SESSION 8A

GENETIC VARIATION WITHIN WEED SPECIES – ITS INFLUENCE ON BIOLOGY AND CONTROL

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THE INFLUENCE OF INTRASPECIFIC VARIATION ON THE BIOLOGY AND CONTROL OF AGRICULTURAL WEEDS

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ABSTRACT

A review is provided of the major factors which affect levels of genetic variability in biological attributes within agricultural weed species. Examples are given of intraspecific variation in weeds in life history trait response to selection pressure from current agricultural management practices and in allozymes which in general measure genetic variation in the absence of direct selection. The relationship between life-history and allozyme studies of genetic variation of weedy species will be compared. In conclusion, the potential influence of intraspecific variation of weeds in relation to sustainable agriculture are discussed.

INTRODUCTION

Agricultural ecosystems are often artificial monocultures of genetic uniformity where the additional homogenizing effects of chemical applications often result in reduction in genetic diversity at both the specific and intraspecific levels. With the increased emphasis on more sustainable agricultural crop production strategies, variability in biological systems, including the weed or pest component of agro-ecosystems, will have increasing significance in determining effective control programs (Altieri, 1991). Genetic differences within weeds can cause phenotypic and behaviourial differences among populations and even between individuals of the same species in their response to external factors. The more heterogeneous the weedy population, the more likely that it can adapt to current as well as proposed alternative agricultural methods. Genetic diversity within agricultural weeds can affect control strategies, as evidenced most obviously with herbicide resistant biotypes, but genetic differences in other life-history attributes, such as dormancy, germination, may also influence control measures.

Weeds can be defined as colonizing plant species that grow in habitats markedly disturbed by man and are commonly classified according to habitat-type, e.g. agrestals - agricultural land; ruderals - waste places, roadsides (Baker, 1974; Holzner, 1982). In spite of their habitat and taxonomic diversity, weeds exhibit many similarities as summarized in the growth characteristics of an "ideal" weed (Holzner, 1982). These include non-specific germination requirements, rapid development to flowering, high seed productivity, ability to self, well developed seed dispersal, and tolerance of a wide range of environmental conditions. However, as indicated by Barrett (1988) and others, not all weeds conform to the above ideal traits.

Studies of genetic variation in weeds and other colonizing species

(see reviews Brown & Marshall, 1981; Barrett, 1982, 1988; Warwick, 1990a) have indicated a number of shared genetic features for weeds (Table 1) that contribute to their success and spread in disturbed environments. The mating system is the most important factor influencing the distribution of genetic variation within and among plant populations (Loveless & Hamrick, 1984; Brown & Burdon, 1987; Hamrick & Godt, 1990). Selfing, important in the establishment of weed populations, results in fewer genotypes and reduced levels of heterozygosity. As a result populations of predominantly selfing weeds tend to be genetically uniform but highly differentiated from one another, in contrast to outcrossing species, where population differentiation is less apparent and most of the variation characteristic of the species is found in a given population.

TABLE 1. List of shared genetic features for weeds that contribute to their success and spread in disturbed environments.

i)	self-fertilization,
ii)	polyploidy,
iii)	a reduced number of genotypes resulting in genetically depauperate populations,
iv)	substantial interpopulation differentiation, and
v)	high levels of phenotypic plasticity.

Agricultural weeds are especially susceptible to the processes that lead to a loss of genetic variation in populations (Barrett, 1988; Barrett & Shore 1990; Warwick, 1990a). The size of original weed contaminations is usually small, resulting in genetic bottlenecks and/or genetic drift, and reduced population variability, particularly in selfing species (Brown & Marshall, 1981). The transient nature of many weed populations due to control practices can also prevent the buildup of large stable population systems capable of maintaining large stores of genetic variation (Barrett & Shore, 1990).

The mating system and differences in the degree of spatial and temporal isolation of weedy populations on a more local scale will also contribute to differences in genetic structure. For example, the crop, depending on height and density, may impede gene flow or exchange of pollen between weedy plants in cultivated fields and adjacent roadside populations. Studies indicate that pollen flow and hybridization between individuals of Chenopodium album growing in a maize field is one-tenth that observed in a pure stand of the weed (Warwick, 1991). In addition, genetic differences in the mating systems have been observed in different parts of the species range, i.e. newly colonized or marginal areas (Brown & Burdon, 1987).

Both genotypic differentiation and phenotypic differences within weedy species are likely responses to selection imposed from common agricultural practices. Phenotypic plasticity is used to describe variation in phenotypic expression of the genotype which enhances the survival and reproduction under diverse conditions (Sultan, 1987) and was considered essential in weeds (under the concept "general-purpose" genotype - Baker 1974) in conferring their wide tolerance to fluctuating

environments. The relationship between genetic differentiation and plasticity is often complex and in fact, genetic variation among individuals for plastic response has been documented in weeds. For example, populations of Xanthium strumarium from natural (riverside) and ruderal (urban waste) habitats differed in their plastic response to resource availability, resulting in the enhanced performance of ruderal populations in more-impoverished habitats (Blais and Lechowicz, 1989).

Studies on genetic variation in weeds for the purposes of this paper will be divided into two sections. The first will provide examples of intraspecific variation in life-history traits which are likely to be of adaptive importance and the result of response to direct selection pressure from current agricultural management practices. The second part will describe allozyme studies which in general measure genetic variation in the absence of direct selection. The relationship between life-history and allozyme studies of genetic variation will be compared. In conclusion, the potential influence of intraspecific variation of weeds in relation to sustainable agriculture will be discussed.

LIFE-HISTORY TRAIT VARIATION

Studies documenting genetic variation in life history traits of agricultural weeds involve the comparative growth of populations from areas subject to different agricultural practices (there may or may not be obvious patterns of morphological or physiological differentiation) under standard cultivation or preferably a range of stress conditions. The distribution of genetic variability among and within weed populations in life-history traits can then be assessed from statistical analyses. Populations which maintain their adaptive differences may be referred to as biotypes or ecotypes, although a continuum in response is likely along a selection gradient. Typical life history traits which may be affected by agricultural management practices include: rates and time of seed germination and dormancy, seed and plant size, growth rates at different stages of maturity, time to flowering and other physiological characteristics, and allocation to reproductive/vegetative components.

Examples of genetic differentiation in weeds in relation to a number of selective factors including: latitude, light regime, soil type, cutting/seasonal mowing, grazing/trampling, herbicides, biotic factors, and cultivation practices are given in Barrett (1982, 1988) and Warwick (1990a). However, with the exception of herbicide resistance, studies investigating genotypic differentiation of agricultural weeds in response to the selection pressures imposed by current agricultural practices, such as cultivation methods, fertilizer applications, herbicides, crop associations, are very limited. A few examples will now be considered.

Variation patterns in seed dormancy and germination profiles have probably received the greatest attention in weed population studies. Jana and Thai (1987) showed that different agricultural conditions could affect variation patterns for seed dormancy in Avena fatua, in particular summer-fallowing of cropland was found to enhance the presence of dormant genotypes. Hume (1990) evaluated the ecological effects of early and late flowering strains of Thlaspi arvense and fall and spring emergence on the phenology, seed production and germination patterns in natural field situations. The strain was found to influence the time at which

plants emerged and the time at which plants will flower the next season and that even within strains there was evidence for genetically-based variation influencing the number of days that plants require to flower.

The most obvious example of genetic variation in agricultural weeds is in response to herbicide selection. There are over 100 weed species with biotypes known to be resistant to herbicides (see reviews Warwick, 1991; Gasquez & Darmency, this conference). It is, therefore, surprising that very little is known about genetic variability for herbicide tolerance in field populations of weeds. The few studies that have been conducted have indicated significant amounts of inter- and intra-population variability for herbicide reaction. For example, studies of Avena fatua populations in Canada (Thai et al., 1985) demonstrated significant differences in variability for triallate reaction within and between exposed and unexposed populations. There was a positive relationship between tolerance of a population and degree of herbicide exposure, and within population variation was higher in unexposed than in exposed populations.

In addition to herbicide control measures, long term weeding pressure in horticultural and arable areas is likely to be a potent selective force favouring the early development of annual weed species. For example, populations of Senecio vulgaris and Stellaria media from intensively weeded habitats develop and fruit more quickly than those from less intensively weeded habitats (Briggs et al., 1991).

ALLOZYME VARIATION

Studies of allozyme variation have become very important in documenting levels of genetic variation in all plant species, including weeds. Allozyme data provide an estimation of genotypic diversity in the absence of direct selection pressure, are considered to be one of the most objective analyses of the genetic structure of populations, and generally reflective of overall genetic variability as independently measured by morphological variation. In addition allozyme data can be used to provide information on the mating system of weeds, and when used as genetic markers information as to the number and likely source of weed introductions. Allozymes can also be used to trace the evolutionary history of a particular trait, i.e. whether multiple occurrences represent the spread of a single genotype or the result of separate events, as used for example in triazine resistance studies in different regions of Canada and France (Warwick, 1991).

Allozymes, first viewed as bands on an electrophoretic gel, are direct gene products, usually with simple Mendelian patterns of inheritance. It is possible to document their genetic basis through classical segregation studies and obtain data for an exact number of coding loci and their allelic products (see reviews: Tanksley & Orton, 1983; Soltis & Soltis, 1990). In addition because of the conservation in numbers of isozymes in plant tissue, it is easier to ensure direct homology of loci when comparing different populations or even different species. The range of enzymes regularly surveyed are coded for by structural genes and the enzymes are primarily concerned with glycolysis and the photosynthetic pathway and are therefore usually expressed. There is no weighting of characters and all loci are given equal value.

Several genetic parameters can be estimated, including number of polymorphic loci, average number of alleles per locus, various indices of genetic diversity and genetic distance, and levels of heterozygosity. These allow a quantitative assessment of inter- and intra-populational differentiation in a given species.

TABLE 2. Examples of allozyme studies in weeds (from Barrett & Shore 1990; Warwick 1990a). Ploidy - diploid (D) or polyploid (P). Mating system - primarily inbreeding (I) or outbreeding (O).

Taxa	Location	Ploidy	Mating	References
Low levels of allozyme variation				
Abutilon theophrasti	Canada	P	I	Warwick, 1990b
Avena barbata	U.S.A.	P	I	Brown and Marshall, 1981
Cyperus esculentus	U.S.A.	P	O	Horak and Holt, 1986
Datura stramonium	Canada	D	O	Warwick, 1990b
Echinochloa crus-galli	Australia	P	I	Barrett and Shore, 1990
Emex spinosa	Australia	D	I	Marshall and Weiss, 1982
Panicum miliaceum	Canada	P	I	Warwick, 1990b
Setaria faberi	Canada	P	I	Warwick, 1990b
Sorghum halepense	Canada	P	I	Warwick, 1990b
Xanthium strumarium	Australia	P	I	Brown and Marshall, 1981
High levels of allozyme variation				
Apera spica-venti	Canada	D	O	Warwick et al., 1987
Carduus spp.	Canada	D	O	Warwick, 1990a
Echium plantagineum	Australia	D	O	Brown and Burdon, 1983

Nearly all the world's most serious weeds are polyploids (Brown & Marshall, 1981, Table 2) and patterns of allozyme variation are strongly influenced by polyploidy. With increased genome copy number and resulting gene duplications, it is possible for a single individual plant to express stable multiple allelic forms at these duplicated gene loci. Consequently in polyploid weeds considerable within-individual variation is often evident in the form of enzyme multiplicity, even though populations may contain only a few genotypes (Barrett & Shore, 1990; Warwick, 1990a,b). Various proposals have suggested that such multi-enzyme phenotypes could potentially result in increased biochemical variability in an individual and permit adaptation to a wider range of environments. This pattern of few, but potentially highly variable, multilocus "allozyme genotypes", may well correspond to Baker's general purpose genotype described earlier. However to date, few studies documenting the ecological or adaptive significance of such phenotypes exist (Soltis & Soltis, 1990). García et al. (1991) examined the relationship between genetic diversity (13 allozyme loci) and adaptedness in the tetraploid Avena barbata and its diploid ancestors A. hirtula and A. wiestii and found that the relationship varied from locus to locus. Superior adaptedness was associated with genetic uniformity for five loci and with fixed multiple allelic diversity for eight loci, suggesting that the latter was an important factor in the evolution of adaptedness in A. barbata.

In general, levels of allozyme variation in weedy species are much lower than those for late successional plant species, 30 and 63% average percent polymorphic loci, respectively (Loveless & Hamrick, 1984; Hamrick & Godt, 1990). However, allozyme variability in weeds ranges from very low levels, one to a few genotypes, to species with extremely high levels of genetic variation (Table 2). The amount and organization of allozyme variation differs among species with contrasting life histories, but also within and among populations of a single species (Barrett & Shore, 1990). In general, predominantly selfing agrestal weed populations have an extremely restricted number of genotypes throughout the sampled range, with most populations containing a single genotype (several North American agricultural weeds in Table 3); while in contrast, the high levels of allozyme variation cited in Table 2 are usually observed in predominantly outcrossing weed species.

TABLE 3. Allozyme variation in five self-fertilizing agricultural weeds of maize and soybean monocultures from eastern Canada (Warwick 1990b).

	Abutilon theophrasti	*Panicum miliaceum*	*Setaria faberi*	*Sorghum halepense*	*Datura stramonium*
Chromosome No.	2*n* = 24	2*n* = 36	2*n* = 36	2*n* = 40	2*n* = 24
No. of population	39	39	8	13	9
No. of loci	27	19	24	21	22
No. (%) of loci monomorphic	25 (93%)	18 (95%)	21 (88%)	18 (86%)	22 (100%)
No. (%) of loci polymorphic	2 (7%)	1 (5%)	3 (12%)	3 (14%)	0
No. (%) of duplicated loci with enzyme multiplicity	14 (52%)	8 (42%)	13 (54%)	3 (14%)	2 (9%)
No. of multilocus genotypes	4	2	9	10	1

As indicated earlier, agricultural habitats are generally considered less heterogeneous environments because of their simple biotic structure and the high level of predictability associated with land use patterns. As a result limited allozyme variation is expected in agrestal as compared with ruderal weedy species or populations (Barrett, 1982, 1988). However, few weed studies have been conducted which test this hypothesis and conclusions to date are somewhat conflicting. Warwick and Black (1986) found little evidence for habitat-correlated differences in levels of genetic polymorphisms in ruderal and agrestal populations of *Chenopodium album* and *Amaranthus retroflexus*, from Ontario, Canada (with the exception of triazine-resistant agrestal populations which showed very low genetic diversity in both species). Isozyme studies in *Chenopodium album* populations in France (Al Mouemar & Gasquez, 1983) have demonstrated greater allozyme polymorphism in garden populations compared with those from cultivated fields. In Europe, Bosbach and Hurka (1981) found greater levels of genetic heterogeneity in populations of *Capsella bursa-pastoris* from recently cultivated sites, compared with those from lawns and other habitats which had not been recently disturbed.

Allozyme data have proved useful for comparing levels of genetic

variation in both native and introduced ranges of a weed and for determining whether weed introductions are the result of single or multiple events (Table 4). For example in studies of the outcrossing weed Apera spica-venti (Warwick et al., 1987), high levels of genetic variability in both life history traits and allozymes were evident in both European and introduced Canadian populations. In contrast in the predominantly selfing weed species, Polygonum lapathifolium (Consaul et al., 1991), very low levels of allozyme variation were observed in populations from both North America and Europe and evidence was provided for a native North American and a European component of the complex in North America.

TABLE 4. Comparisons of genetic variation in native European and introduced Canadian populations of two weeds.

A. Apera spica-venti (from Warwick et al., 1987)

	Canada	Europe
No. of loci surveyed	17	17
Percent polymorphic loci	57	62
Mean no. of alleles	2.54	2.53
Mean heterozygosity	0.23	0.23
Total species diversity	0.211	0.208
Mean population diversity	0.209	0.203
Inter-populational gene diversity	0.010	0.024

B. Polygonum lapathifolium (from Consaul et al., 1991)

	N. America	Europe
No. of loci surveyed	23	23
Percent polymorphic loci	13	26
Mean no. of alleles	2.00	2.00
No. mutilocus genotypes	7	8

GENETIC VARIATION IN WEEDS - ALLOZYME DATA VERSUS LIFE HISTORY TRAITS

The relationship between allozyme variation and genetic variation in life history characters in weedy species is a complex one (Price et al., 1984). Several studies have indicated a lack of concordance between allozyme variation and that seen in life history traits (Barrett 1988; Barrett & Shore, 1990; Warwick, 1990a,b). This would indicate that high levels of genetic diversity as estimated from allozymes are not a prerequisite for a successful weedy species. Allozyme variation would appear to reflect with reasonable accuracy the genetic and adaptive diversity of predominantly outcrossing weedy plants. However, the value of allozyme variation in predicting other types of genetic variation in predominantly selfing agricultural weed species is more limited. Analyses by Loveless and Hamrick (1984) indicated the correlations of several life-history characteristics and ecological factors with allozyme variability and the genetic structure of plant populations; the most important of which was the breeding system. This finding was confirmed in a recent study by Wolff (1991) which compared levels of allozyme and morphological variability in three weedy species of Plantago with contrasting breeding systems.

The differences in adaptive life history traits, evident in populations of weeds, may represent recent divergence in response to selection, a divergence which has not been paralleled in the allozyme characters. The latter are generally considered to be selectively neutral, although studies of population differentiation of wild barley (Nevo et al., 1986) and Plantago major (Van Dijk, 1984) indicated close linkage of allozyme markers with life history differences, suggesting the possibility at least for indirect selection of allozyme characters.

INTRASPECIFIC VARIATION IN RELATION TO SUSTAINABLE AGRICULTURE

With an increasing emphasis being laid on sustainable agriculture, an understanding of the variations within and between individuals, and populations of weedy species and of the genetic diversity manifested in life history traits and allozyme polymorphisms has become of increasing importance. We need to know more about variation in weeds to optimize weed control in current agricultural systems inorder to predict how individuals of a weedy species may respond to the porposed changes in agricultural systems. There will be a need to assess the altered conditions or "stresses" associated with more sustainable or alternative agricultural practices on weeds, such as greater crop rotation, intercropping, reduced use of chemicals and increasing use of genetic pest resistance, and increasing biological control of weeds, insects, diseases.

For example, the proposed reduction in fertilizer use for crops is certainly a management practice which is likely to affect the genetic structure of weed populations since several studies have shown the potential for rapid genetic change in response in weedy species to soil fertility, for example in Taraxacum ssp. (Hommels et al. 1991). Similarly practices which involve greater crop diversity, either as a result of crop rotation and/or increasing within crop diversity (Altieri, 1991), will affect the competitive environment of the weed and crop. Intercropping measures to reduce weed problems and the decreased use of herbicides will also change competitive interactions. Increasing use of green manure and farm manure will have a direct effect on the weed seed bank dynamics and likely genetic composition of weed populations.

The successfulness of biological control programs as an alternative to herbicides, such as with fungal pathogens as bioherbicides or natural insect pests, will also depend on the genetic makeup of both the target weeds as well as the potential control agent. If the weed host consists of a genetically heterogeneous population, it may vary in susceptibility to the biocontrol agent within its geographic range as seen for example among Australian ecotypes of the skeletonweed (Chondrilla juncea) to the rust Puccinia chondrillina.

It has also become clear from recent studies that greater genetic variability in the weed population is not necessarily all bad. For example, population models describing the evolution of herbicide resistance in weeds (Warwick, 1991) recognize that the presence of susceptible plants is important in reducing the likelihood of establishment of resistant populations. Past experience indicates that weedy species do show rapid and considerable adaptive strategies to current agricultural practices, and therefore we can predict similar adaptation to the altered agricultural ecosystems of the future.

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INTRASPECIFIC VARIATION AMONG POPULATIONS OF CLEAVERS (*GALIUM APARINE* L.)

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ABSTRACT

Genetic variation among populations of *Galium aparine*, each collected from field centres and adjacent field boundaries, was determined by means of spaced plant trials. Various morphological and phenological attributes were assessed including leaf characteristics, internode length and flowering date. Although considerable morphological variation was observed between populations, these differences were not, with the exception of internode length, related to habitat. Plants from populations derived from within cropped fields had shorter internodes than those from adjacent field boundaries. Likewise, although there were differences in susceptibility to mecoprop between populations they were not affected by habitat of origin. However, variation in reproductive capacity, seed size and dormancy characteristics differed markedly between habitats. Isozyme analysis failed to provide convincing evidence for separate origins of populations from the contrasting habitats, but evidence of genotypic differentiation was confirmed. The results are discussed in relation to ecotypic selection within a species and implications for migration between habitats.

INTRODUCTION

Population differentiation among weed species is well documented and often exemplified by differences in life strategy including relative allocation to vegetative versus reproductive development. That weeds are genetically disparate is evident from intraspecific variation in susceptibility to herbicides. Variation in response to herbicides has been reported for populations of *Galium aparine* L. (Lovegrove *et al.*, 1985; Lutman & Lovegrove, 1985; Hill & Courtney, 1991), whilst variability in growth and competitive ability have also been documented (Wilson & Wright, 1987). Ecotypic differentiation with regard to germination requirements have been reported for populations of *G. aparine* derived from contrasting ruderal and arable habitats (Froud-Williams, 1985). This paper provides evidence of morphologically distinct ecotypes with differing developmental strategies from such contrasting habitats as well as confirming variation in susceptibility to herbicides.

METHODS

Populations of *G. aparine* were collected between 1983 and 1985 from a number of geographic locations *viz* High Mowthorpe (N.Yorks.) Boxworth (Cambs.) Drayton, Kineton (War.) and North Moreton (Oxon.). Where possible, collections were made from both within crop infestations (field) and adjacent uncropped field boundaries (hedge). The minimum distance between collections from these two contrasting habitats was 10m but more usually >30m. Data are

presented here for five paired populations each from the two contrasting habitats, although a greater number of populations were investigated.

Morphological variation

The extent of genetic variation both between locations and habitats (field and hedge) was determined by means of a spaced plant trial. The experiment consisted of 6 fully randomised blocks, each measuring 6 x 5m and surrounded by a discard row of *G. aparine*. Spaced plants were established 0.5m apart, with four replicates per block providing a total of 24 replicate plots/population. The experiment was sown on 16/9/85. At intervals, plants were assessed for various morphological characters including leaf shape, leaf number per whorl, mean internode length and seed size. The latter attribute was determined at final harvest between 31/7/86 and 8/8/86. The data were subjected to univariate and multivariate analyses of variance.

Phenological variation

In a separate spaced plant trial phenological development and reproductive capacity were determined. Seed of four populations were sown at intervals of 21 days between 27/9/85 and 4/4/86. The experiment was replicated four times and plants spaced at intervals of 0.5m. Flowering date, seed number, size and weight together with dormancy characteristics were assessed between July and August '86.

Herbicide sensitivity

Response to herbicides was investigated during the autumn of 1987. Seeds of the various populations were pre-germinated on filter paper and transferred to pots (10cm) containing a mixture of sandy loam and John Innes potting compost. Seedlings were raised to the second fully expanded leaf whorl stage and then sprayed with mecoprop (570g a.i. ha^{-1}) at four rates (0.35, 0.70, 1.40 & 2.8 Kg a.e. ha^{-1}). Treatments were applied using an Oxford Precision Microsprayer (MDM Engineering Ltd. Waterlooville, Hants). Spray delivery was through a single Allman No.00 nozzle in a volume of 250 l ha^{-1} at a pressure of 2.1 bar. Fresh weight determinations were made fourteen days after spraying and ED_{50} values calculated.

