movement

Susceptible kochia plants produced pollen 4 d and 21 d earlier than the resistant plants in 1991 and 1992, respectively. In 1991, the maximum number of crosses on any one plant was 13%. In 1992, the maximum number of crosses was 4%. In both years crosses were found at the outer limit of the study, 30 m from the resistant plants. As expected, the maximum number of crosses was found in the direction of the prevailing winds on the susceptible plants nearest the resistant plants.

Seed Movement

In 1991, 62 of the 72 plants were recovered. Secured plants lost 3 to 10% of their total weight while the tumbling plants lost 10 to 68% and moved from 0.4 to 4 km. In 1992, 69 of 72 plants were recovered. Secured plants lost 4 to 25% while tumbling plants lost to 6 to 94% of their biomass and moved up to 3.5 km. Plant movement was stopped by physical obstructions, such as ditches and fences.

DISCUSSION

The surveys provide baseline information so that the increase or decrease of resistant populations can be documented with time. The fact that resistant weeds were found over wide geographical areas and in high numbers after less than 10 years of herbicide use, suggests the seriousness of the sulfonylurea resistant weed problem and how quickly resistant weed populations can develop. It was surprising that over 50% of the sites in Idaho contained resistant kochia, since there had been little sulfonylurea use for weed control in crops. However, ALS inhibitors were used to some extent for weed control on road sides and along railroad right-of-ways and resistant weeds may have been selected there and served as a pollen and seed source for resistance to spread into cultivated areas.

Pollen flow may play a major role in the spread of resistance in kochia and Russian thistle. This is particularly true since these weed species are open-, wind-pollinated and the trait is dominant or semi-dominant (Saari, *et al.* 1993). In the tumbleweeds, seeds are responsible for long distance movement of the resistant trait.

The surveys and gene flow studies provide evidence that in a highly mobile, outcrossing species, gene flow is important to the spread of resistance in weeds and may play a greater role in the increase in resistant populations than might have been predicted by simulation models (Maxwell *et al.*, 1990). Management strategies based on the results of these studies

indicate that it will be important to prevent the emigration of the resistant gene into susceptible populations and that the immigration of susceptible genes into resistant populations will not be great enough to prevent the spread of resistance. This has important implications for the individual who is making an effort to use integrated weed management strategies to control or prevent resistance but is adjacent to areas that are not being managed responsibly.

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