Isozyme variation

Isozyme variation was investigated by means of starch gel electrophoresis employing a modification of the system used by Rick *et al.*, 1977), full details of which are given by Ferris-Kaan (1988). A total of fifteen replicate samples were run per gel and six separate enzyme systems studied. They were Glutamate oxaloacetate trasaminases (GOT), Glycerate-2-hydrogenases (G_2DH), Leucine aminopeptidases (LAP) Malic enzymes isomers I & II, (ME I^1&I^2) and Malate dehydrogenases (MDH). R_f values were calculated as the ratio between the migration distance of the most active isozyme band and the 'front' marker (bromophenol blue).

RESULTS

Morphological variation

Univariate analysis indicated significant differences for all morphological attributes assessed, with the exception of seed weight.

Differences in leaf shape (Table 1a) and leaf number/whorl (Table 1b) were highly significant P<0.001) but independent of habitat. Although there was no significant correlation between leaf size and number, there was a negative relationship between these attributes for individual paired comparisons. In contrast internode length (Table 1c) was closely correlated with habitat, with mean internode lengths being greater for four of the five hedgerow populations (P<0.001). The High Mowthorpe populations were not significantly different from one another. Longest internodes were recorded for the hedgerow population at North Moreton and shortest for the field population at Drayton.
No consistent pattern was observed between populations for the various attributes, as the ranking orders differed appreciably.

TABLE 1 Variation in the morphology of *G. aparine* plants derived from seed from five locations and two habitats

a) Variation in leaf shape (spathulate-ensiform 1-5 scale)

Population	High Mowthorpe	Drayton	Boxworth	North Moreton	Kineton	LSD (P<0.05)
Field	3.42	3.61	3.51	3.41	3.65	0.23
Hedge	3.34	3.57	3.63	3.52	3.48	

b) Leaf number per whorl

Field	7.32	7.25	7.62	7.03	7.51	0.29
Hedge	7.36	7.65	7.59	6.97	7.43	

c) Mean internode length (mm)

Field	51.94	45.68	46.22	55.60	49.39	4.72
Hedge	51.72	50.32	58.60	63.35	61.35	

Differences in seed weight were non-significant but differences in seed size were highly significant (P<0.001). Seeds of three of the hedgerow origin populations were of greater diameter than those of their field counterparts (Table 2). A greater proportion of seeds derived from hedgerow populations were >2.80mm diam. whereas a greater proportion of those of field origin were <2.80mm diam. The Kineton populations demonstrated the opposite distribution.

Dormancy as indicated by days to 50% germination was consistently less marked in seeds derived from all the hedgerow populations (Table 3).

TABLE 2. Variation in seed size distribution of *G. aparine* plants from five locations and two habitats

Population	High Mowthorpe	Drayton	Boxworth	North Moreton	Kineton
>2.80mm					
Field	64.4	47.5	47.2	66.4	75.4
Hedge	62.6	55.1	64.6	80.2	66.5
<2.80mm					
Field	35.6	52.5	52.8	33.6	24.6
Hedge	37.4	44.9	35.4	19.8	33.5

TABLE 3. Days to 50% germination at 15°C constant

Population	High Mowthorpe	Drayton	Boxworth	North Moreton	Kineton	LSD (P<0.05)
Field	12.0	26.2	27.7	14.3	17.6	1.40
Hedge	9.5	5.7	10.4	6.6	11.1	

Phenological development

Hedgerow populations flowered slightly earlier than those of field origin for all dates of sowing with the exception of the last (Table 4). Nonetheless, delayed sowing reduced the number of days to flowering irrespective of origin. However, some populations failed to flower from the last sowing date whilst the more southerly populations flowered earlier than those of more northerly provenance.

Mean seed output was consistently greater for field populations than those of hedgerow origin. Mean seed output per plant ranged from 660-1623 for the four field populations investigated and from 441-1208 for the hedgerow populations.

TABLE 4. Days to flowering (Mean of 4 populations)

Sowing date	27/9	18/10	8/11	29/11	20/12	10/1	31/1	14/3	4/4	LSD (P<0.05)
Field	257	232	210	191	176	154	137	100	83	2.5
Hedge	248	229	207	185	169	151	134	99	101	

Herbicide sensitivity

Although all populations tested were adequately controlled with mecoprop, susceptibility differed between populations. However, susceptibility was not consistently affected by location (field *v.* hedge) and response to mecoprop varied between populations (Table 5). ED_{50} values ranged from 0.27 kg a.e. ha^{-1} to 2.0kg a.e. ha^{-1}. The least susceptible populations were from Kineton.

TABLE 5. Doses of mecoprop required to achieve 50% reduction in fresh weight of *G. aparine* (Kg a.e/ha)

Population	High Mowthorpe	Harwell	Boxworth	North Moreton	Kineton
Field	0.42	0.70	1.51	0.44	2.00
Hedge	0.76	1.25	0.27	0.90	1.72

Isozyme variation

In general, differences in isozyme positions were not significantly affected by habitat (Table 6). However, GOT activity differed significantly ($P<0.001$) between the hedge and field populations from Drayton while G_2DH and MEI^2 activity differed significantly ($P<0.001$) between habitats for the High Mowthorpe populations. In contrast to the High Mowthorpe populations, which had two isomeric forms of ME, only one isomer was evident for the North Moreton samples.

TABLE 6. Variation in isozyme activity (Rf values)

Population	High Mowthorpe				Drayton		
Enzyme	Field	Hedge	LSD ($P>0.05$)	Enzyme	Field	Hedge	LSD ($P<0.05$)
G_2DH	0.523	0.500	0.007	GOT	0.624	0.616	0.004
MEI^1	0.663	0.654	0.010	MDH	0.705	0.704	0.006
MEI^2	0.265	0.275	0.004	LAP	0.769	0.763	0.010

DISCUSSION

Morphological variation among populations of *G. aparine* from contrasted habitats has been reported elsewhere (Groll & Mahn, 1986; Berkefeld, 1988 and Niemann, 1988). These authors have variously reported ecotypic differentiation with respect to cotyledon shape, hairiness of stems, internode length, biomass productivity and reproductive capacity. Cotyledon

shape was observed to be the most constant characteristic differentiating between ecotypes (Niemann, 1988). Cotyledons of populations derived from arable ecosystems were more elliptical than those of non-arable habitats (Groll & Mahn, 1986; Niemann, 1988). Although not documented, such differences were also observed in the present investigation. Differences in leaf shape and number/whorl were not significantly affected by the habitats in which the parent plants had been growing.

Both habitat and site of origin (location) influenced internode length in our experiments. Marked variation in internode length between populations of contrasted habitat has been reported previously (Bain & Attridge, 1988). They observed that although the potential height of both field and hedgerow populations was similar, it was achieved in different ways. The older, i.e. first-formed, internodes of hedgerow populations were significantly longer than those of field populations, whereas the converse situation was observed for younger (later-formed) internodes. Such a strategy would confer an adaptive advantage to populations establishing under highly competitive or shaded conditions. Similar observations are reported by Groll & Mahn, 1986). Despite the relatively longer internodes of hedgerow populations, these ecotypes appear less sensitive of shade conditions, rich in far-red light, than their field counterparts (Bain & Attridge, 1988).

Under hedgerow conditions of intense competition and potentially early soil moisture deficit, an early date of maturity would be advantageous. The earlier flowering date of hedgerow populations is consistent with this hypothesis. In contrast to the results reported here, Niemann (1988) observed earlier flowering of field ecotypes. The lower fecundity of these ruderal populations is also consistent with the observation of reduced seed output from populations characteristic of shaded ruderal habitats relative to cropped land, albeit reproductive potential was greater for populations derived from unshaded ruderal habitats (Groll & Mahn, 1986). The larger seed size of hedgerow populations is compatible with the necessity for greater nutritional reserves during seedling establishment under shade conditions.

These differences in morphological and phenological development suggest the existence of two contrasting strategies and are clearly related to ecotypic differentiation. Such a view has been expressed elsewhere enabling populations which experience severe stress or competition to complete their life cycles more rapidly, but with reduced reproductive capacity (Groll & Mahn, 1986). Such conditions may be experienced in shaded field margins or following spring germination in autumn-sown crops. Conversely, those populations of arable ecosystems subject to low initial competition exhibit limited internode extension, forming a rosette habit which affords frost protection. Subsequent extension growth is associated with considerable biomass accumulation and great fecundity.

The protracted dormancy associated with field ecotypes allows these populations to avoid the detrimental effects of autumn cultivations whereas for hedgerow populations rapid germination and establishment would be of advantage. It is likely that cultural practices have selected for seed dormancy and delayed germination within field populations enabling the formation of a persistent seedbank. In the undisturbed field boundary, seed persistence is facilitated through prolonged attachment to the parent plant.

Susceptibility to mecoprop as recorded by the ED_{50}'s varied between populations by a factor of 4-5 but was not correlated with habitat. Such

observations are in agreement with those of Niemann (1988). Thus there does not appear to be evidence for increased tolerance of field populations resulting from selection pressure associated with previous exposure to herbicides. Other workers have indicated that increased tolerance to herbicides is not dependent on previous history of herbicide use (Lutman & Lovegrove, 1985; Courtney & Hill, 1988). Nonetheless, it is noteworthy that the least susceptible populations were from Kineton, an intensively farmed arable field.

Despite the observed differences between hedge and field populations, it is surprising that relatively little isozyme variation was detected between populations. Nevertheless, the spaced plant trials provide evidence for genetic differences between populations from contrasting habitat within particular locations and between locations. It is of interest that least morphological variation was observed between the hedge and field populations collected at High Mowthorpe. At this site, the field boundary consisted of an uncultivated strip, devoid of a hedgerow. Consequently, it is likely that phenotypic plasticity may be of considerable importance in the natural habitat, enabling hedgerow populations to be invasive of arable land. Nonetheless, it is suggested that the ecotypic differentiation reported here and elsewhere (Froud-Williams, 1985) may restrict the distribution of individual populations to specific habitats within arable ecosystems.

Current investigations are continuing to determine whether the origin of field populations is derived from ingress from the field margin or from some separate source, e.g. contaminated grain. The lack of genetic disparity would suggest not, but more conclusive isozyme data is required to validate this. Ecotypic differentiation would favour separate origins, although selective forces have undoubtedly contributed to the existence of two distinct types of cleavers.

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THE RELATIVE INFLUENCE OF GENETIC VARIATION AND PROVENANCE ON THE MORPHOLOGY AND HERBICIDE RESPONSE OF SELECTED POPULATIONS OF *GALIUM APARINE*

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ABSTRACT

Seeds of *Galium aparine*, collected from locations in five European countries, were grown in Germany and Northern Ireland. Plants grown from seed from these sites were then compared along with plants from the original seed collection. A significant effect of provenance, population and an interaction between the two were found with respect to seed size, percentage germination, and some seedling characters. Significant population differences in response to fluroxypyr were unaffected by the provenance of the seeds.

INTRODUCTION

Intraspecific variation within *Galium aparine* has been reported for growth habit and life cycle (Moore, 1975), seed biology (Froud-Williams, 1985) and herbicide response (Courtney & Hill, 1988; Hill & Courtney, 1989). In this investigation, seed of five populations of *G. aparine* collected from Europe were propagated in Germany and Northern Ireland, and compared along with the seed from the original site of collection.

METHODS AND MATERIALS

Germination Test

Seeds of fifteen populations of *Galium aparine* (details of provenance are given in Table 1) were sown at a depth of 1 cm in seed trays (21 x 4 x 34 cm) filled with equal quantities of peat based compost (Bord na Mona) mixed with sand and gravel in a ratio of 2:1:1 respectively. Five hundred seeds of each population were sown in four blocks which were watered daily, and the numbers germinated were assessed every two days.

Table 1 - Details of Populations and Provenance

Population	Provenance (Site)	Year	Seed Storage Conditions
Basel	Wheat field, Switzerland	1987	Polythene bags in sealed plastic
Billingsbear	Hedgerow, England	1987	containers at 4° C.
Bologna	Loam-clay soil, Italy	1987	"
Waringstown	Field, N. Ireland	1986	"
Wihr au val	Laneside, France	1987	"
Basel	N. Ireland*	1988	Polythene bags
Billingsbear	"	"	in sealed plastic
Bologna	"	"	containers at 4° C.
Waringstown	"	"	"
Wihr au val	"	"	"
Basel	Germany+	1988	Paper bags
Billingsbear	"	"	in sealed plastic
Bologna	"	"	containers at 4°C.
Waringstown	"	"	"
Wihr au val	"	"	"

*N. Ireland - Seeds were sown at a depth of 2 cm in clay soil fertilized with 20:20:20 NPK in 25.4 cm pots in November 1987. The plants were maintained in an unheated glasshouse and seeds were harvested from them in August and September 1988.

+Germany - Seeds were sown in 10 litre pots filled with a sandy loam soil containing 1.23% organic matter, 47 mg/100 g soil of P_2O_5 and 27.5 mg/100g soil of K_2O. Nitrogen was applied three times with a total of 1.2 g N per pot. The plants were maintained under outdoor conditions and seed harvested from them in July and August 1988.

Herbicide Response

Seed from five populations of *Galium aparine* that had been grown for seed production in N. Ireland and Germany during 1987/88, plus those from the original site were sown in seed trays (21 x 4 x 34 cm) and maintained under natural light in a glasshouse at an average temperature of 10°C. They were sown between February 24th and March 1st according to the population to take into account differences in germination times. When germinated (March 28th), the plants were transplanted into seed trays prepared as in the germination test and were located with a matrix of 23 x 14 shallow holes 15 mm apart using a template. Ten plants from each of five populations were transplanted into three adjacent rows across each tray with a one row gap in between populations. The positions of the populations were fully randomized. Each sub-plot, consisting of three trays containing three rows of each of the 15 populations, was randomly ascribed one of five rates of herbicide. At the time of spraying, five plants of each population were cut off at the soil surface and plant height, plant leaf area, cotyledon area, fresh

and dry weight were measured. Fluroxypyr (200 g/l) was applied at 5 rates - 120, 100, 80, 60 and 40 g ai/ha plus a control treatment. The plants were sprayed (April 6th) when they had two fully expanded whorls. The herbicide was applied using a pot spraying system at a spray volume of 315 l/ha applied through a 8003 Teejet at a pressure of 200 kpa. The plants were returned to the glasshouse after spraying. Five weeks after spraying (May 11th), the plants were assessed for numbers of live and dead plants.

RESULTS

Seed Size

The seed size (1000 grain weights) of the populations from all the sites show significant differences (Figure 1a). The Waringstown population had the biggest seeds with a 1000 seed weight of 9.5 g and Wihr au val the smallest with 5.6 g. The Wihr au val seeds were significantly smaller than all the other populations.

The effect of site of seed provenance on the seed size (Fig. 1b) was also significant and showed that seed propagated at the German site was much smaller than that from the other sites, which did not differ from each other.

The interactions of the site of seed provenance on the different populations, with the exception of Waringstown where the original seed was largest and the German seed the smallest, were small and inconsistent.

Germination

Each population differed significantly from each other with respect to mean germination time ie. time to 50% total germination (Figure 2a). Across all sites, the Basel population germinated the fastest (mean germination time - 13.4 days) and the Bologna population germinated the slowest (16.5 days). Similarly, each provenance site differed significantly from each other (Figure 2b), the seed grown in Northern Ireland germinating the fastest (mean germination time - 13.5 days) and the seed from Germany the slowest (15.9 days).

The interaction between population and site was again inconsistent, although four of the populations germinated fastest from seed from N. Ireland. Moreover, Basel, Waringstown and Wihr au val germinated faster from seed from Germany than from seed grown in the original site.

Morphology

There was no single population which consistently exhibited the largest observations in all the morphological parameters (Fig. 3). Waringstown appeared to produce the biggest plants in terms of fresh and dry weight but Bologna seemed to have the largest plant and cotyledon areas, whilst Basel had the tallest plant height. However, the only significant differences were in cotyledon area and dry weight. Bologna and Wihr au val had cotyledons which were significantly larger than those of Waringstown and Basel but Wihr au val had the smallest plants

Figure 1a Seed Size of Selected Populations of *G.aparine*

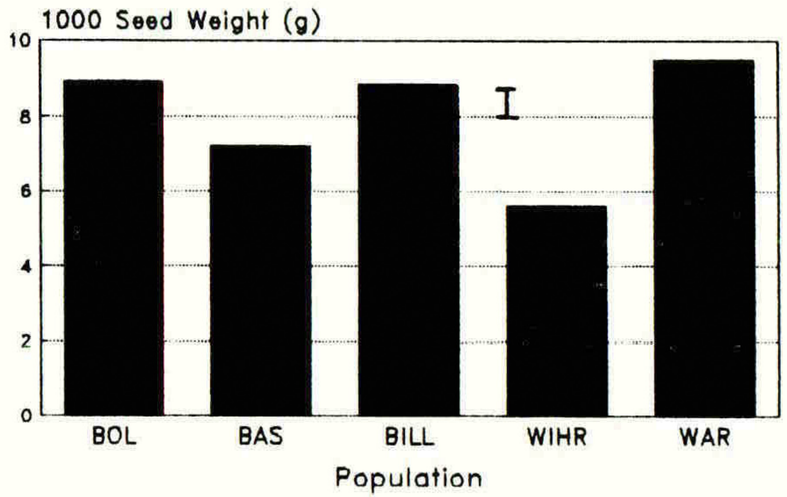


Figure 2a Mean Germination Time of Selected Populations of *G.aparine*

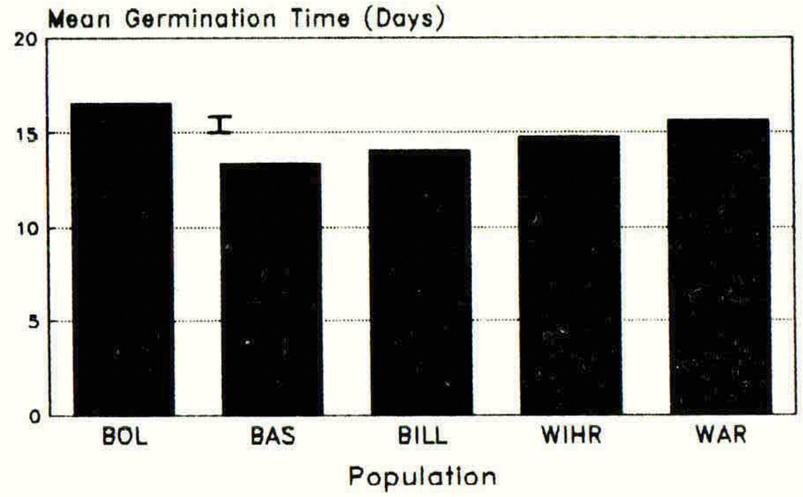


Figure 1b Effect of Site of Seed Propagation on Seed Size of *G.aparine*

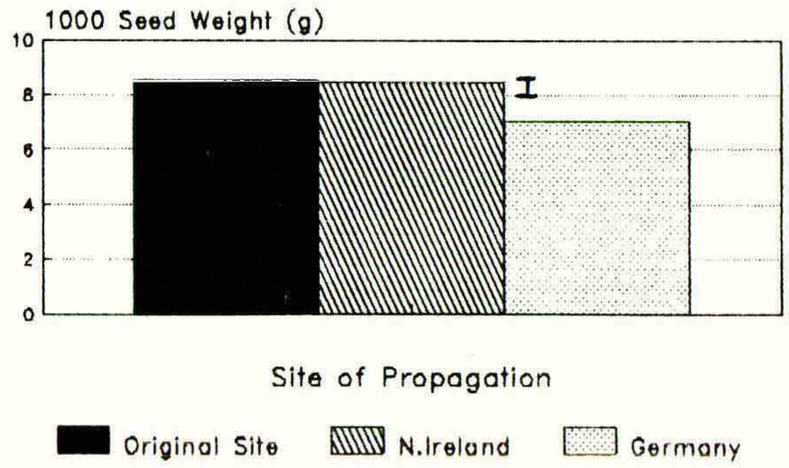


Figure 2b Effect of Site of Propagation on Germination of *G.aparine*

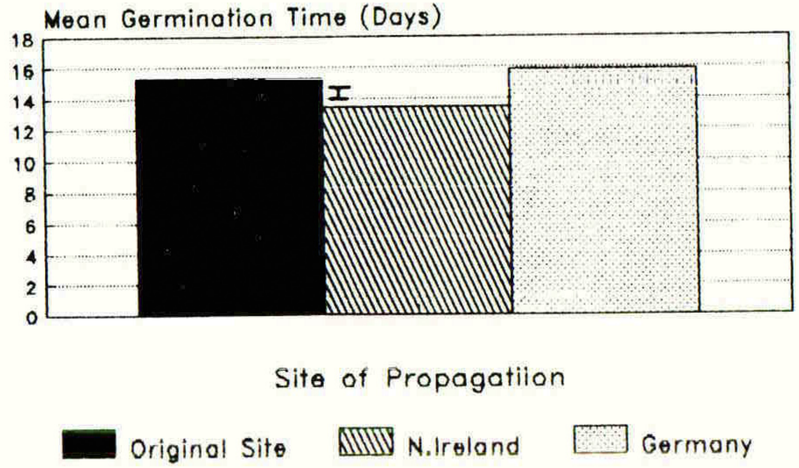


Figure 3 Morphology of Selected Populations of *Galium aparine*

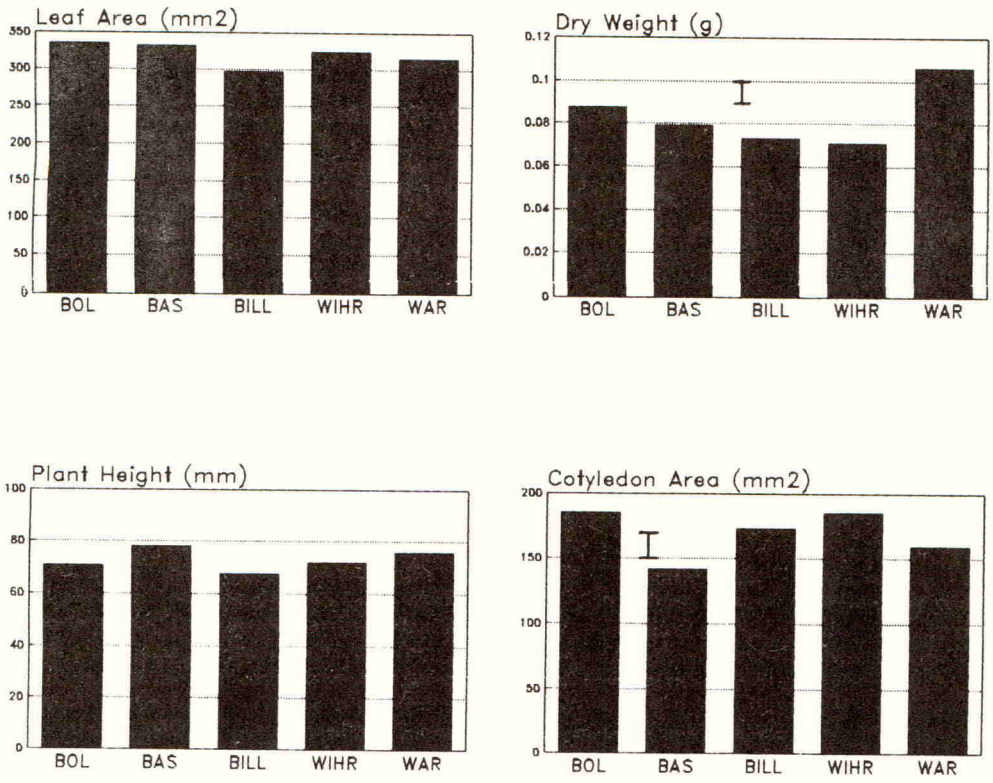
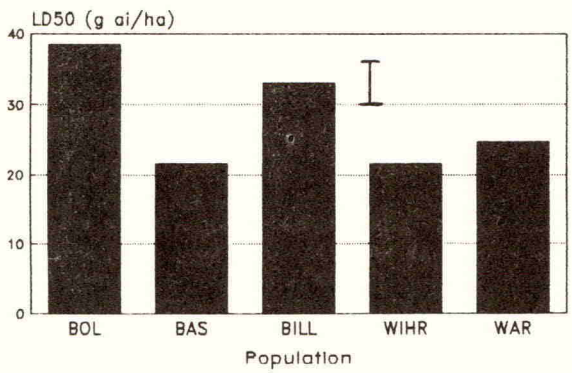


Figure 4 Response to Fluroxypyr of Selected Populations of *G.aparine*



(0.071 g/plant) with Bologna (0.087 g/plant) and Waringstown (0.104 g/plant) being significantly larger. In fact, Waringstown was significantly heavier than all the other populations.

The effect of site of seed provenance on the morphology of *G. aparine* was highly significant with seed propagated at the West German site producing the smallest seedlings in all parameters measured.

The interaction between the site of seed provenance and the populations was again inconsistent. However, the small German seed did produce the smallest plants.

Herbicide Response

The values presented are the doses of fluroxypyr calculated to achieve 50% kill. The response of the populations (Figure 4) show that Bologna was the most tolerant (LD50 38.5 g ai/ha) and Wihr au val the most susceptible (21.6 g ai/ha). Both Bologna and Billingsbear (LD50 33.1 g ai/ha) were significantly more tolerant than Wihr au val. No significant differences were observed between sites of propagation with the LD50 doses of the three sites only differing by 1.4 g ai/ha. The effect of site of seed provenance on the response of the populations to fluroxypyr showed that there was no significant interaction between the sites of provenance and the populations.

DISCUSSION

Although only five populations were compared in this study, each population differed significantly from each other in terms of seed size and mean germination time. Intraspecific variation in these characters has been shown for many species including *Galium aparine* (Froud-Williams, 1985), and the variation shown here gives a measure of the genetic variation between populations of *G. aparine*.

The significant differences in seed size and mean germination time between the sites of seed provenance shows a marked influence of the environment in which the seed was produced on seed size and germination. Environmental factors during seed formation have been shown to influence the germination of the seeds produced. Factors such as temperature (Akpan & Bean, 1977), daylength (Gutterman, 1973), drought (Izzeldin *et al.*, 1980) and nutrient availability during seed production (Szirtes *et al.*, 1981) have been shown to exert an effect on the subsequent germination of the seed. Because it was not possible to record the precise environmental factors during seed propagation at each site, it is not possible to identify the factors which have influenced *G. aparine* germination and development.

The differences between the populations and sites show that both genetic and provenance factors influence both the speed of germination and seed size of *G. aparine*, and the interaction suggests that populations may react differently to different provenance factors. Naylor and Abadalla (1982) concluded that the germinability of *Poa annua* was a population rather than a habitat character. Similarly, Froud-Williams (1985) implied that dormancy and germination characteristics of *G. aparine* were genotypic rather than phenotypic in origin. If

population or genotypic factors predominated in the speed of germination and seed size of *G. aparine* then the populations would tend to have the the same profiles across the sites. Though Waringstown, Billingsbear and perhaps Basel are similar, Bologna and Wihr au val differ, not just from the other three but from each other. This suggests that both ecotype and environmental variation plays a significant part in the phenotypic expression of germination in *G. aparine*.

The effect of the site of seed propagation on the morphology of the populations is more pronounced. The seedlings grown from seed propagated at the German site were significantly smaller in all parameters measured than seedlings from all other sites. The precise environmental factors that differed between the sites are not known, hence it can be suggested that a number of environmental factors operating during seed formation influence subsequent seedling growth to a varying degree. Factors such as nutrient level (Austin, 1966;), water deficit (Izzeldin *et al.*, 1980), temperature (Fussell & Pearson, 1980) and sunshine hours (Szirtes *et al.*, 1981) during seed formation have been shown to influence the growth of resulting seedlings. Any of these factors, alone or in combination during provenance could influence the growth of *G. aparine* seedlings.

Froud-Williams (1985) found that differences in germination behaviour between field and hedgerow populations were unaltered when grown under identical conditions, thus implying that differences between populations were of genotypic rather than phenotypic origin. However, this appears not to be the case with morphology. If dry weight is taken as an example, one would expect Bologna to always be heavier than Wihr au val at each propagation site if differences were only genotypic in origin. In fact Bologna is only heavier at two sites of propagation. Hence it appears that genotypic and provenance factors both affect the morphology of *G. aparine*.

Although it seems that both genetic and provenance factors influence the seed biology and morphology of *G. aparine*, this does not appear to be the case with response to fluroxypyr. Significant differences were found between the populations. However, no significant differences were found between the sites of seed provenance and the interaction between the populations and sites. This suggests little or no influence of site of seed provenance on the response of the seedlings to fluroxypyr. Hence it seems that the response of *G. aparine* populations to fluroxypyr is genetically determined and the environment in which the seeds were produced has little influence.

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VARIABILITY IN HERBICIDE RESPONSE WITHIN WEED SPECIES

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ABSTRACT

The increase in the reported cases of resistance to various herbicides within several weed species emphasizes the need for a genetic approach to the understanding of how differential herbicide responses evolve. The genetic variability of a species in its response to a herbicide depends on variation in morphological and physiological characteristics and on polymorphisms that may occur in genes encoding structures involved in herbicide activity. These genes will be selected according to the herbicide selection pressure, their cost to the plant in the absence of herbicides, and the mating system of the species. The build up of a resistant population will depend on these conditions and also on the number of genes confering the resistance. Some studies report polymorphisms that are as yet only confirmed in laboratory tests without any practical consequences in the field. In others, variability has led to the spread of resistant biotypes responsible for serious economic losses.

INTRODUCTION

Although wild species are highly adapted to their environment, it is well known that all species are actually polymorphic for many characteristics. This has been particularly deaply demonstrated since the introduction of isozyme separation in population genetics studies. These non selected characters are polymorphic within almost all species. But due to natural selection, variability is very different from one character to another (Warwick, 1991). Obviously herbicide response of weed species is not an exception to this rule.

Variation in herbicide susceptibility in a weed species is due to both environmental conditions and to genetic variability. The former relate to the growing conditions of the plants, climatic conditions during the treatment and spray characteristics. These variations are responsible for almost all treatment failures for which the surviving weeds are said to be resistant. As a consequence, in the following year herbicide performance returns to normal. However even in good environmental conditions, some plants may still survive the treatment because of various genetically inherited characters.

Plants may avoid herbicide activity by general characteristics such as thicker cuticules, presence of hairs, narrower leaves, longer roots and many other morphological traits. When the herbicide is absorbed, it may be differently translocated and detoxified before reaching its target. The plant may also increase the production of target sites enzymes of may mutate the target site so that the herbicide is no longer effective. Generally the consequence of a mutation is reduced fitness of the resistant plant introducing new possibilities of variation. Furthermore, the inheritance of these characteristics combined with the mating system variability, produces a continuum in the herbicide response within a species from susceptible to resistant genotypes. In addition, as the different families of herbicides affect a

multiplicity of metabolic pathways, many mechanisms are available for differential response or resistance to herbicides, giving rise to further sources of variation.

In this paper we will review different sources of genetic variability according to the consequences arising from their mechanisms, their heredity and their cost (i.e. fitness) for the genotypes. This genetic variability can be considered in different ways. Firstly, one can study the genetic structure of populations through their response to herbicide treatments. Secondly, different genotypes (selected or not by herbicides) can be studied in laboratory tests and thirdly, once resistance has appeared in the field, one can measure the extent of variability in herbicide response from susceptible to resistant. An arbitrary division has been made in this paper between cases of variability that have been studied from an academic aspect but without any consequences in the field, and cases that have led to the spread of herbicide resistant biotypes in fields that require some changes in the cultural practices.

VARIABILITY NOT YET CAUSING PROBLEMS IN THE FIELD

Herbicides used in cultivated areas do not kill all plants. They must be selective to the crops. These treatments always leave a few weed plants which are still able to produce enough seeds for recruitment in later years. These plants may survive either by chance because of environmental variability or because they have some characteristics enabling them to withstand the treatment.

Indirect measurements

In population genetics studies, the inheritance of foliar isozymes can be determined and the bands likened to alleles. The polymorphism of these alleles allows one to estimate the structure of the populations. During such an investigation within *Chenopodium album* several populations were collected in a large range of situations (Al Mouemar & Gasquez, 1983). Some populations were collected in private gardens obviously never treated, while others were collected in different fields with various herbicide pressures. Garden populations showed a high variability (up to 28 phenotypes/100 plants). A population from a non-treated hop field had 12 phenotypes, whilst at the extreme, only one phenotype remained in triazine resistant populations from maize fields. Furthermore in a field experiment the densities occuring in non-treated plots permitted a high rate of allogamy which would maintain a high polymorphism. So the low phenotypic variability in agricultural fields is probably an actual effect of herbicide selection (Gasquez, 1984). But none of the few phenotypes remaining in the fields (except maize fields) was resistant to the herbicides used. Thus, the selection was apparently acting at various steps in the life cycle of the species, only selecting for vigorous plants.

In the same way, we had previously reported with *Echinochloa crus galli*, that populations from maize monocultures were less polymorphic than populations collected in non cultivated places (Gasquez & Compoint, 1977). In this case the frequency of progenies surviving an atrazine treatment was higher for maize field populations. This indicated an effective selection due to the repeated use of the same herbicide. As *Echinochloa crus galli* can detoxify atrazine, one may assume that the selected plants had the highest level of herbicide metabolism.

Search for differential herbicide responses

In order, to test the variability of the response of weeds to herbicides some authors studied biotypes differing in morphological traits. In 1964, Withworth

established the existence of a variable susceptibility to 2,4-D within several clones of *Convolvulus arvensis*. This shown not to be due to morphology but to physiology (Withworth & Muzik, 1967). Moreover, field bindweed biotypes have been studied for morphological and physiological characteristics and have also been tested for response to glyphosate (DeGannaro & Weller, 1984). Each biotype showed a particular response even if the less susceptible biotypes could not survive high rates of herbicides. Environmental studies showed that the differential susceptibility was maintained under various growing conditions, suggesting that, at least, a part of the response was under genetic control. Heritability studies showed that glyphosate susceptibility was inherited by additive gene action. Hence, glyphosate resistance in a field bindweed population could be enhanced by selection pressure of repeated herbicide spray which will result in the death of the more susceptible biotypes and allow the surviving tolerant plants to cross and concentrate the alleles for resistance (Duncan & Weller, 1987).

In some cases, investigations have been made to determine whether populations were becoming resistant to the herbicide regularly applied. Samples of three species (*Senecio vulgaris*, *Chenopodium album* and *Capsella bursa-pastoris*) from non-treated fields and from fields where simazine had been used for up to twelve years showed a good correlation between the number of years of treatment and the decrease of mortality to low rates of simazine (Holliday & Putwain, 1974). These results clearly demonstrated the selective effect of the herbicide, even though there was no biotype resistant at field rates.

Many studies have dealt with variability of herbicide response in *Avena fatua*, although it is only very recently that farmers have experimented problems with herbicide resistant wild oats (Powles & Howat, 1990). Differential responses of wild oat lines to herbicides were first observed with several carbamates. The difference in response to diallate and triallate between resistant and susceptible lines was 2 to 2.5 fold, and to barban was greater than 10 fold (Rydrych & Seely, 1964 ; Jacobsohn & Andersen, 1968). The same authors were unable to establish a relationship between herbicide response and varieties of *A. fatua* identified on the basis of seed morphology. Further studies showed that leaf surface area, flowering time and height were not different in untreated conditions. Only tillering was lower in plants tolerant to difenzoquat, M.S.M.A. and flamprop (Somody *et al.*, 1984), and leaf area was lower in the case of diclofop resistance (Olufummilayo *et al.*, 1990). Whatever the mechanism, variability for herbicide reaction was found within and among herbicide exposed populations as well as unexposed populations. However, populations which had a long history exposure to the herbicide produced significantly higher frequencies of tolerant plants (Jana & Naylor, 1982 ; Thai *et al.*, 1985). In addition, non exposed populations were also more variable in response to stress. A tentative hypothesis would be that selection due to herbicide spray had led to the loss of genetic variability and increase of tolerant genotypes. Therefore, adaptation of populations is possible in response to repeated herbicide spray and would constitute a potentially serious problem in wild oat control. The level of genetic variation for tolerance has been quantified using calculation of genotypic and phenotypic variances. The observed heritabilities in a study of 11 populations ranged from 0.12 to 0.63 which indicated high potentiality for wild oats to develop tolerance to herbicides (Price *et al.*, 1983).

The presence of tolerant phenotypes in most wild oat populations collected from habitats never exposed to triallate raised the possibility of natural selection favouring the corresponding resistance genes (Price *et al.*, 1985). In wild populations of emmer wheat in Israel, difenzoquat tolerance was shown to be in the "wild type" and polymorphism was also found for chlorotoluron in never previously treated populations (Snape *et al.*, 1991). Polymorphism for herbicide response of previously unexposed populations may be associated with favourable morphological or

physiological characteristics. Associations with discrete characters such as isozymes may also exist in wild oats, that may explain part of the loss of isozyme polymorphism of selected populations (Price *et al.*, 1985). These data emphasize that weed populations from areas never treated have generally variable responses to herbicides depending on the different genotypes growing in these fields. Even if there is no evidence for any resistance, populations from regularly treated fields are less polymorphic thus indicating a selection of some genotypes. But whether only vigorous plants or the tolerant to the herbicide are selected, their characteristics are almost quantitative, suggesting that many independant genes may be involved. Thus, whatever the mating system, the spread of selected plants could be very hazardous. As an example, in an experiment on a population of *Alopecurus myosuroides* which had been selected for four years with simazine at the tillering stage, the surviving plants were shown to have more dry matter and longer roots than never treated plants (Darmency, 1981). This illustrates the potential of populations to evolve, thanks to morphological variability, towards phenotypes that should escape the herbicide which remains near the soil surface, but so far no simazine resistant *A. myosuroides* has been reported.

VARIABILITY LEADING TO RESISTANCE

Although there may be a variable response to a herbicide from the different genotypes of a population, the control may be quite acceptable, because the field rate of herbicide is high, killing almost all the plants. But in some cases, the frequency of non controlled plants gradually increases leading the field advisors to an empiric notion of resistance when they consider that control is no longer satisfactory. The appearance of such plants is only due to the selection of one or more genotypes within the population by the repeated use of only one herbicide. But in this case, the selective pressure is so strong that genotypes with new mechanisms due to rare mutations can be selected. The frequency of mutated plants in populations before any treatment is very variable depending on the cost to the plant of possessing this new characteristic, as well as the inheritance of the mutation and the mating system of the species. This frequency is thought to be generally very low. But as, after the very first treatment, these biotypes are so highly fitted to these conditions they can lead to the build up of massive resistant populations. When the cost is insignificant, the mutated genotypes can be quite numerous just by chance, especially when the species is highly autogamous. Thus in some cases, it has been shown that the repeated use of the herbicide is not always the only reason for the presence of mutated genotypes strains. For example *Stellaria media* resistant to mecoprop is present in non (or not intensive) treated areas (Lutman & Lovegrove, 1985).

Resistance due to non specific mechanisms

Many reports of resistant biotypes to various herbicides do not discuss the mechanism of resistance. Nevertheless the repeated use of the same herbicide is the cause an actual selection pressure (LeBaron & Gressel, 1982). For example *Poa annua* resistant biotypes to paraquat in Great Britain survive higher than normal doses of herbicide (LeBaron & Gressel, 1982). Populations of *Ranunculus acris* and *Carduus nutans* in New Zealand regularly treated by MCPA now resist up to five or six times the rate which kills susceptible plants (Bourdot *et al.*, 1989). In U.S.A. after 15 years of treatment with D.S.M.A. and M.S.M.A. in cotton fields, *Xanthium strumarium* is lightly damaged by a twice normal dose of herbicide (Haigler *et al.*, 1988). After 10 years of treatment *Lolium rigidum* is now no longer controlled by aminotriazole in railroads in Australia (Powles & Howat, 1990). *Erigeron canadensis* from carrot fields in Switzerland can survive high rates of linuron but this resistance is unrelated to the commoner triazine resistance (Beuret, 1988).

Other studies give more information on the mechanisms and genetics of resistance. In Switzerland, *Amaranthus lividus* from maize fields can be up to eight fold more resistant than susceptible plants but this cannot be correlated with psII activity of isolated chloroplasts, even where there is a variability in psII activity (Beuret, 1988). Biotypes of *Erigeron bonariensis* which are five times more resistant than susceptible plants could survive as a result of a sequestration mechanism preventing paraquat from entering symplast (Fuerst *et al.*, 1985). In Australia paraquat resistant biotypes of *Hordeum glaucum* which are 250 times more resistant than susceptible ones, seem to restrict paraquat in the apoplast of leaves. Crosses with susceptible plants have shown that the inheritance of this resistance is monogenic with incomplete dominance (Islam & Powles, 1988).

Many of the resistant biotypes reported above are not very widespread and of little economic importance. This could be the consequence of the mechanisms which give rise to only moderately resistant plants but this is probably also due to polygenic inheritance which delays the spread of resistance as even selfed resistant plants produce a variable proportion of susceptible progenies. In contrast, the simple inheritance and the high rate of resistance within *Hordeum glaucum* are responsible for large widespread resistant populations although they are confined to specific areas (Islam & Powles, 1988).

Resistance due to herbicide detoxification

Successful detoxification of the herbicide can be achieved by only one enzyme. Within *Abutilon theophrasti* the resistance to atrazine seems to be due to a three or four fold increase of the amount of glutathion-S-transferase (Gronwald *et al.*, 1989b). This characteristic is inherited by only one dominant gene. But in many cases, several enzymes are certainly involved. For about ten years, *Alopecurus myosuroides* resistant to chlorotoluron has become increasingly frequent in winter cereals in Great Britain. It has been established that resistant plants display a higher activity of the enzymes of the monoxygenase family which detoxify the herbicide (Kemp *et al.*, 1990). The resistant populations are actually composed of plants ranging from susceptible to less 1000 g/ha a.i. to plants surviving up to 17000 g/ha a.i.. The progenies of plants surviving a field treatment produce both susceptible and resistant plants at very variable frequencies from one mother plant to another. These data and hybridizations indicate that this resistance is nuclearly inherited by two or more genes (Chauvel, 1991). Furthermore the genetic structure of resistant and susceptible populations is similar. This is probably due to its polygenic inheritance, a high allogamy and a similar fitness of resistant and susceptible biotypes (i.e. no cost for the resistance) (Chauvel, 1991). Despite a potentially high level of resistance, all these genetic characteristics lead to a slow increase in the resistance of plants in treated populations (resistant biotypes ranging from 25 % to 45 % within ten years (Moss & Kemp, 1990)). This is certainly one of the best documented examples of wide genetic variability related to the detoxification of the herbicide.

Other resistant biotypes may detoxify the herbicide through monoxygenase enzymes. *Lolium rigidum* biotypes from Australia can withstand up to eight times the dose of diclofop-methyl which kills susceptible plants (Powles *et al.*, 1990). Once more the response of the populations is very variable, as probably the inheritance of the mechanism is not simple. Similarly paraquat resistant biotypes of *Conyza bonariensis* produce two or three times more enzymes which detoxify peroxides produced by paraquat (Shaaltiel & Gressel, 1986). It was concluded that probably one dominant nuclear gene controls the levels ot the enzymes involved in superoxide detoxification (Shaaltiel *et al.*, 1988).

The selection of resistant biotypes with enhanced detoxification seems to be particularly common within weed species and herbicide families. Since the biochemical mechanisms generally involve several metabolites, the inheritance is often polygenic leading to very polymorphic populations. But despite these characteristics these resistant biotypes are not very widespread.

Resistance due to mutation of the herbicide target

Unlike the detoxification resistance, there are few cases of target mutation. The acetyl CoA carboxylase within *Lolium multiflorum*, is 15 fold less susceptible to diclofop than that of susceptible plants (Gronwald *et al.*, 1989a). Resistance to MCPP in *Stellaria media* could be due to less affinity between the herbicide and a target (Barnwell & Cobb, 1989) but little is known concerning the origin, the inheritance and the consequences of these resistances.

Resistant biotypes to trifluralin have appeared within two species (*Eleusine indica* and *Setaria viridis*) after more than ten years of repeated treatments. At least within *Eleusine indica* it has been shown that the trifluralin target (ß tubuline) is mutated (Vaughn *et al.*, 1990). This new molecule has no affinity with the herbicide giving a high resistance level, but the resistant plants grow very slowly probably because the mutation affects cell division. This characteristic seems to be inherited by a linked group of four genes. Perhaps because of the high cost of this mutation and to the restricted area where trifluralin is used regularly as the only herbicide, these resistant plants are also not very widespread.

The resistance to sulfonyl ureas and especially chlorsulfuron appeared very quickly within some weeds (*Kochia scoparia*, *Salsola iberica*, *Lactuca serriola*) in winter cereals in USA and *Stellaria media* in Canada. After four years of treatment a field in Idaho had up to 400 *Lactuca serriola*/m². This resistance is due to one mutation of the gene encoding for acetolactate synthase and is therefore inherited by only one gene (either dominant or semi dominant (Mallory-Smith *et al.*, 1990)). This rapid spread of resistant populations suggests that the frequency of mutated plants was quite substantial before the treatment. This could be due to the fact that this mutation seems to have a very low cost as we have observed in a mutated *Cichorium intybus* (unpublished data).

Since the report of the first triazine resistant biotype of *Senecio vulgaris* (Ryan, 1970) up to 50 species have produced at least one resistant population (LeBaron, 1991). All the species have the same point mutation on the gene encoding the target of the triazines. This is the only resistance which is completely maternally inherited. The advantage of the resistant biotype is so high in the treated fields that there are only resistant biotypes in the population. Thus this mutation produced in a few years massive weed populations in fields or areas repeatedly treated with triazine. Despite this important similarity, this mutation has not led to genetic monorphism in all species. In order to test the possibility of such variability we have studied isozyme polymorphism in *Chenopodium album* from various resistant populations (Gasquez & Compoint, 1981). A resistant population had only one genotype and other populations from the same area had the same genotype. However there are considerable genotype differences between populations from different areas. This indicates a genetic heterogeneity associated with the resistance. Furthermore in a population which had never been treated a low rate treatment of atrazine selected 17 phenotypes within 90 surviving plants, but further selection left only one.

In 1981 Zanin *et al.* reported that resistant populations of *Solanum nigrum* in Italy were not affected by 6 kg/ha a.i. of atrazine but had differential responses at higher rates. Some populations were completely killed by 32 kg/ha a.i., others had still

70 % of surviving plants at the same rate. These data suggest that there are different resistant biotypes, even within populations with variable response to the herbicide, despite the same mutation.

Within *Alopecurus myosuroides*, Chauvel & Gasquez, 1988 showed that a triazine resistant population had the same polymorphism with the same alleles in the same phenotypes at the same frequency as a susceptible population from the same area. These data emphasize that the mutation for triazine resistance is distributed over all the genotypes of the population.

Despite an apparent uniformity due to the same mutation within all species and similar high levels of resistance, some genetic variability remains even within some populations. The unique mutation of a herbicide target is very specific, generally very small and distributed within all the mutated species, leading to large populations of similar resistant biotypes. But despite the high advantage of these plants in treated fields these plants are not clones or progenies of only one strain. One must remember that the resistant gene has the same behaviour as any other gene and will be distributed in populations according to the mating system of the species and its own advantage in each genetic background. So the remaining variability will be more linked to the genetic characteristics of the species than to the characteristics of the resistance.

EVOLUTION OF RESISTANCE

Although the resistant plants are highly fitted to the herbicide, selection is always present producing new variability even in stable environmental conditions. For example, within a triazine resistant population of *Poa annua*, a new biotype more fitted to the local conditions appeared very quickly. This was probably due to crosses with surrounding susceptible plants (Darmency & Gasquez, 1983). In Hungarian vineyards, paraquat and triazine co-resistant populations of *Erigeron canadense* have appeared as well as triazine resistant populations (Pölös *et al.*, 1988) suggesting that, even with extreme selection pressure there is enough variability in the genetic pool to allow further evolution.

In Australia a population of *Lolium rigidum* developed successively resistance to four herbicides, maintaining the resistance to the previous herbicides (Powles & Matthews, 1992). The chlorotoluron resistant biotypes of *Alopecurus myosuroides* display many cross resistances to a wide range of herbicides (Moss & Kemp, 1990 ; Chauvel, 1991). But these resistances are very variable from one herbicide to another and from one population to another (Moss, 1990). This could be due to the presence of different biotypes at different frequencies in each population or to different mechanisms coexisting in the same plant. This heterogeneity could be due to the selection pressure which selects not one enzyme but at least one or two enzyme families.

CONCLUSIONS

Thus, species are in the constant process of producing genetic variability, including for herbicide response, so that the farmer will certainly continue to face problems originating in genetic variability.

a) Variability in herbicide susceptibility exists in unselected populations. Consequently, the concept of a standard "wild type" representing the entire species has no sound basis, is useless and misleading because it cannot represent and predict the range of variation occuring for each character within a species (Darmency et al., 1991).

b) Some populations can be shown by genetic studies to exhibit variation in response without it obviously affecting field control, but the potential for problems in the future is increasing, because nobody knows why their frequency did not increase and what minor change may start their spread.

c) Resistance that causes field problems is not necessarily the result of selection of one genotype. The herbicide may be selecting for a range of different mechanisms in a population. As long as different means to overcome the herbicide effects have similar consequences (i.e. similar fitness of resistant and susceptible biotypes in treated conditions) they could be selected according to their initial frequency. But if there is a substantial difference between two mechanisms they will be selected one after the other according to the fitness level.

d) Basic genetic theory would show that using mixtures and alternating herbicides will not stop the development of resistance although this strategies greatly help to slow up it. However these strategies are better than continuing with single products.

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VARIABILITY IN SUSCEPTIBILITY OF ALOPECURUS MYOSUROIDES TO IMAZAMETHABENZ-METHYL AND ISOPROTURON

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ABSTRACT

Alopecurus myosuroides plants were raised from seed under standard growing conditions and sprayed with below recommended field doses of imazamethabenz-m and isoproturon. Efficacy against 16 replicates ranged from kill to complete recovery. Analysis of shoot weight data suggested control plants came from a single population with a normal distribution, but herbicide treated plants showed considerable deviation. Clones of 72 biotypes were treated with the two herbicides and distribution of vigor scores on a grid confirmed the wide spread of susceptibility, but log-linear analysis showed no evidence for the tolerance of one herbicide being associated with the tolerance of the other. Herbicide treatment of four of the above clones confirmed clones and their mother plants had similar responses to the herbicides. Although biotypes differed widely in root and shoot characteristics, these did not appear to play a key role in determining their herbicide tolerance.

INTRODUCTION

Alopecurus myosuroides Huds. is one of the most important grass weeds of autumn sown cereals in England and it is normally controlled with chlorotoluron and isoproturon (Davis et.al., 1990). The performance of these and other herbicides is often inconsistent against A. myosuroides (Flint, 1985). Soil type, cultural practices (Moss, 1979), straw and ash (Nyffler and Blair, 1978) and soil moisture (Blair, 1985) all influence herbicide activity. Since 1982 an increasing number of chlorotoluron-resistant biotypes of A. myosuroides have been detected, introducing a further source of variability in herbicide performance (Moss and Cussans, 1991).

Even in pot studies, conducted under standard soil and environmental conditions, more plant to plant variation is seen in A. myosuroides than in Avena fatua as judged by spray retention and imazamethabenz-m activity (Pillmoor & Caseley, 1984). The aim of this study is to investigate variability in A. myosuroides response to imazamethabenz-m and isoproturon.

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MATERIALS AND METHODS

Plants

Alopecurus myosuroides seeds were obtained from the Weed Research Organisation stockbed which had no herbicide treatments since its establishment in 1975.

Seeds (1984 harvest) were germinated at 20°C and after four days those seeds with the radicle just emerging were planted 1cm deep in moist Begbroke sandy loam soil contained in 9.5cm diameter pots. Two seedlings were planted in each pot but were thinned to one. The plants were kept in controlled environment rooms set at 16/10°C, 75/85% r.h. (day/night) conditions under a 14h photoperiod of approximately 100 Wm^{-2}.

Clones of selected biotypes were obtained by growing plants in Levington compost until they had developed 3-4 leaves and 4-10 tillers. Tillers with 1-2 leaves and visible roots were detached and grown in Begbroke sandy loam soil contained in 3.5cm diameter pots for approximately two weeks when they were transplanted into 10cm diameter pots.

Herbicides

The herbicides were applied as overall sprays using a laboratory sprayer fitted with a Spraying Systems Teejet 8001 nozzle calibrated to deliver 200 l ha^{-1}. at an operating pressure of 207 KPa. Flowable formulations of imazamethabenz-m and isoproturon containing 300 and 500 g AI ha^{-1} respectively were used. Additional formulation components were added to maintain surfactant concentrations found in recommended doses of 0.625 and 2.5 kg AI ha^{-1} for imazamethabenz-m and isoproturon respectively.

In all experiments the plants were set out in a randomised block design.

Experiment 1. Plants, raised from seeds, were sprayed at the 2-3 leaf and 1-2 tiller growth stage with doses of herbicide shown in Table 2. Plants treated with both herbicides were sprayed with isoproturon up to 2h after imazamethabenz-m. Plants treated with a single herbicide were also sprayed with the blank formulation of the other herbicide, thus in all treatments the formulation component was the same. Each treatment was applied to 16 plants and following spraying, the plants were watered from above, avoiding the foliage, to maintain the soil close to field capacity. Visual assessments, where 7 = untreated control and 0 = dead (Richardson and Dean 1973), were made 2, 4 and 6 weeks after treatment and fresh and dry weights were recorded after 6 weeks.

Experiment 2. Four clones of 72 biotypes were grown to the 2-3 leaf, 3-4 tiller growth stage and single clones of each biotype were sprayed with 0.325 and 1.25 kg AI ha^{-1} f imazamethabenz-m and isoproturon respectively. The untreated pair of plants served as controls and as future stock plants. The plants were watered once from above after spraying and by sub-irrigation thereafter. After six weeks the vigor of the plants was assessed using scores as shown in Table 1.

Experiment 3. Clones of four biotypes selected from Experiment 2 were sprayed as described above with 5 doses of two herbicides (See Table 3.), when they had 2-3 leaves, 3-4 tillers. Single plants were sprayed for each treatment and were assessed after six weeks as described in Experiment 2.

Spray retention was estimated by recovery of sodium fluorescein from the foliage of eight plants by washing with 0.005M NaOH following spray application of 0.1% W/V sodium fluorescein contained in 0.3 kg AI ha^{-1} imazamethabenz-m solution.

TABLE 1. Vigor assessment

score	growth	imazamethabenz-m	isoproturon
0	none	necrotic	chlorotic
1	slight	leaves short & twisted	leaves chlorotic
2	stunted	tillers short & twisted	tillers chlorotic
3	moderate	----------most tillers as above----------	
4	vigorous	----------few tillers----------	
5	as untreated control		

Experiment 4. Twelve replicate clones of the four biotypes selected from Experiment 2 were grown for seven weeks and the root and shoot characteristics shown in Table 4 were recorded.

RESULTS

Experiment 1.

The mean scores for herbicide damage to A. myosuroides (Table 2) indicated progressive phytotoxicity with increasing dose, but a similar trend with respect to time after spraying was only seen with the high dose of isoproturon and the high dose of the combined treatment. The range of scores for each mean reflected biotype variability which tended to be less following imazamethabenz-m treatment compared to isoproturon, particularly at the 2 week assessment.

TABLE 2. Effects of imazamethabenz-m (IM), isoproturon (IPU) and both compounds on 16 replicates of A. myosuroides.

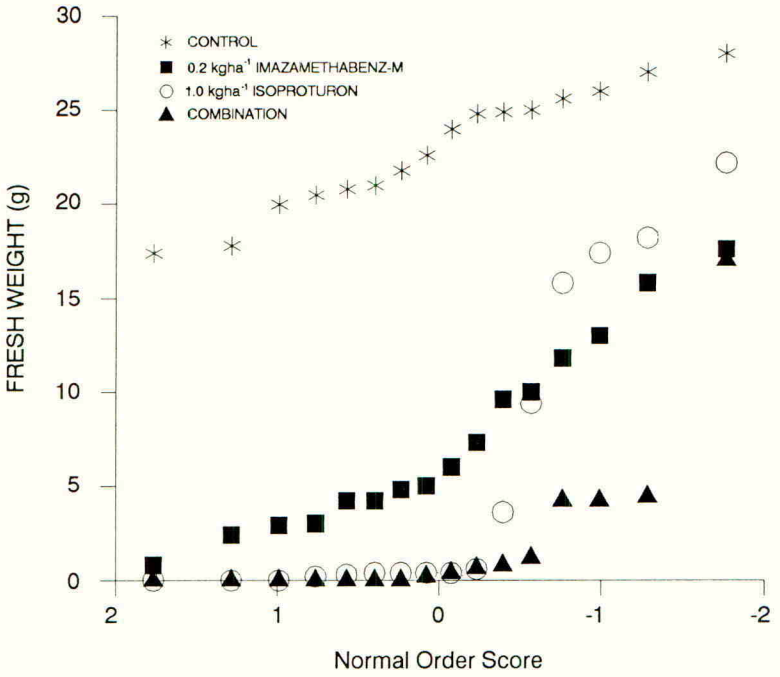
Dose kg AI ha^{-1}	0	IM 0.1	IM 0.2	IPU 0.5	IPU 1.0	IM 0.1 IPU 0.5	IM 0.2 IPU 1.0
Time				Visual assessment[a]			
2 weeks		4 (3-5)[b]	3 (3-4)	6 (4-7)	4 (1-6)	4 (2-6)	3 (1-7)
4 weeks		4 (2-6)	3 (2-5)	6 (2-6)	2 (0-6)	5 (0-5)	1 (0-6)
6 weeks		4 (2-6)	3 (2-6)	6 (2-6)	2 (0-6)	5 (0-6)	1 (0-6)
				Dry weight as % of Control[c]			
6 weeks	100 (4.85g)	43.4	19.8	76.6	16.8	47.2	7.3

[a] mean score [b] range of scores

[c] LSD (P=0.05) for dry wt. = 13.8%

The high dose combination killed the most plants achieving a mean score of 1 at 4 weeks, however dry weight of foliage at 6 weeks did not differ significantly from the high dose of the individual herbicides.

FIGURE 1. Response of 16 replicates of A. myosuroides to imazamethabenz-m and isoproturon alone and in combination, 6 weeks after spraying.



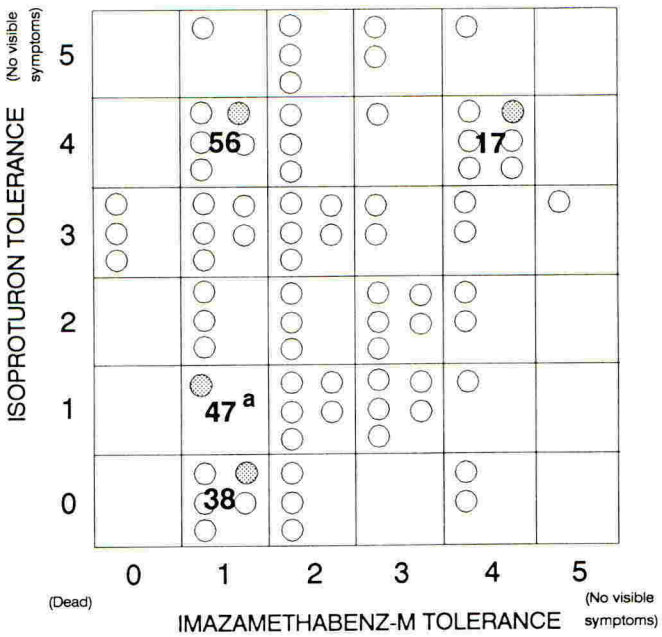
Fresh weights of untreated control and the high herbicide dose values for all replicates in ascending order of magnitude are plotted in Figure 1 against the 'normal order' scores (Nelder 1971). The control plants lie on a gently sloping straight line suggesting the replicate samples come from a single population with a normal distribution. The herbicide treatment points, particularly for isoproturon, lie on lines with generally much steeper slopes than the controls indicating they have a greater variability; also there is some evidence of the lower values having a different, lesser slope, indicating these replicates may not come from a single normally distributed population. Control and high dose of isoproturon fresh weights ranged from 18 to 28g and 0.2 to 23.2g, respectively.

Experiment 2.

The distribution on a grid of vigor scores for clones of 72 biotypes of A.myosuroides treated with imazamethabenz-m and isoproturon are shown in

Figure 2. Biotypes such as 17 were tolerant to both herbicides while others such as 38 and 47 were susceptible to both compounds. Other biotypes were susceptible to one herbicide and tolerant to the other. Thus isoproturon had little effect on biotype 56 which is controlled by imazamethabenz-m. A log-linear model, with Poisson Error Distribution and log link function, was fitted to the table of scores for the 72 biotypes with respect to herbicide tolerance (McCullagh, Nelder, 1983). The residual variance after fitting the two 'main effect' terms for isoproturon and imazamethabenz-m independently was 29.22 on 25 df. As the tabulated 5% value for x^2 on 25 df is 37.65, the residual deviance is not significant and so there was no evidence for the tolerance of one herbicide being associated with the tolerance of the other. The correlation between the two sets of data (r =+ 0.062) provides further evidence of the independence of the tolerance of the two herbicides.

FIGURE 2. Response of 72 cloned biotypes of A. myosuroides to imazamethabenz-m and isoproturon at 0.325 and 1.25 kg ha^{-1} respectively, 6 weeks after treatment.



[a] numbered biotypes used in subsequent experiments

Experiment 3.

Biotype 17 showed a marked tolerance to both herbicides with scores of four following application of 0.5 and 1.4 kg AI ha^{-1} of imazamethabenz-m and isoproturon respectively (Table 3). The former herbicide caused transitory inhibition of leaf development and some leaf twisting for two weeks following treatment, but the plants soon recovered. Isoproturon at 1.7 kg AI ha^{-1} caused chlorosis two weeks following spraying but subsequent growth was without symptoms, but less vigorous than the controls. Biotypes 38 and 47 were very susceptible to imazamethabenz-m and moderately

susceptible to isoproturon which at the higher doses caused severe chlorosis and necrosis but some leaves were unaffected. Bictype 56 was highly susceptible to imazamethabenz-m but tolerant to isoproturon. Biotype 38 retained more spray solution than biotypes 17 and 56.

TABLE 3. Visual assessments of imazamethabenz-m and isoproturon activity against susceptible and tolerant cloned biotypes of A. myosuroides, 6 weeks after treatment.

	imazamethabenz-m					isoproturon					Spray retention
$kgha^{-1}$	0.1	0.2	0.3	0.4	0.5	0.5	0.8	1.1	1.4	1.7	μlg^{-1} dry wt.
Biotype						Score					
17	5	4	4	4	4	4	4	4	4	3	118±22.4[a]
38	3	1	2	0	2	3	4	2.5	2.5	2.0	218±12.9
47	3	1	2	0	0	2.5	4	1.5	2.5	2.5	-
56	0	1	0	0	0	4	4	2	4	4	132±15.1

[a] = standard error

TABLE 4. Root and shoot characteristics of A. myosuroides cloned biotypes susceptible (s) and tolerant (t) to imazamethabenz-m (IM) and isoproturon (IPU) assessed seven weeks after propagation.

Characteristic	Biotype							
	17		38		47		56	
	IM-t	IPU-t	IM-s	IM-s	IM-s	IM-s	IM-s	IPU-t
	Shoots							
Max foliage ht. cm	30.5		39.7		29.7		22.2	
Tiller no.	7.3		6.3		8.7		6.8	
Tiller Angle from vertical °	27		53		56		35	
	Roots[a]							
Main root depth cm	4.8		3.5		3.1		4.8	
Main root no.	intermediate		many		many		few	
Fine root no.	few		many		many		intermediate	

[a] roots visible when pot removed

Experiment 4.

Biotype 17 which is resistant to both herbicides was intermediate in foliage height and tiller number, but was much more erect in habit than the herbicide susceptible biotypes 38 and 47 (Table 3). Main root distribution in 17 was concentrated at 4.8cm towards the base of the pot while in the susceptible biotypes most main roots were found higher in the profile. Fine roots were more abundant in the susceptible biotypes. Biotype 56, resistant to isoproturon and susceptible to imazamethabenz-m, was slightly less erect than 17 and had broadly similar root characteristics. The biotypes differed in other features such as anthacyanin expression with biotype 56 having the most and 17 the least pigment.

DISCUSSION

In these experiments inconsistent herbicide activity due to soil and weather factors was minimised by using standard soil and growing conditions.

The fresh weight of control plants (Figure 1) lay on a straight line suggesting that they come from a single population with normal distribution, but following herbicide treatment considerable deviation occured as indicated by the steep slope of the herbicide treated plant values. Both herbicides have some foliar activity, but phytotoxicity results primarily from uptake from the soil (Pillmoor; Caseley, 1984 , Blair, 1978). Initial cessation of shoot development with imazamethabenz-m and chlorosis and necrosis with isoproturon results from combined foliar and root uptake while sustained phytotoxicity and death depend on soil activity. Thus variability between plants could be related to shoot and root characteristics that influence herbicide uptake.

Treatment with each herbicide of clones of 72 biotypes confirmed the wide spread of susceptibility, but there was no evidence of the tolerance of one herbicide being associated with the tolerance of the other. However, some biotypes such as 17 (Figure 2) which were resistant to both herbicides and showed similarity to the chlorotoluron resistant Peldon population which is cross-resistant to other herbicides from different chemical groups and with differing modes of action (Moss & Cussans, 1991). Other chlorotoluron populations are not cross-resistant (Moss & Orson, 1988) a feature demonstrated by biotype 56 which showed resistance to isoproturon but not imazamethabenz-m.

The scores in Table 3 describe the herbicide response of biotypes 17, 38, 47 and 56 and confirmed that these clones respond similarly to their respective mother plants (Figure 2). Cross-resistant biotype 17 retained significantly less spray than susceptible biotype 38 and this may be associated with the prone habit of 38 compared to the more erect form of biotype 17 and this is reflected in the tiller angles (Table 4). Furthermore the position and abundance of roots in the susceptible biotypes 38 and 47 probably favoured uptake of herbicides from the soil. However the shoot morphology of biotype 56 was intermediate and its root system resembled that of biotype 17, but it was still susceptible to imazamethabenz-m and tolerant to isoproturon. Thus the significance of shoot and root morphology is uncertain. Studies on the mechanism of chlorotoluron resistance in Peldon populations have shown that enhanced herbicide degradation is of major importance (Kemp & Caseley, 1987; Caseley et.al. 1990) and it is probable that differential metabolism played a role in the variability of herbicide activity shown here. Differences in

shoot and root form and morphology can contribute to inconsistency in herbicide performance, but their role in the evolution of herbicide resistant populations remains to be determined.

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THE OCCURRENCE OF HERBICIDE RESISTANT *ALOPECURUS MYOSUROIDES* (BLACK-GRASS) IN THE UNITED KINGDOM AND STRATEGIES FOR ITS CONTROL.

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ABSTRACT

An additional 26 populations of *Alopecurus myosuroides* (black-grass) resistant to chlorotoluron were identified in 1989/90. This brings the total identified since 1982 to 46 farms in 19 counties of England. Of the 267 samples collected on a random basis between 1988 and 1990, 76% were susceptible, 16% were marginally resistant and 7% were resistant to chlorotoluron. Of 137 (non-random) samples tested in 1990, 27 showed resistance to fenoxaprop-ethyl. Of these only 20 also showed resistance to chlorotoluron, indicating that cross-resistance patterns are not consistent. In a field experiment, annual or rotational ploughing, used in conjunction with herbicides, was demonstrated to be an effective control measure for resistant *A. myosuroides*. Herbicide dose response experiments demonstrated a good correlation between the efficacy of chlorotoluron in the field and ratings obtained from glasshouse screening experiments. Cross-resistance was demonstrated to several other herbicides but, in contrast, some herbicides were effective on one resistant population. A strategy for the prevention and control resistance is discussed.

INTRODUCTION

Resistance to chlorotoluron was first detected in a UK population of *A. myosuroides* in 1982 (Moss & Cussans, 1985). By 1988 resistance to chlorotoluron had been found on 20 farms in 7 counties of England (Clarke & Moss, 1989). This paper presents results from further screening tests for resistance, not only to chlorotoluron, but also to fenoxaprop-ethyl (+ safener), a herbicide introduced into the United Kingdom in spring 1990. Results are also presented for experiments designed to assess the effects of cultivations on the control of resistant populations and the effects of resistance on herbicide efficacy under field conditions.

TESTING OF SEED SAMPLES FOR RESISTANCE

Materials and Methods

Seed samples were collected in July 1989 and 1990 from winter cereal fields and tested in glasshouses at ADAS, Cambridge, or IACR sites (Long Ashton Research Station or Rothamsted Experimental Station). The test procedure was that described by Moss & Orson (1988) in which seeds were sown in pots of soil and plants sprayed at the 2-3 leaf stage with 2.5-2.75 kg AI/ha chlorotoluron. Three standard reference populations were used in all

tests: Rothamsted (susceptible); Faringdon (partially resistant, 2*); Peldon A1 (resistant, 5*). There were 5 replicates. In addition, 2 pots (1990 ADAS) or 5 pots (1990 IACR) per population were treated at the 2-3 leaf stage with 150 g AI/ha fenoxaprop-ethyl + safener ('Cheetah R').

In both tests the % reduction in foliage fresh weight was calculated by relating weights in treated and untreated pots for each sample.

Sources of Seed Samples

In 1989 a total of 101 populations were collected and subsequently tested by ADAS. Seeds were collected from 49 fields within a 50 mile radius of Peldon, Essex as part of a Home-Grown Cereals Authority (H-GCA) funded random survey. A further 38 samples were collected from fields, selected at random, from the ADAS Winter Wheat and Winter Barley disease surveys of England and Wales. Additionally 14 samples were received from fields where clients had requested a test for resistance. The 108 samples collected in 1990 were from the ADAS disease survey (101 randomly selected samples) and from clients (7 samples).

Forty-five samples were tested by IACR in 1989 and 29 in 1990. Most of these samples were from farms where resistance had previously been detected and were included to determine whether resistance occurred on other fields on these farms. Samples from 15 new farms were tested (1989=11; 1990=4).

Results and Discussion

For the chlorotoluron test the classification described by Clarke & Moss (1989) was used. Samples were classified by a star rating from 1* to 5* or as S (susceptible). Only samples classified as 2* or more were deemed resistant and the higher the star rating the greater the degree of resistance.

Resistance was detected on 26 new farms (Table 1), and was confirmed in 18 other fields on 10 farms already identified as possessing resistant *A. myosuroides* in these, and previous IACR tests. None of the 12 populations, previously found to be resistant, had reverted, and become susceptible. This demonstrates that the screening technique used, is a reliable method for detecting resistance.

TABLE 1. Classification of resistance levels of 1989 and 1990 seed collections from farms not previously sampled.

		Number of samples						
	Source	TOTAL	S	1*	2*	3*	4*	5*
					---- Resistant -----			
1989	H-GCA survey	49	30	15	3	1	0	0
	Disease survey	38	25	8	5	0	0	0
	Others (ADAS)	14	9	2	3	0	0	0
	Others (IACR)	11	7	2	2	0	0	0
	TOTAL	112	71	27	13	1	0	0
1990	Disease survey	101	81	13	6	0	0	1
	Others (ADAS)	7	2	3	1	0	0	1
	Others (IACR)	4	0	1	3	0	0	0
	TOTAL	112	83	17	10	0	0	2

Since 1982, seed samples from a total of 531 fields have been tested for resistance to chlorotoluron. Resistance has now been detected in 67 fields on 46 farms. These farms are widely distributed in 19 counties of England: (Bedfordshire (1 farm), Buckinghamshire (3), Cambridgeshire (3), Dorset (1), E. Sussex (2), Essex (13), Gloucestershire (1), Hertfordshire (1), Kent (1), Leicestershire (1), Lincolnshire (2), Norfolk (1), Northamptonshire (1), Nottinghamshire (1), Oxfordshire (6), Suffolk (5), Surrey (1), Warwickshire (1), Worcestershire (1)).

Most of the resistant populations were ranked 2*, and therefore exhibit partial resistance to chlorotoluron. However more severe resistance, at the 3-5* level, has been recorded in Buckinghamshire, Essex, Lincolnshire, Leicestershire, Oxfordshire and Suffolk.

In random surveys an average of 76% of the 267 samples tested between 1988 and 1990 were classified as S, 16% as 1* and 7% as resistant (Table 2).

TABLE 2. Resistance level classification for samples from random collections.

Year	Number of samples	% in each category S	1*	2* or more
1988 #	79	86	9	5
1989	87	63	26	10
1990	101	79	13	8
Mean	267	76	16	7

= from Clarke & Moss, 1989

However, in 1989 and 1990, seed collected from 36 non-random sources, where herbicide performance in the field had been inadequate, resulted in 28% of samples being classified as resistant and 22% as 1*. This shows that poor field performance gives an indication of resistance. However, it is important to note that 50% of samples where a problem in A. myosuroides control was identified were not classified as showing any degree of resistance. This highlights the need to examine all reasons for poor control and not to assume resistance as the only cause.

The results for the control by fenoxaprop-ethyl were analysed statistically and samples were classified as resistant only if the % reduction in fresh weight was significantly less ($P \leq 0.05$) than the Rothamsted susceptible reference population. Of the 27 populations showing resistance to fenoxaprop-ethyl, 20 showed at least a 2* level of resistance to chlorotoluron. In contrast, 11 of the 137 samples tested were resistant to chlorotoluron but showed no evidence of resistance to fenoxaprop-ethyl. The relative response of the 1990 IACR samples to chlorotoluron and fenoxaprop- ethyl was studied by conducting a regression analysis, based on the data for foliage fresh weight of treated pots. The correlation coefficient ($r=0.47$) was low, but statistically significant at $P \leq 0.05$.

There appears to be some relationship between resistance to these herbicides. However, these results support other studies that show that the pattern of cross-resistance is not consistent between populations, either in

terms of the specific herbicides affected or the degree of resistance (Moss, 1992).

FIELD EXPERIMENTS

Two field and one container experiment were conducted on resistant *A. myosuroides* populations.

Cultivation Experiment

Materials and Methods

Experimental details and the first four years results of an experiment started in 1985 at Peldon, Essex (4* population) have been reported previously (Orson & Livingston, 1987; Clarke & Moss, 1989). After two years with a range of herbicide treatments superimposed on the cultivation treatments, the experiment was changed in autumn 1987 so that herbicides were applied in line with the normal farm practice to the entire 24 m x 18 m cultivation plots. This included sequences of at least three herbicide applications active against *A. myosuroides*. Herbicides used have included isoproturon, trifluralin, chlorsulfuron/metsulfuron methyl and tri-allate. The experiment was of a randomised block design with three replicates. In the final year, autumn 1989, the plots ploughed in 1985 were again ploughed. This allows the comparison of ploughing every year, every four years and annual minimal cultivation.

Results and Discussion

The benefit of ploughing was again demonstrated (Table 3). Although the infestation level of *A. myosuroides* with rotational ploughing was higher than with annual ploughing, especially in 1988, the level had been reduced to zero by 1990. The experiment was re-drilled (mid November 1989) and this, as well as intensive use of herbicides, accounts for the high levels of control.

TABLE 3. Effect of cultivation on the numbers of *A. myosuroides* heads/m^2.

	Autumn treatments					*A. myosuroides* heads/m^2 (July)				
	1985	1986	1987	1988	1989	1986	1987	1988	1989	1990
Annual Plough 25 cm	P	P	P	P	P	107	1280	44	10	0
Rotational Plough	P	mc	mc	mc	P	107	1394	883	114	0
Mincult 5 cm	mc	mc	mc	mc	mc	915	3667	2162	378	16
					LSD (P≤0.05) ±	360	1344	306	145	10.3

Note: 1986 and 1987 counts refer to untreated plots, 1988, 1989 and 1990 refer to treatment as farm practice.

Population/Herbicide Dose Response Experiment

Materials and methods

Seeds of 5 populations of *A. myosuroides* with a range of ratings of resistance to chlorotoluron (Table 4) were sown in 4m long paired rows (10

cm apart, 30 cm between populations) in a chalky boulder clay soil at Boxworth EHF, Cambridgeshire on 19 October 1989. There were 4 replicates. Three herbicides, each at 4 doses, were sprayed on 16 February at the 2 tiller stage using a modified Van de Weij pressurised knapsack sprayer in 200 l water/ha using 02-F80 nozzles at 200 kPa. A. myosuroides control was assessed by counting the number of plants one month after treatment, and expressed as the % reduction of the number, in that plot, before treatment.

Results and Discussion

The results demonstrated that resistance can cause substantial reductions in herbicide performance, even at higher than recommended dose rates (Table 4). There was a good correlation between the efficacy of chlorotoluron in the field, and the * ratings obtained from the glasshouse screening experiments. At equivalent doses, isoproturon gave better control than chlorotoluron and again there was a good correlation with the * ratings. There was a poorer correlation between field performance of diclofop-methyl and * rating.

Control of the Boxworth population (1*) was poorer than Rothamsted (susceptible) when treated at the label recommended rate of chlorotoluron (3.5 kg AI/ha). This result is of concern since 16% of the populations of A. myosuroides were ranked 1* in the random surveys (Table 2). The results for H/121 confirm that it shows a high level of resistance to chlorotoluron and isoproturon, but no resistance to diclofop-methyl. The apparently higher level of control of H/121 by diclofop-methyl than the susceptible standard (Rothamsted) warrants further study to establish whether negative cross-resistance exists. Other glasshouse studies have confirmed the contrasting pattern of cross-resistance, relative to the Peldon population, but have showed no evidence of negative cross-resistance (Moss, 1992).

TABLE 4. Percentage reduction of A. myosuroides plants/m²: Population/herbicide dose response experiment, Boxworth 1989/90.

Herbicide	Rate of AI kg/ha	Population Rothamsted (S)	Boxworth (1*)	Faringdon (2*)	H/121 (4*)	Peldon (5*)
chlorotoluron	1.75	43.9	18.6	5.3	34.7	12.2
chlorotoluron	3.5 @	86.9	60.5	42.0	38.2	28.1
chlorotoluron	7.0	95.5	82.7	80.8	41.2	12.4
chlorotoluron	14.0	100.0	95.5	88.1	74.9	29.4
isoproturon	1.25	60.0	42.7	38.0	38.5	16.6
isoproturon	2.5 @	95.5	94.9	84.0	44.5	53.7
isoproturon	5.0	100.0	95.6	98.2	70.9	94.1
isoproturon	10.0	100.0	100.0	100.0	84.7	100.0
diclofop-methyl	0.567	22.6	7.8	0.0	27.9	6.9
diclofop-methyl	1.134 @	53.4	46.1	29.8	84.5	9.7
diclofop-methyl	2.268	64.3	58.9	46.6	88.4	56.1
diclofop-methyl	4.536	93.7	91.5	71.7	92.7	41.1
Mean (LSD (P≤0.05) ±16.81)		78.0	68.5	59.6	58.2	42.2

@ = maximum label recommended rate
* = ratings for resistance to chlorotoluron based on glasshouse screening tests)

Effect of Resistance on Herbicide Activity in Simulated Field Conditions

Materials and Methods

The method used has been described by Moss (1987). Seeds of 3 populations (Table 5) were sown in containers of silty loam soil (3% o.m.) on 4 October 1990 and placed in an outdoor plunge bed at LARS. There were 4 replicates in a randomised block design, and 3 untreated containers per replicate for each population. Seven herbicides, each at 3 doses, were applied on 19 November 1990 at the 3½ leaf stage using a laboratory sprayer delivering 242 l water/ha at 210 kPa through a single 'Spraying Systems' 8001 Tee-jet nozzle. Foliage fresh weight was recorded on 11 April 1991, when untreated plants were at the two tiller stage.

Results and Discussion

The Rothamsted population was controlled very effectively by the recommended rate of all herbicides (Table 5). With the exception of chlorotoluron and tralkoxydim, the lower rates also gave over 90% control.

TABLE 5. Percentage reduction in foliage fresh weight in simulated field conditions.

Herbicide	Dose kg AI/ha		Population Rothamsted (S)	Peldon (5*)	Bucks C1 (4*)
chlorotoluron	1.17		53.0	-9.6	-12.6
chlorotoluron	3.50	@	99.0	-3.6	4.0
chlorotoluron	10.50		99.9	11.9	12.7
isoproturon	0.83		92.3	-3.9	5.6
isoproturon	2.50	@	98.9	14.7	22.8
isoproturon	7.50		99.3	73.3	61.7
fenoxaprop-ethyl (+ Safener)	0.04		98.3	47.2	45.8
fenoxaprop-ethyl (+ Safener)	0.12	@	97.7	61.0	45.8
fenoxaprop-ethyl (+ Safener)	0.36		97.9	66.8	66.8
fluazifop-P-butyl + Agral 0.1%	0.042		99.6	88.2	-
fluazifop-P-butyl + Agral 0.1%	0.125	@	98.8	99.2	-
fluazifop-P-butyl + Agral 0.1%	0.375		99.0	99.6	-
quizalofop-ethyl + Fyzol 11E 2l/ha	0.025		97.9	94.5	-
quizalofop-ethyl + Fyzol 11E 2l/ha	0.075	@	99.5	97.3	-
quizalofop-ethyl + Fyzol 11E 2l/ha	0.225		99.9	99.9	-
sethoxydim + Adder 1%	0.097		99.4	99.4	-
sethoxydim + Adder 1%	0.290	@	99.4	99.7	-
sethoxydim + Adder 1%	0.869		99.7	99.9	-
tralkoxydim + Adherb 0.5%	0.083		75.4	23.4	-
tralkoxydim + Adherb 0.5%	0.250		93.6	47.1	-
tralkoxydim + Adherb 0.5%	0.750		98.9	69.0	-
				LSD (P≤0.05) ± 9.60	

@ = label recommended rate

In contrast, all rates of chlorotoluron, isoproturon, fenoxaprop-ethyl and tralkoxydim gave substantially poorer levels of control of the Peldon population. This population was well controlled (>95%) by the recommended rates of fluazifop-P-butyl, quizalofop-ethyl and sethoxydim. The lower rate of quizalofop-ethyl and sethoxydim also gave a high level of control but the lower rate of fluazifop-P-butyl was significantly poorer. This supports previous studies which indicated that there is partial resistance to fluazifop-P-butyl in the Peldon population (Moss, 1987). It appears there was no evidence of differential efficacy between the Rothamsted and Peldon populations from sethoxydim. This is consistent with unpublished glasshouse dose response studies. Control of the Bucks population by the 3 herbicides tested was also poorer than Rothamsted, but similar to Peldon.

The experiment showed that, while resistance does not cause complete inactivity of herbicides, substantial reduction in activity can occur. However the scale of the reductions in herbicide performance varies substantially between herbicides for reasons that are, at present, poorly understood.

STRATEGIES TO COMBAT RESISTANCE

These recent results have highlighted the need to develop strategies to combat both the spread of resistance and to aid control of resistant populations. So far only populations rated 2* or more have been classified as resistant. The field dose response experiment at Boxworth indicates that 1* populations need to be considered as partially resistant at least to some herbicides. The strategy outlined here will reduce the dependence on herbicides and is applicable both to prevention of the development of resistance and the control of existing resistant populations. Detailed advice for the control of resistant populations using herbicides requires a knowledge of the pattern of resistance to the whole range of herbicide options. It is assumed here that there is cross-resistance, but research is showing a wide range of patterns of resistance.

Cultural Aspects

The strategy is designed to reduce the reliance on herbicides for A. myosuroides control. The worst cases of resistance have so far been associated with continuous, or near continuous, winter cereal cropping where the main cultivation has not included ploughing and where herbicides to control A. myosuroides have been used regularly. The following points are listed in order or priority, but it is clear that not all points are practical in every circumstance.

1) Where possible plough at least once every 4 or 5 years.
2) Include spring sown crops in the rotation.
3) Include autumn sown broad-leaved break crops, such as beans or oilseed rape, in the rotation. This will permit the use of herbicides which are effective on resistant populations.
4) Delay autumn sowing to allow a high proportion of seedling emergence to occur prior to sowing. Destroy as many weed seedlings as possible before cultivation and sowing using a non-selective product such as glyphosate or paraquat.

Herbicide Choice

1) Herbicides should be used to maximise their efficacy. Ensure that they are applied at the correct dose, timing and water volume, and in optimal climatic and soil conditions.
2) Where resistance has been confirmed, do not apply more than the equivalent of the maximum recommended dose of any single active ingredient to any single crop.
3) Because of the potential risk of rapid development of severe resistance to the aryloxyphenoxypropionate and cyclohexanedione herbicides, it is recommended that members of these groups (diclofop-methyl, fenoxaprop-ethyl, fluazifop-P-butyl, quizalofop-ethyl, sethoxydim, cycloxydim) are not used as the sole, or main, means of A. myosuroides control in consecutive crops. Resistance to substituted-urea herbicides, such as isoproturon and chlorotoluron, appears to develop less quickly.

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ESCAPE OF ENGINEERED GENES FROM RAPESEED TO WILD BRASSICEAE

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ABSTRACT

Artifical hybridization has been achieved between transgenic rapeseed and *B. adpressa*, which produced *in vitro* hybrids at a low rate (2-3 %). In the field, the use of a male sterile rapeseed led to the production of 600 seeds per m² of the suspected hybrid. Hybrid seeds germinated as quickly as rapeseed, without dormancy. Some of these plants had half the sum of the chromosome number of the parent species. Subsequent growth of the hybrid plants showed a wide morphological and developmental variability. Most but not all hybrids were sterile. These preliminary results and the use of a computer model may help to understand how the spread of a recombinant gene in a weed population may evolve.

INTRODUCTION

No transgenic plants have yet been registered as new crop varieties but there are numerous research programs in progress in this area (Gasser & Fraley, 1989). The most advanced work is on soybean, cotton, rice, sugarbeet, potato, lucerne, tobacco and different *Brassica* crops with the aim to develop resistance to insects, viruses and herbicides, induction of male sterility for hybridization programs and modification of seed storage proteins (Botterman, 1989; Mariani *et al.*, 1990; Guerche *et al.* 1990).

Some of the genes encoding for such characteristics could probably confer some adaptative advantage if they were introduced accidentally into weeds. This could lead to more invasive, agressive and competitive weeds in cultivated fields, and cause a shift in the ecological balance. So, it is important to study possible interaction between the newly engineered "crop-gene" and the closely related weed species. Herbicide resistant plants were obtained and are of potential use in the field to control weed infestations. Genetic engineering has already created crop plants resistant to a range of herbicides including, resistance to amino acid biosynthesis inhibitors like sulfonylureas, imidazolinones, glyphosate and phosphinothricin, to photosynthesis inhibitors like atrazine or bromoxynil, and to growth regulators such as the 2,4-Dichlorophenoxyacetic acid (Mazur & Falco, 1989 ; Lyon *et al.*, 1989). In the case of oilseed rape, the development of herbicide resistance is a valuable method of preventing contamination of the harvested rape seed with seeds from Cruciferous weeds, such as wild mustard, and from double hight or single low rapeseed volunteer, which are difficult to control with normal herbicide treatments.

In the framework of a "risk assessment" program of the C.E.C. relating to the release of genetically engineered organisms (Biotechnology Action Program, 1989), gene dispersal from transgenic crops was studied using the phosphinotricine resistance gene isolated by Plant Genetic System (P.G.S.; Gand, Belgium). Different research laboratories in Europe collaborate in this program, working on potato (C.P.O. Wageningen, Netherlands), lucerne (I.P.S.R. Norwich, United Kingdom) and oilseed rape (P.G.S., I.P.S.R. and I.N.R.A. at Rennes and Dijon, France). Within this program using P.G.S. herbicide resistant material, INRA has led the program estimating the risk of interspecific crossing between oilseed rape and wild brassiceae like *Sinapis arvensis*, *Raphanus raphanistrum*, *Brassica nigra*, *Brassica adpressa* and wild strains of *Brassica oleracea* (Kerlan *et al.* 1991).

In risk assessment studies, there are practical advantages in using herbicide resistance as a tool to detect interspecific weed-crop hybrids, as it is relatively easy to detect gene transfer when treating large number of plants. From a theoretical point of view, herbicide resistance is also a particularly suitable character to be tested in pioneer risk assessment studies, because its main risk, if transmited to wild plants, is to confer a beneficial adaptative trait only in herbicide treated areas. If such an accident occured, the damage could be limited by banning the relevant herbicide for a long time from the affected area, so that there was no more selection in its favour, thus rendering the resistance gene ineffective. This would not be the case with traits conferring pathogen and insect resistance or for genes improving the use of habitat resources: once introduced, they still confer an advantage, even in uncultivated areas.

In our study, we have special interest in the intergeneric cross between rapeseed (*B. napus*, 2 n = 38) and hoary mustard (*B. adpressa* Boiss. / *Hirschfeldia incana* (L.) Lagrèze-Fossat, 2 n = 14), an annual mediterranean weed present in south and western France, in sandy places in Jersey and Alderney and casualy in South England and the Netherlands (*Flora Europaea*). It flowers from May to September, so that its flowering period overlaps with spring rape and partially with winter rape. As far as we know, there are no previous reports on the occurence of a natural interspecific hybrid between these two species.

We report here on the different approaches we have used to estimate the possibility of such a cross, using laboratory techniques as well as field experiments. The work to obtain hybrids has involved manual crosses followed by *in-vitro* techniques and also natural hybridization in fields. Secondly, to characterise the hybrids and their development, we have performed cytogenetic and electrophoretic studies, and observed germination and growth. Finally, computer simulations have been used to improve our understanding and predict the behaviour of the resistance gene in wild populations and its effect on weed infestations.

HYBRIDIZATION

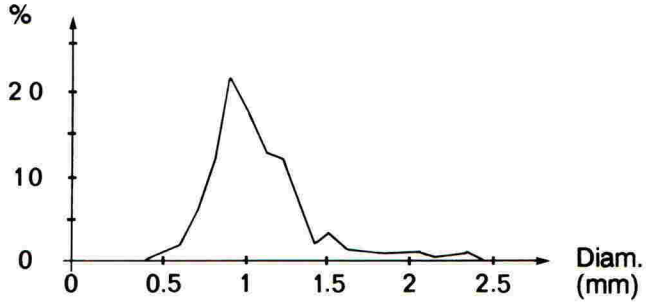
Field trials

A 640 m² trial was set up in Rennes INRA in 1990 with alternate rows of *B. adpressa* and *B. napus* var. *oleifera* cv. Brutor male sterile line. This trial was more than 500 m distant from any rapeseed field. The hybrid seed yield obtained on the male sterile rapeseed was 600 seeds/m². This is very low compared to a normal yield of rapeseed with up to 34 000 seeds/m² in the same conditions of alternate rows of normal and male sterile lines. Since there was no competition between the pollen of rapeseed and hoary mustard, this is the upper limit of the natural frequency of the interspecific cross. This represents 0,3 % of hybrid seeds in the potential harvest.

Obviously, this will be dramatically reduced when using a male fertile rapeseed cultivar as rapeseed auto-pollination will impair cross-fertilization by pollen of hoary mustard. In addition, the weed/crop ratio will not be 1:1 in a true cultivated field, so that the pollen of wild plant will be at low concentration and will lead to still fewer interspecific crosses. However, the results obtained here are an important feature because they indicate that spontaneous crosses may occur although hybridization barriers exist between the two species.

The seeds harvested on the male sterile rapeseed were very heterogeneous in size : from very small seeds similar to *B. adpressa* (mean diameter : 0.73 mm) to seeds of the size of normal rape seeds (mean diameter : 2 mm).

FIGURE 1. Mean diameter distribution of hybrid seeds



We divided the seed lot into 3 groups using 0.8 and 1.4 mm sieves for further studies.

TABLE 1. Analysis of the size distribution of hybrid seed lots

Seed lot	mean diameter (mm)	percent of the total hybrid seed lot
Small	0.76	14
Medium	1.10	84
Big	1.90	2

Manual pollination and *in-vitro* techniques

To avoid problems of incompatibility between species, we chose immature buds on the female parent. After opening and removal of anthers, we exposed the flower to pollen of a freshly openned flower. One week after pollination, the pods were initiated but as there was a risk of abortion of the interspecific crosses, we removed them and put them on a sterile, growth regulator free culture medium in petri dishes (Delourme *et al.*, 1989).

The fertilised ovaries were able to develop on this medium and the seeds obtained had the capacity to germinate about two months later. Plantlets were grown first *in vitro* in tubes and then in soil in a greenhouse.

TABLE 2. Hybrid production by *in-vitro* techniques

Brassica adpressa	*Brassica napus*	No. of pods	No. of Embryos	No. of plants	Yield
M	F	262	38 (14,5%)	8 (21 %)	3,05 %
F	M	1 117	36 (3,0 %)	15 (41 %)	1,34 %

It is clear from Table 2 that the hybridization cross is easier with rapeseed as female parent. We obtained more hybrid embryos on rapeseed (14 %) than on hoary mustard (3 %). In contrast, it seems to be easier to grow a plant from a hybrid embryo born on hoary mustard (41 %) than from one born on rapeseed (21 %). Finally, we obtained a better hybridization yield with rapeseed as female parent (3,05 %) than with the opposite cross (1,34 %).

If this result corresponds to what may occur in spontaneous crosses, it indicates that resistant hybrids may appear more frequently within the seed crop production than outside the field in uncultivated areas.In the field trial mentioned above, we have noted on the male sterile rapeseed a mean of 0.016 pod formed per flower and 0.023 seeds harvested per flower. If all the harvested seeds germinate and if all the plants obtained are hybrids, we have in the field a hybridization yield of 2/3 of potential hybrids obtained by manual methods. Fortunately, most of the crop production is harvested (seed shattering represents 1 to 3 %), so the most important way of producing resistant hybrids results in few gene escape.

The resistance gene was characterized in six hybrid plants obtained with *B. adpressa* as female parent, by studying its presence with the polymerase chain reaction (P.C.R.) and its expression after phosphinothricin treatment to small parts of the leaves. After PCR, these six hybrids had some amplification products indicating that they have inherited of the inserted gene. However, only five of these hybrids showed a resistant phenotype, the last one being susceptible to phosphinothricin. Perhaps regulation of the inserted gene can occur in an interspecific hybrid genome. This suggests that studiing these regulation problems could lead to prevent from gene expression in foreign organisms.

SEED GERMINATION AND GROWTH OF THE HYBRIDS

Germination rate

Germination of three lots of 100 hybrid seeds was estimated in optimal conditions : on wet blotting paper, in petri dishes, in a regulated growth cabinet at 25-20 °C (16 h photoperiod) (see Table 3).

It is clear that the germination rate of the hybrid seed is quite close to rapeseed. It is possible that the dormancy, generally a characteristic of weeds, has disappeared in these hybrids either because of dominant non-dormant genes inherited from the cultivated parents (dormancy at the embryo level) or simply because of maternal effects from the cultivated mother plant (in case of extra-embryonic control of dormancy).

TABLE 3. Percentage germination of hybrid seeds of the three size classes and *B. adpressa* (Figures followed by the same letter are not significantly different (p = 0.05) according to the Newman and Keuls test)

Seed lots		Germination (%)	N-K group (5 %)	Confidence interval at 95 %
Hybrids:	Big	96.0	A	94 - 97
	Medium	88.5	A	84 - 93
	Small	88.5	A	85 - 92
B. adpressa		54.5	B	47 - 62

Growth studies

One month after germination, we made three observations to characterize the growth of the different classes of plants

TABLE 4. Comparative growth of young plants of *B. napus*, *B. adpressa* and plants from the three seed size classes of the hybrids (10 replicates)

Classes of plants		3rd leaf length Mean	NK group	3rd leaf width Mean	NK group	no. of leaves Mean	NK group
B. napus		8.15	A	6.20	A	5.2	B
Hybrids:	Big	8.00	A	6.90	A	5.4	B
	medium	7.05	B	4.60	B	5.9	AB
	Small	6.15	C	3.20	C	6.0	AB
B. adpressa		5.15	D	2.50	C	6.5	A

The leaf length, of plants from rapeseed and hybrids with big seeds were not different whereas those from the other class were smaller. It appears that hybrids issued from big seeds tended to have leaves as large as rapeseed ; hybrids from small seeds had small leaves like hoary mustard whilst leaves from medium seeds were intermediate but with greater variation than the other two hybrid groups.

For the development stage (number of leaves), rapeseed and hybrids from big seeds were clearly differentiated from hoary mustard, the two others hybrid groups being intermediate.

Considering the overall conclusions of the growth studies, some of the supposed hybrids (from big seeds group) have a crop-type development : slower plastochrome and leaves as big as the rapeseed parent. These plants, representing 6% of the total hybrid seed lot (cf. table 1), are clearly not hybrid plants. Plants from the others hybrid seed lots present all the intermediate forms between the weed type and the crop type and are thought to be hybrids.

The observation of morphological characteristic traits like the hairyness of leaves present in *B. adpressa* and the shape of the basal part of the leaves also results in forming intermediary classes as in the preceding study.

ELECTROPHORESIS STUDY AND CYTOGENETICS OF THE HYBRIDS

Electrophoresis was done using discontinuous PAGE system or starch gels. Seven enzymatic systems were shown to distinguish the two parent species: leucine aminopeptidase (LAP), 6 phosphoglucodeshydrogenase (6PGD), aconitase (ACO), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI) esterase and superoxyde dismutase (SOD).

Hybrids show a combination of the two parental patterns, with in some cases a new band that may be interpreted as a heterodimeric band. As expected from the results above, some of the supposed hybrids had the *B. napus* pattern for all isozymes. Therefore, some of the seeds obtained on the male sterile rape are due to unexpected long distance pollination by foreign rape pollen.

Some of the plants studied here were tested cytogenetically. Plants with hybrid isozyme pattern had 26 chromosomes (expected hybrid formula) and plants with rapeseed pattern had 38 chromosomes (normal rapeseed formula).

REPRODUCTION OF HYBRIDS

A proportion of the hybrids demonstrated female fertility but male fertility was rare. From 89 plants observed, 50% had 0 to 10% of their pods formed and 12% had 10 to 20% (maximum observed). As far as pollen production was concerned, 74% were sterile and 9% had around 30% of viable pollen grains (maximum observed). We must emphasize here, that these hybrids were produced on male sterile rapeseed plants (Cybrids from Ogura cytoplasm) (Pelletier *et al.*, 1983).

We have shown that, by using these procedures fertile hybrid plants can be produced. Now that this most difficult obstacle has been cleared, several backcrosses with *B. adpressa* will be necessary to confirm that introgression from genome of rape in hoary mustard could occur .

COMPUTER SIMULATIONS

The complexity of biological processes that influence the transfer of herbicide resistance to wild relatives, requires a research approach that focuses on the interaction between life history processes and population genetics. The model developed here takes into account gene flow and factors influencing fitness. It predicts the local evolution, spreading and subsequent dynamics of a resistance gene from a cultivated species (oilseed rape *Brassica napus*) to its related weed (*Brassica adpressa*).

In the scenario tested here, the resistance depends on a single nuclear dominant locus introduced in the rapeseed. The field is treated with the herbicide (efficiency of 97% on sensitive plants) and the resistance ensures a 100% survival to the treatment. A hedgerow is full of the wild relative. The model tests the effect of the recombination rate (rec) of the resistance gene between the crop and the weed and the different factors controlling the characteristics of the cultivated species, the barrier to hybridization (Hb) between rapeseed and its wild relative and the cost of the resistance gene (cost) in the absence of treatment, on the level and speed of transfer of the resistance gene from the crop to the wild relative. Simulations have been run on

computer changing these various parameters. The observed variance in the amount of resistance transfer mainly depends on the hybridization barrier and the cost of resistance. Note that there is an important positive interaction between the two parameters (Table 5).

TABLE 5. Analysis of variance of the frequency of resistant plants in wild type from simulation data

Model : Resistance Frequency = Constant + recombination effect (rec) + Hybrid barrier (Hb) + Cost of resistance effect (cost) + Hb* cost interactions + residual

Source	Sum of squares	df	Mean square	F-ratio	P
rec	209.806	5	41.961	2.427	0.035*
Hb	11682.044	5	2336.409	135.151	0.000***
cost	9521.223	6	1586.870	91.794	0.000***
Hb*cost	24557.337	30	818.578	47.351	0.000***
residual	7174.250	415	17.287		

Models can be used as a tool for identifying out different situations according to their degree of risk of transfer. This, of course, requires that relevant parameters be measured in experimental designs. The simulations also highlight the characters that have the biggest effect on gene transfer efficiency. The simulation reported here illustrates the major importance of the hybridization barriers, that could be built up in the transgenic crop using suicide genes. Other genetic events like recombination have lower effects, indicating that the place of the insertion of the new gene on one or other chromosome may be of secondary importance. Looking for high cost resistance gene which will disadvantage the weed-crop hybrid has no sense, because plant breeders need low cost beneficial genes in crop varieties.

CONCLUSION

In this preliminary study, the series of biological steps giving the rise to the transfer of a gene from a transgenic rapeseed crop to a closely related weed are discussed. Spontaneous hybridization between the two studied species is possible especially with the rapeseed as female parent but also with hoary mustard. It will inevitably occur at much lower rate in true field condition than in our experiments. Even in our optimised field conditions, hybridization was affected by long distance rapeseed pollination. Germination of hybrid seeds is closer to rapeseed than hoary mustard, i.e. without dormancy, which is probably disadvantageous in a natural habitat. However, we know that rapeseed volunteers may occur six to ten year after in the same field due to seed persistance in the soil. Hybrid variability for growth and morphology is also a way to adapt successfully to different environments. Finally, although pollen fertility is reduced, backcrosses with wild plants remain possible, which might lead to the transfer of the gene to the genetic resource of weed populations, if no chromosome segregation problems occur in plants obtained from the backcross. Knowing these facts, and using simulation models to predict the combined role of the different biological parameters, it will be possible to estimate on a case by case basis the risk of gene dispersal from transgenic crops.

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INTEGRATED ANALYSIS OF THE POPULATION STRUCTURE OF A CLONAL PERENNIAL WEED (*RUBIA PEREGRINA*)

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ABSTRACT

The aim of this paper is to show the need for the development of integrated analysis of population biology in order to improve weed management. The emphasis is put on the study of spatial distribution of plants because of its interaction with other processes generating variability. Results of the integrated analysis of the population structure of *Rubia peregrina*, a clonal perennial weed of herbicide treated vineyards, based on the modelling of its spatial distribution and on the analysis of its genetic diversity are reported. They are discussed in relation to the generation of a general population model that includes the variability due to individual performance.

INTRODUCTION

The most popular studies on population biology of weeds which were performed in order to improve weed management strategies were linked to the emergence of herbicide resistance. But, separate analysis of genetic and physiological mechanisms responsible for herbicide resistance have not resulted in development of new control strategies. Recently, Roush *et al.* (1990) suggested that such a development will require integrated analysis of the genetic, physiological and ecological processes that influence the dynamics of weed population. More generally, such integrated analysis of population dynamics of weeds are needed to assist the development of integrated weed management system, based on optimum herbicide control strategies (Dirzo & Sarukhan 1984).

Studies on weed population biology have been concerned with the analysis of only one or two processes influencing weed population dynamics, many of the reported experiments being concerned with the analysis of genetic diversity or of global demographic variations (Navas, 1991). More recently, emphasis has been put on the importance of weed patchiness for the prediction of crop yield losses (Hughes, 1990) and on population dynamics *via* seed dispersal patterns, relative distance to unmanaged zones and relationships between neighbouring individuals (e.g. Van Groenendael, 1988; Marshall, 1989; Navas & Goulard, 1991). Moreover, the functioning of populations has been shown to be strongly related to their spatial distribution through the pattern of pollen flow (Gasquez, 1985), intraspecific competition (Brain & Cousens, 1990) and susceptibility to pests and pathogens (Hasan & Ayres, 1991). Thus, modelling the spatial structure of a population and of its variability is a basic and fundamental feature of population dynamics : in the short term, it is involved in prediction of crop damage and infestation levels, while in the long term, it is involved in adaptive and evolutionary responses of genotypes.

In the present paper, an integrated approach is used to study the population dynamics of *Rubia peregrina* (wild madder), a clonal perennial weed of herbicide treated vineyards of southern France. Results of modelling its spatial distribution

pattern and of quantification of interactions between neighbouring plants (Navas & Goulard, 1991) are summarized and integrated with results of the analysis of its genetic diversity in the field (Navas & Gasquez, 1991). A general evaluation of the functioning of *R. peregrina* populations in a vineyard is proposed, knowing their demographic diversity.

EXPERIMENTAL DETAILS

General

The species

Rubia peregrina (Rubiaceae) is native to Mediterranean Europe. During the last decade, due to the decreasing use of ploughing and the increasing use of herbicides, to which it is tolerant (Guillerm & Maillet, 1984), *R. peregrina* has become a major weed in herbicide treated vineyards established near shrublands and woodlands. In vineyards, it typically grows around vine-stocks and spreads preferentially by vegetative growth within rows under vine cover. It is a clonal perennial herb with persistent whorls of four to eight leaves. During winter, numerous branched rhizomes are produced which give rise to aerial shoots the following spring. For *R. peregrina*, $2n=44$, and it is considered to be tetraploid. In southern France, the hermaphroditic flowers appear in June. Ripe fruits containing one or two seeds are found from late October to January. The fleshy, black berries are eaten by birds which disseminate the seeds.

The study site

The vineyard was located 10 km northwest of Montpellier (43° 38'N,3°46'E), in a sub humid Mediterranean climate. Vine stocks were located on a 1.90 x 1.30 m^2 grid. The vineyard was chemically weeded twice a year with simazine and aminotriazole in April and May and glyphosate in June. It was not fertilized during the study. *R. peregrina* was the most abundant species of weed. The other weeds were also perennials : *Sanguisorba minor*, *Phyllirea angustifolia*, *Rosa canina*.

Spatial distribution of *Rubia peregrina*

The clumping dynamics of *R. peregrina* was studied using a two-dimensional approach, which quantified interactions between plants in an anisotropical way, according to their relative positions. Collection of field data, statistical and modelling methods are precisely described in Navas & Goulard (1991).

Methods

Maps of *R. peregrina* distribution were made in April 1986 and January 1988 in the vineyard by recording the presence or absence of the weed in 1.90 x 1.30 m^2 quadrats, centred on the vine-stocks. This division in quadrats was made to define a lattice, used for calculations. Four zones were defined : two were located in the middle of the vineyard and were very invaded by *R. peregrina* whereas the other two ones were located near the edge of the field and were less invaded. For both years and each zone, the following calculations were performed :

(1) the observed distribution pattern was compared with a random pattern and its departure from randomness was described using Monte Carlo procedures. To do that, its intensity λ was calculated and the cumulative function p(k) of the probability of having at least one plant within intervals of various lengths ($[x-k,x+k]$) centred on x, a random site of the lattice, where k is a number of lattice units was estimated such that :

$p(k) = 1/L(k)$ # {x such that a plant is in [x-k,x+k] which is on the lattice}

where $L(k)$ = number of sites such that the interval [x-k,x+k] is in the lattice.

With the random model,

$$p(k) = po(k) = 1-(1-\rho)^{2k+1} \text{ for } k > 0$$

$p(k) < po(k)$ when the set of plants is clustered and $p(k) > po(k)$ for a regular pattern.

(2) when the observed pattern was nonrandom, the probability of a plant being found at a site in the vineyard, taking the surrounding context into consideration, was calculated, using a Gibbs model, and the model parameters were estimated in order to quantify the interactions between plants. These parameters were : α which is related to the probability that a plant will appear assuming that no other plants are in the neighbourhood; ß/ which measures the interaction between a site and its corresponding neighbours located ± I apart. Various models corresponding to increasing interaction configurations were fitted to observed data. The model used was the simplest as the subsequent more complex relationships did not differ significantly.

(3) the model was validated by calculating p(k) using estimated parameter values and Monte Carlo simulations.

Results

Results are reported only for two zones located near the edge (first zone) or in the middle (second zone) of the vineyard because of similar behaviour for the two other ones. The random pattern was clearly rejected : for both zones and years, the estimated probability of the appearance of a plant in a row was less than the Monte Carlo intervals of a random model which corresponded to an aggregated spatial pattern (Fig. 1). In the first zone, spatial pattern intensity increased significantly from 1986 to 1988, which resulted in a significant increase of α (Table 1). In 1986, the pattern was highly aggregated (Fig 1a) with an average clump size of three plants because of a strong influence of the two immediate neighbours on a plant appearance at a site (Table 1); in 1988, the pattern was less aggregated (Fig. 1b) and characterised by a weaker influence from the four immediate neighbours i.e. an average clump size of five plants (Table 1). In the second zone, the spatial pattern did not differ between the two years inducing no variation of α. For both years, the pattern was aggregated (Fig. 1cd) and characterized by a weak influence from the six immediate neighbours in the row (Table 1).

TABLE 1. Gibbs model simulations of interactions between *R. peregrina* plants. Only significant values of ß are shown (see text for precisions).* = number of significantly interacting neighbours on the row.

zone	year	number*	α	ß1	ß2	ß3
1	1986	2	-3.10	2.10		
1	1988	4	-1.90	0.78	0.58	
2	1986	6	-2.00	0.42	0.69	0.53
2	1988	6	-1.89	0.54	0.24	0.73

Simulated and observed estimations of plant appearance were in close agreement for both years.

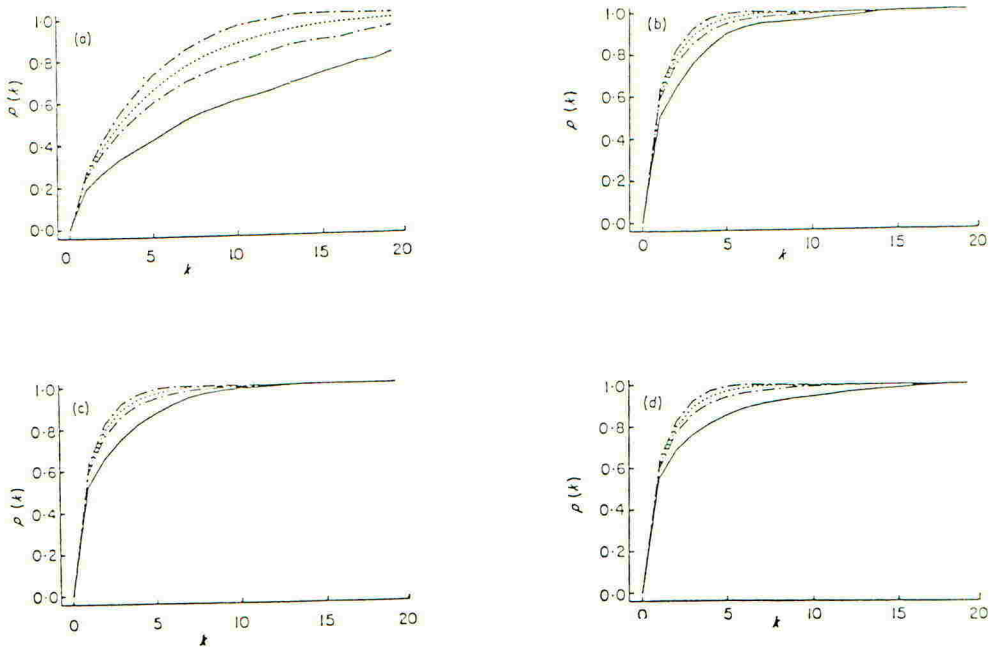

FIGURE 1. Probability p(k) of a plant appearance within a 2k interval, centred on a random point of a row. The dotted line represents a pattern produced by a Poisson process, dashed lines represent 0.05 significance levels around the line representing a Poisson process; the bold line represents p(k) for observed data. (a) first zone in 1986; (b) first zone in 1988; (c) second zone in 1986; (d) second zone in 1988.

Estimation of the genetic diversity

The genetic diversity of the population was estimated by the detection of isozyme phenotypes, using different scales of sampling. Collection of samples, methods and analysis are more precisely described in Navas (1989) and Navas & Gasquez (1991).

Methods

Samples, each including a rhizome fragment and an aerial shoot, were collected in April 1987 (coarse and row samplings) and February 1988 (plot sampling). These were grown in a greenhouse prior to electrophoresis. In part of the vineyard which included ten adjacent rows where *R. peregrina* was abundant, samples were collected at 50 cm intervals along 15 m lines along the rows (coarse sampling). Along a row, samples were collected in every clump, and at 40 cm intervals within clumps longer than 40 cm (row sampling). Within four 0.5 x 0.6 m^2 plots 10 to 50 m apart, all ramets present were mapped and excavated preserving rhizome connections. Samples of one ramet per excavated clone were analysed by electrophoresis.

Foliar isoenzymes of plants regenerated from collected samples were separated by electrophoresis on polyacrylamide gel, using the method described by

Gasquez & Compoint (1976). Three enzyme systems showed clear activity and variability : the alpha and beta esterases and the leucine amino peptidase. Each regenerated plant was analysed at least twice in order to reduce the probability of raw band variation due to the effect of environmental conditions. The amount of phenotypic polymorphism was estimated using P index described by Kahler *et al.* (1980).

Results

More than 70% of isozyme phenotypes detected using the coarse and row samplings were unique i.e. they were detected in only one population (Table 2). This resulted in high values of phenotypic polymorphism.

Line and plot samplings gave apparently contradictory results. In the row sampling, five diffuse clumps were longer than 0.40 m and consequently were sampled two or three times : within the five apparently homogeneous clumps, all detected phenotypes were distinct. In contrast, in each of the four small plots, few phenotypes were detected (1 to 4), they were composed of a high number of closely packed ramets (10 to 39 ramets per phenotype) and characterized by distinct clonal territories (Fig. 2).

TABLE 2. Estimation of the genetic diversity of *R. peregrina* using different samplings

Sampling	Number of samples	Number of phenotypes	% of unique phenotypes	P index of Kahler *et al.*
coarse	40	31	77	77
row	39	28	72	63
plot	127	8	0	*

* : no value because no electrophoresis was performed when only one clone was excavated in a plot.

Discussion

These data show that the spatial distribution of *R. peregrina* may be explained on the basis of seed dispersal pattern since only local extension is due to clonal spread.

1. Foundation of the population

Previous results have shown that the vineyard population of *R. peregrina* was established by seeds originating from the surrounding unmanaged habitat and that there was no relationship between the seed rain and the distance to the edge (Navas, 1989). Thus, inter-field seed dispersal was characterized by a scattered pattern (*sensu* Howe, 1989) and was not responsible for clumped distribution of adult plants.

2. Establishment or juvenile phase

R. peregrina seeds are dispersed by small birds (e.g. *Erithacus rubecula*), which deposit only one or two seeds at once and which usually move on the ground under vine cover (Debussche *et al.*, 1982). It can be suggested that, as soon as founder plants produce fruits, within-field seed dispersal may occur and induce a clumped distribution of *R. peregrina* along the rows. This stage corresponds to what was observed in the first zone in 1986. Such a dispersal pattern induces a directional

dispersal of siblings belonging to a same family, which may lead to a decrease of global genotypic diversity.

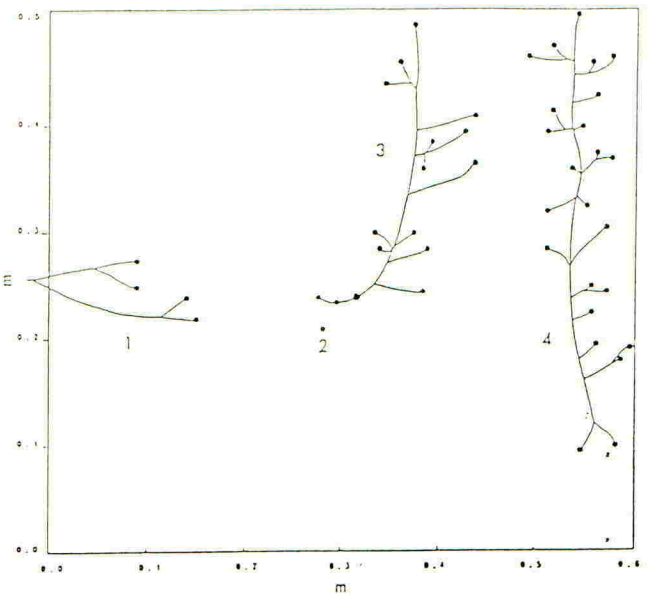

FIGURE 2. Spatial distribution of clones on a plot. • represent ramets and connections between ramets are drawn. Figures correspond to distinct isozyme phenotypes.

3. Expansion phase

New clumps are created by inter-field dispersal which induces an increase in the genotypic diversity whereas "old" clumps are spread both by clonal growth over a short distance (less or equal to 0.40 m) and within-field dispersal. This assumption is based on results on isozyme phenotype diversity of the row sampling. This stage corresponds to what was observed in the first zone in 1988, which was characterized by an increase in both number and size of clumps.

4. Maturity stage

When resource depletion zones (*sensu* Harper, 1985) of neighbouring clumps overlap or when founder plants of clumps become senescent, the maximal clump size has been reached. At this stage, the population is at its saturation size. This stage corresponds to what was observed in the second zone for both years.

5. Senescent stage

No direct observation has been done on the behaviour of *R. peregrina* populations after the maturity stage but senescent stages have been very frequently described for clonal perennial plants (e.g. Thompson *et al.*, 1991). During this stage, both the number and size of clumps diminish appreciably with a strong decrease in growth and clonal spread. These observations are generally explained by an increase in fixed somatic variation with aging.

This scenario suggests a general explanation of the spatio-temporal distribution of phenotypes through the analysis of clumping dynamics. Integrated

analysis is thus useful to characterize the spatial localization of foci of colonization and to detect the spatial fluxes of phenotypes. However, the susceptibility of weeds to control strategies and their level of damage on crop plants also depend on the variability of individual performance due to either physiological, developmental or morphological differences which are under genetic control (Mortimer, 1984). Furthermore, for clonal perennials, differences may be significant between rhizome derived and seed derived plants. For *R. peregrina*, under greenhouse conditions, differences in response to shortage of either water, light or nutrients were significant between seed and rhizome plants : the former were more performant but less plastic than the latter. These differences in performance and plasticity may alter the general functioning of populations as these two kinds of plants are more often separated in time with a primary establishment due to seeds, the colonization and maintenance in the fields being explained by vegetative spread (Kigel & Koller, 1985). For *R. peregrina*, clonal integration and high performance of seed plants allow rapid establishment in newly colonized habitats whereas high plasticity of rhizome plants represent advantages for extensive foraging in more unstable habitats. Such co-occurrence of genotypic variation and phenotypic plasticity as means of adaptation to variable environments, may be of importance if husbandry practices are changed.

CONCLUSION

This example demonstrates the influence of population variability, and especially of spatial distribution, on population dynamics. It is clear that in an overall model of population dynamics, processes generating variability must be classified. I suggest the following classification :

(1) spatial distribution of seeds in the soil bank, of seedlings and adult plants.
(2) inheritance of major adaptive traits; fitness of plants having or not having these traits.
(3) quantification of performance and competitive ability of sexual and asexual offsprings.

These data can be taken into account through submodels of general population dynamics models including quantification of individual numbers par life stage and rates of flux between stages.

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RESPONSE TO SUBSTITUTED UREAS, TRIAZINES AND CHLOROACETANILIDES IN A BIOTYPE OF *ALOPECURUS MYOSUROIDES* RESISTANT TO CHLOROTOLURON.

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ABSTRACT

A chlorotoluron-resistant (R) biotype of *A. myosuroides* has been found in wheat fields in the province of Lérida (Northeast of Spain). Both leaf fluorescence and Hill reaction assays indicated that the tolerance to chlorotoluron of the R biotype was due to metabolic detoxification rather than to herbicide insensitivity of the chloroplast target site. The growth response and ability to detoxify the herbicide of both the R biotype and a susceptible (S) "wild-type" biotype following treatment with 13 other substituted urea, 12 triazine and 3 chloroacetanilide herbicides were compared. There was some evidence of increased resistance and greater detoxification for all 13 ureas (benzthiazuron, buturon, chlorobromuron, chloroxuron, diuron, isoproturon, linuron methabenzthiazuron, metobromuron, monolinuron, monuron, metoxuron and neburon) in the R biotype but there were no differences between the R and S biotypes response to the triazines and chloroacetanilides. Resistance was more marked to some ureas than to others.

INTRODUCTION

Alopecurus myosuroides (black-grass) populations resistant to chlorotoluron have previously been described in Germany (Niemann & Pestemer, 1984) and England (Moss & Cussans, 1985). Resistance mechanism in these cases has been explained by a rapid detoxification process of the herbicide (Jones & Caseley, 1989). Recently a *A. myosuroides* biotype resistant to chlorotoluron has been found in winter wheat fields located in Lérida (Northeast of Spain) that had been treated continuously with this herbicide for at least the last ten years. The aim of the work described was to characterize this biotype, by studying the following aspects:

1) Comparison of growth capacity of resistant and susceptible plants treated with different doses of chlorotoluron.
2) Resistance mechanism.
3) Variability in response to chlorotoluron between individuals of resistant and susceptible biotypes.
4) Cross-resistance to urea, triazine and chloroacetanilide herbicides.

MATERIAL AND METHODS

Plant material

Seeds of the resistant (R) biotype of *A. myosuroides* were collected from winter wheat fields near Lérida (Northeast of Spain) where chlorotoluron had been repeatedly used for at least the last ten years. Seeds of the susceptible (S) biotype were collected from adjacent marginal areas that had never been treated with herbicides. Seeds were germinated on moist filter paper in Petri

dishes which were incubated under continuous ilumination (50 W m^{-2}) in a growth chamber (temp. 25ºC, relative humidity 95%) for 7 days.

Phytotoxicity studies

Pregerminated seeds were planted in 6 cm diameter, 6 cm high perforated plastic pots (3 seeds per pot) containing 150 g of soil treated with a range of doses of chlorotoluron (0.05-4.0 kg/ha a.i.), as described in De Prado *et al.* (1990a). There were three replicates. After 15 days the plants were harvested and fresh weight of the shoots was determined.

Chlorophyll fluorescence

Detached leaves from resistant and susceptible 4-week old plants were incubated in 20 mg/l chlorotoluron or in water. Their fluorescence intensity was measured at intervals for 6 hours thereafter, by using a Hansatech Modulated Fluorescence Measurement System as described in De Prado *et al.* (1990b). Results are given of the ratio of fluorescence at each sampling time (0.5-6 h) (F_T) compared to the fluorescence at the beginning of incubation (F_0). At the end of the 6 h period the samples were removed from the chlorotoluron and placed in water. Their fluorescence was measured 8, 16 and 24 hours after transfer. The ratios of the fluorescence at each of the three sampling times (F'_T), to that at the begining of the incubation with the herbicide (F_0) were calculated.

Hill reaction

Broken chloroplasts were isolated from 4-week old plants of the two biotypes, as described by Ducruet & De Prado (1982). The Hill activity (ferricyanide as acceptor) was measured as O_2 exchange with a Clark-type oxygen electrode (De Prado *et al.*, 1989). The concentration of chlorotoluron required to obtain a 50% reduction in the Hill reaction activity (I_{50}) was calculated from linear plots of inhibition percentages versus logarithm of concentrations.

Population studies

The photosynthetic responses of 60 plants of both biotypes to chlorotoluron were studied. Fluorescence assays were performed on leaf samples as described earlier. Leaf samples were incubated in herbicide for 6 h and then transferred to water for 24 h. Their herbicide detoxifying capacity was calculated by comparing the fluorescence values after 24 h detoxification in water with those at the beginning of incubation in herbicide (F_{24}/F_0 ratio).

Cross resistance

The responses of chlorotoluron resistant and susceptible biotypes to 13 other substituted ureas, 12 triazines/triazinones and three chloroacetanilides (Table 3) was investigated as described earlier for chlorotoluron phytotoxicity studies. Briefly, pregerminated seeds were planted in pots containing soil treated with a range of herbicide doses and after 15 days the number of surviving plants was recorded. As the tested range of herbicide doses resulted in many cases not complete enough for calculating ED_{50} values, the highest dosis at which all plants survived was taken as estimate of herbicide tolerance. At this dosis, however, an ample reduction in growth and phytotoxicity symptoms were generally observed. Secondly, leaf samples were taken and the detoxification capacities of the two biotypes exposed to the urea and triazine herbicides were calculated, as described earlier.

RESULTS

The doses of chorotoluron required to reduce growth of the R and S plants by 50% were calculated from dose response curves of the fresh weight data. The ED_{50} values for the R and S biotypes were 2.80 and 0.12 kg/ha a.i., respectively.

The fluorescence emissions of leaf samples from the resistant and susceptible biotypes for the 6 hours of exposure were similar (Fig 1). In both cases incubation in water did not affect the level of fluorescence emission at any sampling time whereas incubation in herbicide produced a marked increase in fluorescence emission which reached a maximum value after herbicide treatment for 4 h. Thus a similar susceptibility to chlorotoluron of the photosynthetic electron transport in both biotypes can be concluded. In agreement with this conclusion, inhibition of the Hill reaction by chlorotoluron in isolated chloroplasts was similar for both the resistant and susceptible biotypes (Table 1).

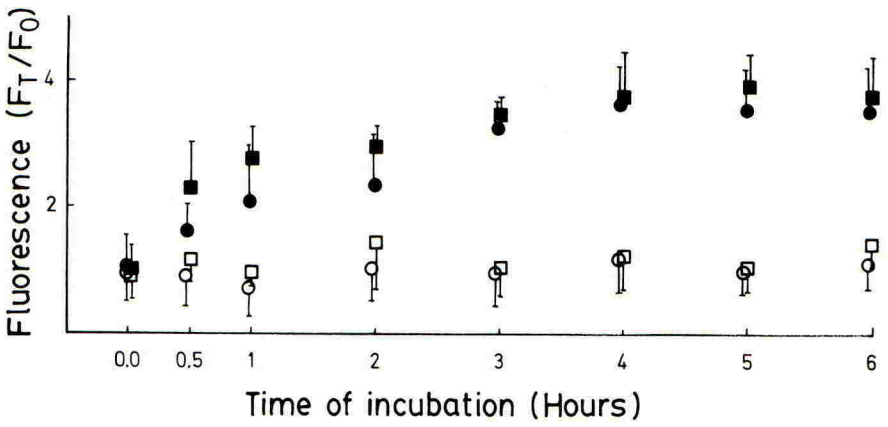

FIGURE 1. Chlorophyll fluorescence of leaf samples of resistant and susceptible biotypes incubated for 6h in chlorotoluron or water: (○,●) resistant biotype incubated in water or chlorotoluron, (□,■) susceptible biotype incubated in water or chlorotoluron. Vertical bars = S.E. of mean.

TABLE 1. Concentration of chlorotoluron required to obtain 50% inhibition of the Hill reaction in isolated chloroplasts from leaves of resistant and susceptible biotypes.

A. myosuroides	I_{50} (µM)		I_{50} R/I_{50} S
Resistant	0.23	0.03	1.21
Susceptible	0.19	0.02	

To explore if enhanced detoxification was the mechanism underlying to the tolerance response showed by the resistant biotype, leaf samples of the two biotypes were transferred from herbicide to water and their fluorescence was measured at intervals for 24 hour thereafter. The photosynthesis, as measured by fluorescence, remained blocked in the susceptible biotype over the 24 h test period. However, the photosynthesis of the resistant biotype completely recovered over the 24 h test period (Fig 2).

In population studies, all the 60 plants of the susceptible biotype responded similarly to the herbicide. In contrast, there were marked

differences in the responses of the plants of the resistant biotype. Three groups of plants were identified, referred to as R1, R2 and R3, with decreasing detoxification capacity (Table 2). However, most plants had a high potential to detoxify the herbicide.

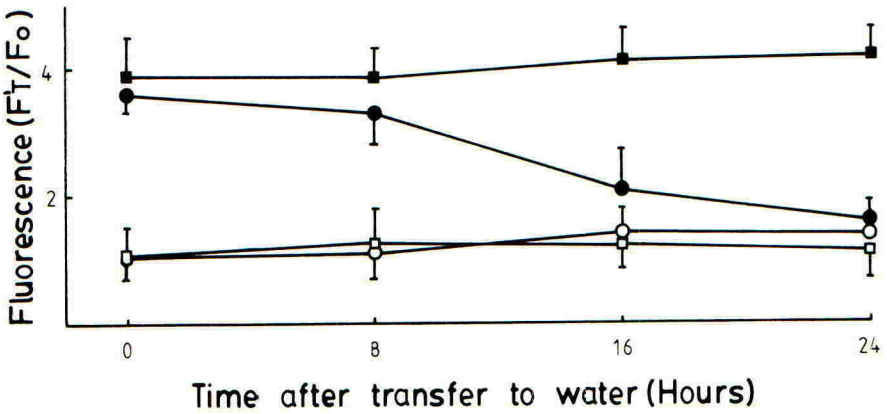

FIGURE 2. Chlorophyll fluorescence of leaf samples of resistant and susceptible biotypes following incubation with chlorotoluron or water for 6 h and transferred to water for 24 h: (o,●) resistant biotype incubated in water or chlorotoluron, (□,■) susceptible biotype incubated in water or chlorotoluron.
Vertical bars = S.E. of mean.

TABLE 2. Response to chlorotoluron in plants of resistant and susceptible populations of *A. myosuroides*

Population	detoxifying capacity F_{24}/F_0	Percentage
Resistant (60 plants)		
R1	1.0 to 1.5	79
R2	1.5 to 2.0	17.5
R3	2.0 to 4.0	3.5
Susceptible (60 plants)	3.0 to 5.0	100

The resistant biotype showed a higher tolerance to many of the urea herbicides tested both in the direct response to the herbicides and in its ability to detoxify them, as shown in the fluorescence tests (Table 3). The phytotoxicity test showed clear resistance to most ureas, particularly buturon, isoproturon, linuron, monuron and metoxuron. Least differences were found with chloroxuron and methabenzthiazuron. The fluorescence test did not always agree precisely with the whole plant differences, but in general the detoxification capacity was less with the susceptible biotype. Chlorobromuron, diuron, linuron, metobromuron and monolinuron showed the greatest ability to detoxify, differing from the susceptible biotype by at least a factor of two. Although there was some variation in the response of the plants to the triazine and chloroacetanilide herbicides the two biotypes responded similarly

(data not shown). There were also no marked differences in the ability of the two biotypes to detoxify the triazines (not shown).

TABLE 3. Herbicide cross-resistance in *A. myosuroides* resistant to chlorotoluron.

Herbicide	Highest surviving rate (kg/ha a.i.)		Detoxifying capacity (F_{24}/F_0)	
	R	S	R	S
Ureas				
Benzthiazuron	2.0	0.25	1.9	2.5
Buturon	1.0	0.05	2.3	3.0
Chlorobromuron	4.0	0.50	1.5	4.7
Chloroxuron	0.5	0.25	3.2	2.9
Diuron	3.0	0.25	1.7	4.1
Isopropuron	0.5	0.01	2.5	3.7
Linuron	7.0	0.25	1.6	4.8
Methabenzthiazuron	1.0	0.25	2.7	3.4
Metobromuron	3.0	0.50	1.3	2.7
Monolinuron	3.0	0.25	1.6	3.9
Monuron	0.5	0.01	2.7	3.9
Metoxuron	0.5	0.01	2.3	3.4
Neburon	5.0	0.50	1.8	3.3
Triazines				
No resistance was found for the following products: Ametryn, Atrazine, Cyanazine, Metribuzin, Prometon, Propazine, Secbumeton, Simazine, Terbumeton, Terbutryn, Terbuthylazine.				
Chloroacetanilides				
No resistance was found for the following products: Acetochlor, Alachlor, Propachlor.				

DISCUSSION

Our results indicate that resistance to chlorotoluron has been originated in the biotype of *A. myosuroides* from Lérida (Spain) as a result of herbicide usage in winter wheat. The resistance response is clearly due to herbicide detoxification, as revealed by the similar inhibitory effect exerted by chlorotoluron on photosynthetic activity of resistant and susceptible plants and the complete recovery of photosynthesis after chlorotoluron exposure and posterior transfer to water in resistant but not in susceptible plants. There was some variation in chlorotoluron response within individuals of a population of resistant plants, although most plants had a high capacity to detoxify the herbicide. The resistant biotype showed clear resistance to most of 13 other urea herbicides tested being this fact probably due to its enhanced ability to detoxify them. In wheat, chlorotoluron is metabolized by ring-methyl hydroxylation and N-demethylation, reactions that are probably catalysed by cytochrome P-450 monooxygenases (Mougin *et al.*, 1990). As such monooxygenases show a broad substrate specificity, it seems feasible that an acquired resistance mechanism to chlorotoluron based on enhanced levels of these enzymes could also confer cross-resistance to some ureas and, inclusively, other herbicide classes. Our resistant biotype did not show any increase in tolerance to several triazine and chloroacetanilide herbicides, but a chlorotoluron resistant bitype of *A. myosuroides* previously found in England showed cross-resistance to several phenylureas as well as to other herbicides belonging to different classes including triazines and anilides

(Moss & Cussans 1991). This seems denote that different patterns of cross-resistance can be developed by different populations of *A. myosuroides* resistant biotypes. A similar situation has been found in different Australian populations of *Lolium rigidum* resistant to diclofop-methyl (Heap, 1991).

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ANNUAL RYEGRASS: AN ABUNDANCE OF RESISTANCE, A PLETHORA OF MECHANISMS.

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ABSTRACT

Herbicide resistance in annual ryegrass (*Lolium rigidum*) has evolved many times in Australia. The mechanism of resistance to a particular herbicide is not necessarily the same in all resistant populations, at least two mechanisms of resistance to diclofop-methyl and chlorsulfuron have been detected. Individuals and populations that contain more than one resistance mechanism have also been detected. In ryegrass, the variety of resistance and the propensity for resistance to develop indicates that mixing or rotating herbicides with different modes of action will not avoid the long-term onset of resistance.

INTRODUCTION

It is a simplistic, but commonly held, view that resistance in any herbicide resistant weed biotype is invariably the result of a single biochemical or physiological mechanism. In reality, herbicides select for every available mechanism, whether specific or non-specific, that endows tolerance to the rate of herbicide used in the field. It is stressed that although a resistant biotype may tolerate very high levels of a herbicide the selection pressure *per se* is not for high levels of tolerance but for mechanisms that allow survival at the rates recommended for agricultural use. The mechanisms of resistance that have been described in the 100 species that have developed resistance since the late 1960s (Holt & LeBaron, 1990) include target site insensitivity, target site overproduction, herbicide detoxification, reduced herbicide entry, reduced herbicide translocation and changes in the intracellular compartmentation of herbicides (LeBaron & Gressel, 1982).

The frequency of different mechanisms in a population may vary during a selection period that spans a number of years. Factors that could influence the relative frequency of different mechanisms within the population include the original frequency of the traits in the unsprayed population and the relative fitness of the resistant plants during the different phases of the agricultural system. Often, growers will increase herbicide rates or change herbicides as weeds become more difficult to control. Presumably such changes in selection pressure would discriminate between different resistance traits.

In contrast to observations of herbicide resistant plants, many examples are known of insects that exhibit more than one mechanism of resistance (Vilani & Hemingway, 1987). There is also evidence from insect studies that different mechanisms of resistance may predominate in a population at different stages during the selection process (Hemingway et al., 1991). Some of these mechanisms may predispose the population to be resistant to other insecticides. Is the possession of multiple-mechanisms of resistance or the phenomenon of successions in the predominance of mechanisms less prevalent in plants or have these processes been overlooked? Overlooking such phenomena would not be difficult as firstly, mechanistic studies of naturally selected populations tend to compare susceptible with resistant individuals and, secondly, most researchers stop looking for resistance mechanisms once a mechanism has been found.

Annual ryegrass (*Lolium rigidum*) is a diploid (2n=28), obligate out-crossing, annual grass that is a weed of crops grown during the cool, winter wet season in the Mediterranean climate regions of southern Australia. Observations with ryegrass will be used to demonstrate firstly, the

plethora of resistance mechanisms that can be selected in a single species, secondly, that independently selected biotypes of one species can exhibit different mechanisms of resistance to a herbicide, and thirdly, that more than one mechanism of resistance to a herbicide can occur within a biotype. We propose that not only can the degree of resistance change during the selection process but the predominant mechanism of resistance may also vary. In effect, the nature and degree of the resistance responses are dynamic.

FREQUENCY AND TYPE OF RESISTANCE IN *L. RIGIDUM*

Annual ryegrass exhibits an abundance of resistance. Resistance has been observed to aryloxyphenoxypropionates, cyclohexanediones, sulfonylureas, imidazolinones, triazines, triazoles, triazinones, phenylureas and to trifluralin (Powles & Howat, 1990). Enhanced tolerance to 2,4-D has also been observed (P. Boutsalis, unpublished). Both multiple-herbicide resistance and cross-resistance have been documented (Heap & Knight, 1986; 1990; Powles & Howat, 1990). The phenomenon of herbicide resistance in ryegrass is now so widespread in southern Australia that occurences of resistance are rarely considered worth reporting to researchers and extension agencies no longer bother to survey for resistance. Recent estimates suggest that over 600 farms contain at least one paddock infested with resistant ryegrass. In the majority of cases resistance does not appear to have been imported, rather it has evolved *in situ*.

The mechanisms responsible for resistance in the small number of ryegrass biotypes so far examined are varied. Resistance to the aryloxyphenoxypropionates and cyclohexanediones can be due to a mechanism possibly associated with differences in membrane characteristics and the capacity to sequester herbicide (Häusler et al. 1991; Holtum et al., 1991) or to the presence of insensitive acetyl Co-A carboxylase. Resistance to sulfonylureas can be associated with increased rates of detoxification (Christopher et al., 1991a; Matthews et al., 1990) and/or with herbicide-insensitive acetolactate synthase (Christopher et al., 1991b). Resistance to substituted ureas and triazine-like compounds is not due to an insensitive photosystem II target site but is due to enhanced metabolism and detoxification probably catalysed by cytochrome P_{450} linked mono-oxygenases (Burnet et al, 1991). The mechanisms responsible for resistance to trifluralin and enhanced tolerance to 2,4-D are unknown. Clearly, the ryegrass is a good generalist in that the genome possesses many potential mechanisms of herbicide resistance.

The reasons behind the propensity of ryegrass to develop resistance have been speculated upon by Powles and Matthews (1991) who suggested the involvement of a combination of factors that include the varied diploid genome, the out-crossing breeding system, rapid generation time, the exposure of large populations to selection pressure, the variable nature of the agricultural system which is infested and the natures of the compounds to which ryegrass is exposed. At present, ryegrass biotypes are classified on the basis of their site of origin and on their resistance spectra. Classification of biotypes based upon their isozyme complements has been unsuccessful due to the variety of patterns observed in individuals within each biotype; this variety is presumably a result of the out-crossing reproduction system. Isozymes systems tested to date include aconitase, aspartate aminotransferase, fumarase, glucose phosphate isomerase, glutathione reductase, isocitrate dehydrogenase, malate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphoglucomutase.

DIFFERENT MECHANISMS OF RESISTANCE IN DIFFERENT BIOTYPES

As most outbreaks of resistance in ryegrass have evolved independently (i.e. resistance has evolved repeatedly) and as resistance can be endowed by more than one mechanism, it is possible that different mechanisms of resistance to the same herbicide may be present in different biotypes. Such differences have been documented. For example, biotypes SLR 31 and WLR 1 are resistant to chlorsulfuron, an inhibitor of acetolactate synthase. SLR 31, like wheat, contains a chlorsulfuron-sensitive acetolactate synthase whereas acetolactate synthase from WLR 1 is

chlorsulfuron-insensitive (Table 1). It is tempting to conclude that, as WLR 1 is much more resistant to chlorsulfuron than is SLR 31, the possession of a herbicide-intolerant target site endows stronger resistance. However, wheat, which possesses a herbicide-sensitive acetolactate synthase, is at least as resistant to chlorsulfuron as is WLR 1. The rates of uptake of ^{14}C-chlorsulfuron into the two resistant biotypes and into a susceptible biotype are similar.

The selection pressures imposed in the field upon SLR 31 and WLR 1 were not the same. SLR 31 is classified as a chlorsulfuron cross-resistant biotype as it had been exposed to diclofop-methyl for 4 years but had never been exposed to an acetolactate synthase-inhibiting herbicide (Heap & Knight, 1990). On the other hand, WLR 1 is defined as being resistant to chlorsulfuron as it had been exposed for 7 consecutive years to chlorsulfuron but had never been exposed to diclofop-methyl (Christopher et al. 1991b). SLR 31 is resistant to diclofop-methyl but WLR 1 is not. Other species within which different biotypes exhibit different mechanisms of resistance include triazine-resistance in *Abutilon theophrasti* (Gronwald et al., 1989) and sethoxydim-resistance in *Zea mays* (Parker et al., 1990a, 1990b). In the latter species the biotypes have been generated under laboratory conditions.

In SLR 31 inheritance of resistance to diclofop and chlorsulfuron both appear to be under nuclear control and are not controlled by single fully dominant or recessive genes. Resistance to both herbicides appears to be quantitatively inherited. The inheritance characteristics of WLR 1 are not yet known.

TABLE 1. Inhibition by chlorsulfuron of acetolactate synthase (ALS) from a susceptible (VLR 1) and 2 resistant (SLR 31, WLR 1) annual ryegrass biotypes, and from wheat cv. Millewa (Christopher, J.T., unpublished)

ryegrass biotype	chlorsulfuron rate* required for 50 % mortality	chlorsulfuron. concn. required for 50 % inhibition of ALS
	LD_{50} (g h^{-1})	I_{50} [nM]
VLR 1	4	12
SLR 31	64	12
WLR 1	> 256	200
WHEAT	> 256	12

* agricultural rate is 8-16 g chlorsulfuron per hectare

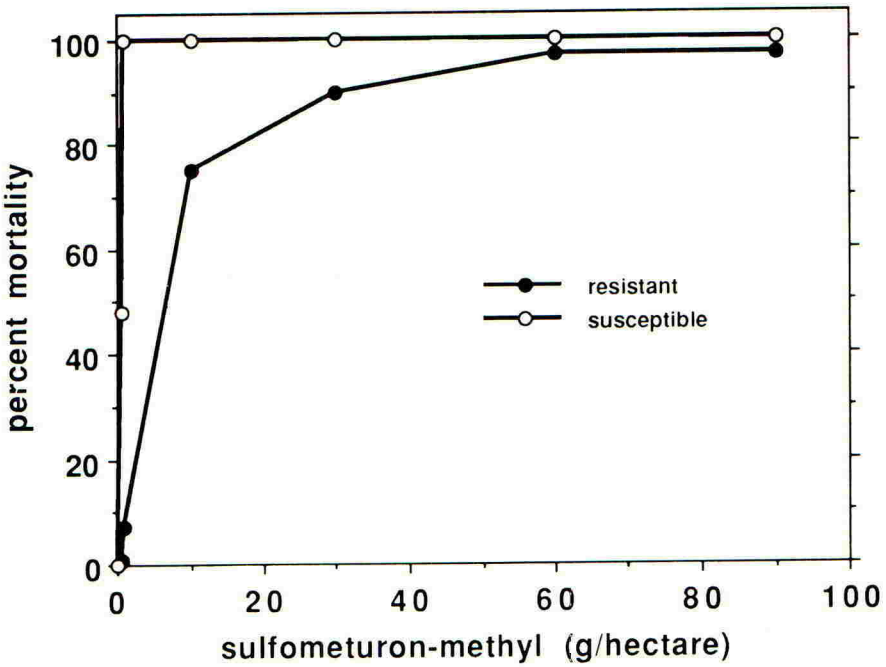
It is surprising that no reports of resistance due to the detoxification of diclofop have been reported in ryegrass. Diclofop is a selective graminicide. It is selective because wheat and, to a lesser extent, barley can degrade the compound using mixed-function oxidases. Susceptible ryegrass also has some capacity to degrade the compound via similar means (Holtum et al., 1991). Some biotypes of ryegrass which have developed resistance to chlorsulfuron following selection with diclofop exhibit enhanced rates of chlorsulfuron degradation. The products of catabolism are similar to those formed in wheat as a result of mixed-function oxidase activity (Christopher et al., 1991a). One might have expected that if an enhanced capacity of these enzymes were selected by diclofop then the enzymes might have some capacity for degrading diclofop. This does not seem to be the case. In wheat, which tolerates both diclofop-methyl and chlorsulfuron, different mixed-function oxidases appear to be involved in the catabolism of these two herbicides (McFadden et al., 1989; Zimmerlin & Durst, 1990).

MULTIPLE MECHANISMS OF RESISTANCE WITHIN A BIOTYPE

Two examples of multiple mechanisms of resistance within a single biotype have been documented in annual ryegrass. We have begun only recently to survey for this phenomenon and undoubtedly will detect other instances.

The first biotype was a multiple-herbicide resistant biotype, VLR 69, that had been exposed to, and had developed resistance to, simazine, diuron, diclofop and chlorsulfuron (Burnet M., unpublished). Mild resistance to cyclohexanediones was also present. The biotype exhibited an asymptotic dose-response curve to the non-selective sulfonylurea herbicide sulfometuron-methyl (Figure 1). Such a curve indicates that the responses of all individuals to the herbicide are not uniform; some individuals in the population are highly resistant whereas most are less so.

FIGURE 1. Effect of sulfometuron-methyl, a non-selective sulfonylurea, on mortality of chlorsulfuron susceptible (O, VLR 1) and resistant annual ryegrass (J, VLR 69) (M. Burnet, unpublished)



Pot-grown plants exhibited 100 % survival in the presence of 200 g chlorsulfuron ha^{-1} but only 5 % survival in the presence of 90 g sulfometuron-methyl ha^{-1}. Acetolactate synthase from the sulfmeturon-susceptible individuals had an I_{50} to chlorsulfuron of about 35 nM. However, acetolactate synthase from the 5 % of plants that survived sulfometuron-methyl was relatively insensitive to chlorsulfuron and had an I_{50} of around 357 nM. Similar results were observed when ryegrass was germinated on agar containing 27 nM sulfometuron and the chlorsulfuron inhibition kinetics of acetolactate synthase from rescued susceptible seedlings was compared with those of acetolactate synthase from resistant seedlings. Clearly, although the population as a whole is resistant to chlorsulfuron but susceptible to sulfometuron-methyl subsections of the population exhibit different degrees of resistance and possess different mechanisms of resistance.

The second example of multiple mechanisms within a biotype can be found in a comparison between biotypes SLR 31 and WLR 96. Both biotypes are resistant to the aryloxyphenoxypropionate herbicides and in both biotypes diclofop-methyl has been the predominant, although not exclusive, selective agent. In comparison to other resistant biotypes SLR 31, VLR 6 and NLR 12, WLR 96 exhibits a far higher resistance to haloxyfop (TABLE 2; Häusler et al., 1991; Heap & Knight, 1990). In common with membranes from the other resistant biotypes, membranes from WLR 96 depolarise in the presence of diclofop acid and repolarise when the herbicide is removed (Häusler et al., 1991). Despite the similarities in membrane responses, the herbicide-inhibition kinetics of acetyl Co-A carboxylases from the biotypes SLR 31 and WLR 96 are very different. Acetyl Co-A carboxylase from SLR 31 is more than 150-times more sensitive to inhibition by diclofop acid and haloxyfop acid than is acetyl Co-A carboxylase from WLR 96 (Table 2).

TABLE 2. Responses of whole plants and acetyl Co-A carboxylase (ACCase) from susceptible (VLR 1) and resistant biotypes (SLR 31 and WLR 96) of annual ryegrass to diclofop and haloxyfop.

ryegrass biotype	diclofop-methyl rate required for 50 % mortality[a]	I_{50}[b] of ACCase to diclofop acid	haloxyfop-methyl rate required for 50 % mortality[a]	I_{50} of ACCase to haloxyfop acid
	LD_{50}[c] (g a.i. ha^{-1})	µM	LD_{50} (g a.i. ha^{-1})	µM
SLR 2	< 150	0.2	< 13	0.4
VLR 1	< 150	0.2	< 13	0.4
SLR 31	9,600	0.3	80	0.7
WLR 96	≈20,000	55	> 1,650	> 55
VLR 6	≈3,000	n.d.[d]	50	n.d.

[a] Recommended rates for diclofop and haloxyfop are 375 and 90 g per hectare, respectively.

[b] I_{50} = concentration required to inhibit acetyl Co-A carboxylase by 50 %.

[c] LD_{50}= amount of herbicide required to kill 50 % of plants 21-28 days after spraying. [d] n.d. = not determined

Although the mechanism, or mechanisms, of resistance in SLR 31 are not known it is clear that the membrane repolarisation response documented by Häusler et al. (1991) is not associated with a herbicide-insensitive acetyl Co-A carboxylase. WLR 96 exhibits a membrane recovery response and possesses a herbicide-insensitive acetyl Co-A carboxylase, therebye indicating that more than one mechanism of resistance is present. The relative contributions of the two mechanisms to the slightly different resistance spectra of the two biotypes is unknown as is the mechanism which allows membranes to repolarise following exposure to aryloxyphenoxypropionate or cyclohexanedione herbicides. An important take-home message of these observations is that in studies of resistance to the aryloxyphenoxypropionate and cyclohexanedione herbicides measurements of the inhibition kinetics of acetyl Co-A carboxylase alone are insufficient for the determination of the mechanism(s). Häusler et al. (1991) have shown that the ability of ryegrass roots to acidify a growth medium may give an indication as to whether ryegrass plants exhibit the membrane repolarisation response.

CONCLUSIONS

Herbicide-resistant ryegrasses exhibit a variety of different traits that endow resistance; the traits include target site insensitivity, enhanced metabolism and variations in membrane properties of unknown function. For the aryloxyphenoxypropionate, cyclohexanedione, sulfonylurea and imidazolinone herbicides studies in our laboratory have shown that more than one trait can be responsible for resistance. The relative importance of different resistance-endowing traits is not the same in all populations. The predominant mechanism in any population probably initially reflects the relative frequencies of potentially useful traits in the original susceptible population. Survival, and thus expression of traits, will be determined by the numbers of plants sprayed, the rate and nature of herbicides used, the heritibility characteristics of the traits and the nature of the agricultural system. In general, agricultural rates of herbicide application are such that any trait that will give protection against two to three times the recommended rate will result in a field infested with 'resistant' weeds. Although individuals in the population may possess resistance mechanisms that give either very high levels of resistance or levels of resistance barely sufficient to allow escapes from the recommended rate, all will be selected. Individuals with either type of mechanism will be resistant. In an outcrossing weed species, such as ryegrass, these traits will be transferred between the progeny of the survivors. We are currently comparing the resistance complements of ryegrass with a self-pollinating species, wild oats.

An important implication of the selection of a variety of resistance traits in a population is that some traits will give resistance to only a single herbicide whereas others may be less specific and may give resistance to a number of herbicides. In all likelihood, more traits will be selected when large populations are sprayed. The exposure of large weed populations to herbicides will concentrate the traits in the surviving weed population. The increased concentration of traits, coupled with the variety of mechanisms they represent, could reduce the time required for a population to respond to further herbicide selection. Resistance avoidance strategies based upon the rotation or mixing of herbicides with different modes of action consequently will have shorter useful lives. It is a general observation with ryegrass that once resistance to one aryloxyphenoxypropionate is present the biotypes appear to be 'sensitised' in that it rarely takes more than two consecutive years of application of cyclohexanediones or sulfonylureas before resistance develops to these compounds.

The experience with annual ryegrass suggests that the herbicide resistance spectrum of weeds from a natural population reflects only the resistance that is present at the time of collection. The extent, spectrum and quite possibly mechanisms of resistance that were present in preceding generations or that might be present in subsequent generations, particularly after further exposure to either the same or other herbicides, will not necessarily be the same.

It is often suggested that the onset of herbicide resistance can be avoided if herbicides with dissimilar modes of action are used either in rotation or in mixtures. The variety of non-target site resistance mechanisms present in ryegrass indicates that such strategies will not provide long-term protection. More complex Integrated Weed Management strategies such as those described by Powles and Matthews (1992) will be needed. In general, our experience in the Australian cropping regions is that such strategies require a reduction in the dependence upon selective herbicides and a move away from continuous cropping systems.

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PROPANIL RESISTANCE IN *ECHINOCHLOA COLONA* POPULATIONS WITH DIFFERENT HERBICIDE USE HISTORIES

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ABSTRACT

Differences in susceptibility to propanil were found in screenhouse tests with *Echinochloa colona* populations from Costa Rican rice fields with different herbicide use histories. Comparison of LC_{50} values in populations not regularly exposed to pre-emergence pendimethalin applications showed that the level of resistance was associated with increasing intensity of propanil use. All of these populations showed some resistance when propanil had been used for more than five years. Twice yearly application over 12 or more years had resulted in an 8 fold decrease in susceptibility. Results from two populations where pre-emergence pendimethalin applications had been rotated with propanil suggested that herbicide rotation might be used as part of a strategy for propanil resistance management.

INTRODUCTION

Potential weed losses in rice are estimated at between 30% and 73% (Rojas and De la Cruz, 1973). In Costa Rica the grasses are the most important weed group and, along with *Rottboellia cochichinensis* and *Ischaemum rugosum*, *Echinochloa colona* is one of the most aggressive species Ocampo, 1985). The principal control for grass weeds is propanil, which has been used for at least twenty years in Costa Rican rice. Currently it is estimated that about 250 mg of active ingredient is applied annually.

Recently, farmers began reporting poor control of *E. colona* with propanil. Higher dosages and more frequent applications were becoming necessary in some fields and resistance to the herbicide suspected. It was therefore decided to investigate if resistance to the herbicide was present, and whether it was related to any particular herbicide use history. Results are reported of the evaluation of the mortality response of seven collections of Costa Rican *E. colona* to propanil.

MATERIALS AND METHODS

Collections of *E. colona* seed were made from mature plants toward the end of the 1988 growing season in seven rice fields in the Central Pacific region of Costa Rica. This area has a long history of rice cultivation, which allowed seed to be collected from fields with herbicide histories ranging from 2 to 15 years of propanil use (Table 1). In recent years, most of these fields have also received applications of pre-emergence herbicides applied soon after the rice has emerged. However, two of the samples, Aguirre-A and Aguirre-B, came from fields to which pre-emergence applications of pendimethalin are routinely made. A single seed sample of *E. colona* not exposed to propanil (and therefore expected to be susceptible) was collected from a field recently under maize and beans in

San Antonia de Belen in the Central Uplands. The seed samples were air dried and stored at 5°C.

Seedlings were grown in the screenhouse in 14 cm diameter plastic pots filled with soil previously sterilized with methyl bromide. Eighteen pre-germinated seeds were planted and later thinned to sixteen plants/pot (one replicate). Pre-germination results in > 90% germination in all samples.

TABLE 1. Herbicide use histories of the *Echinochloa colona* populations evaluated for susceptibility to propanil.

E. colona population	No. years propanil use	No. rice harvests/ year	Detailed use history[1] Propanil	Pendim-ethalin	Other[2]
San Antonio	0	0	N	N	N
Parrita-2	2	1	1/2p	N	N
Garabito	5	2	2/3p	0p	0p
Parrita-12	12	2	2/3p	0p	0p
Aguirre-A	15	1	1/2p	RP/0p	0p
Aguirre-B	15	1	1/2p	RP/0P	0p
Parrita-15/1	15	1	1/2p	0p	0p
Parrita-15/2	15	2	2/3p	0p	0p

[1] Numerals show typical number of applications per harvest; N = no use reported; 0 = occasional use; R = regular use; P = Pre-emergence use p = early post-emergence use

[2] Other herbicides used were benthiocarb, oxadiazon, molinate and butachlor

Stam LV-10 (360 g propanil/l; Lot No. 80.916, provided by Rohm and Haas Co. de Costa Rica) was applied in water to seedlings at the three leaf stage, using an AZ $C0_2$ sprayer calibrated to deliver a spray volume equivalent to 200 l/ha. Five separate bioassays were carried out in which, depending on the *E. colona* populations included, between seven and nine concentrations plus a control of water alone, were applied to six replicates/treatment. The concentrations were between 0.75 and 70 mg a.i./ml and varied between bioassays to take account of the large difference in response encountered in the different populations. Seedling mortality was evaluated 14 days after application, a plant was considered to be dead if it possessed no visible green tissue. Overall control mortality was < 1%. Temperatures in the screenhouse during the tests ranged from 18.9°C to 28.6°C.

Data from the five bioassays were combined and percentage mortalities were analyzed by PROC PROBIT (SAS Institute, 1985), using the "C=" option for control mortality. Since this procedure automatically pools data for all replicates of a particular concentration, for data entry we slightly altered equal concentrations in different replicates so as to force the procedure to treat each replicate separately. This has the effect of

narrowing the confidence intervals but has almost no effect on LC_{50} or slope values (Tabashnik *et al.*, 1987). Two LC_{50}s were considered significantly different if their 95% fiducial limits did not overlap.

RESULTS AND DISCUSSION

Comparison of LC_{50}s suggests that propanil resistance is already present in most of the populations of *E. colona* studied. Six of the seven populations collected from rice fields had significantly higher LC_{50}s than the San Antonio population that was presumed to be susceptible (Table 2 and Figure 1). The least susceptible populations, Parrita-12 and Parrita-15/2, were more than eight times more resistant to propanil than the San Antonio population.

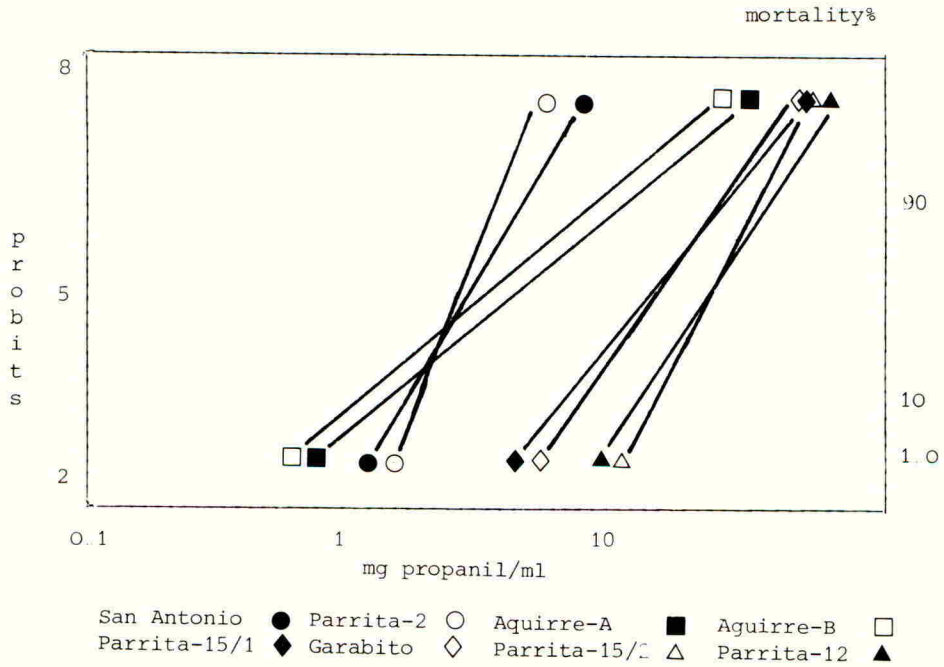

FIGURE 1. Mortality response of Costa Rican *Echinochloa colona* populations to propanil

In populations not regularly exposed to pre-emergence application of pendimethalin (all except Aguirre-A and Aguirre-B), the results suggest that resistance is associated with increasing intensity of propanil use. Parrita-12 and Parita-15/2, the least susceptible populations, were collected from fields planted to rice twice yearly for 12 or more years. Greater susceptibility was detected in the Garabito and Parrita-15/1 populations, which have, respectively, fewer years or less frequent use of propanil. Both populations were more than five times more resistant to propanil than the susceptible San Antonio population. Only the Parrita-2 population, where propanil has been used for only two years, showed no decrease in susceptibility.

Preliminary results from studies on some of these populations suggest that resistance to propanil is associated with elevated levels of aryl acylamidases (Gabriel Macaya & Roberto Guevara, unpublished data). These are the same enzymes involved in propanil metabolism in rice.

The two Aguirre populations, from fields that in addition to propanil in post-emergence have received pre-emergence pendimethalin applications, had LC_{50} values only slightly higher than that of the susceptible San Antonio population. However, they were more heterogeneous, higher concentrations of propanil being needed to kill the most tolerant plants. This suggests that part of these populations possesses increased resistance while the remainder is composed of susceptible genotypes. Pre-emergence pendimethalin use appears either to have delayed resistance development or to have reversed the process in these fields. No inference can be made about which of these possibilities is the case because the resistance status of the populations prior to the commencement of pendimethalin use is unknown.

TABLE 2. Mortality response of *Echinochloa colona* populations to propanil (Stam LV-10).

E. colona population	n	LC_{50} mg a.i./ml (95% FL)	Slope	RR[1]
San Antonio	768	3.35 (3.15- 3.55)	5.63	-
Parrita-2	672	3.19 (3.05- 3.40)	7.87	0.95
Garabito	1920	18.43[a] (17.33-19.48)	4.74	5.50
Parrita-12	1920	27.77[a] (26.02-29.47)	5.38	8.29
Aguirre-A	1344	5.24[a] (4.84- 5.64)	2.83	1.56
Aguirre-B	1344	4.34[a] (3.75- 5.64)	2.83	1.30
Parrita-15/1	1920	17.13[a] (16.23-17.98)	4.17	5.11
Parrita-15/2	1920	28.34[a] (27.07-29.62)	6.48	8.46

[1] RR = Resistance ratio, LC_{50}/LC_{50} San Antonio,
[a] LC_{50} significantly > LC_{50} San Antonio by non-overlap of 90% FL

Pre-emergence application in rice is a non-recommended use of pendimethalin in Costa Rica due to phytotoxicity problems. However, this usage in rotation with propanil in the Aguirre populations appears to have resulted in a less severe resistance problem than its recommended use as a mixture with propanil in post-emergence. This suggests that herbicide rotation might be used as a strategy for propanil resistance management, as has been recommended for other herbicides (Gressel, 1987). Future studies should investigate the effect on propanil resistance of rotations of propanil with other herbicides such as oxadiazon and butachlor, for which pre-emergence use is recommended.

CONCLUSIONS

Propanil resistance in Costa Rica *E. colona* was confirmed by this study and can be expected to become more prevalent the longer dependence on propanil as the major weed control chemical for rice is maintained. The current practice of using propanil in post-emergence as a mix with pendimethalin or other herbicides, does not appear to be effective in preventing build-up of resistance. However, this study suggests that rotation of propanil with pre-emergence herbicide applications might play a part in future resistance management strategies.

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