SESSION 5

HERBICIDE RESISTANCE IN CROPS AND WEEDS

CHAIRMAN MR R. J. MAKEPEACE

SESSION ORGANISER MR H. M. LAWSON

GENETICALLY-ENGINEERED HERBICIDE TOLERANCE - TECHNICAL AND COMMERCIAL CONSIDERATIONS

R. FRALEY, G. KISHORE, C. GASSER, S. PADGETTE, R. HORSCH, S. ROGERS, G. DELLA-CIOPPA, D. SHAH

Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO, 63198 USA

ABSTRACT

Gene transfer and cell selection techniques have been used successfully to create crop plants tolerant to several major herbicides. By the early 1990s, the first modified crops will be marketed - by the year 2000, this new technology will begin to exert a dramatic influence on weed control practices and on the introduction of new herbicide products. The decision to commercialize herbicide-tolerant plants will depend on the performance, cost and environmental acceptability of the herbicide. The nature of the resistance mechanism and the accessibility to commercial germplasm represent other key factors in the decision-making process. Ultimately, herbicide-tolerant crops will accelerate the trend towards commercializing fewer, more effective, less costly and environmentally more acceptable weed control products.

INTRODUCTION

The rapid progress that has been made in the development of gene transfer systems for higher plants has surprised even the most optimistic of researchers in the field. Today, nearly two dozen species of crop plants including vegetables, cotton, oilseed rape, alfalfa and sunflower can be routinely manipulated using available Agrobacterium tumefaciens or free DNA transformation systems (Fraley et al. 1986). Within the next 2-3 years it is likely that all major crop species will be accessible to improvement using this technology. In addition to the tremendous progress that has been made in the transformation of plants, equally dramatic progress has been made in the identification of single gene agronomic traits which confer insect (Fischhoff et al. 1987, Vaeck et al. 1987), viral disease (Abel et al. 1986, Tumer et al. 1987) and herbicide tolerance when expressed in transgenic plants.

Advances have been particularly dramatic in the engineering of selective herbicide tolerance, because existing knowledge of herbicide mode-of-action and metabolism has permitted rapid identification of key target genes. It is quite clear now that within the period of the next 5-10 years, commercial level, selective tolerance mechanisms will be available for major existing herbicides as well as for newly-introduced products. The availability of selective crop resistance to many of today's potent, broad-spectrum herbicides will exert a dramatic effect on weed control practices as well as on the introduction of new chemical products. In this paper, we will focus on the technical aspects of engineering herbicide tolerance as well as on other factors which will influence the commercialization and adoption of this new technology.

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ENGINEERING OF SELECTIVE HERBICIDE TOLERANCE

The biochemical basis for the mode-of-action of several herbicides has now been elucidated through physiological, biochemical and genetic studies. This research has facilitated the identification and molecular cloning of genes encoding herbicide-sensitive and insensitive target proteins from both microbes and higher plants. The enzymes which detoxify herbicides have also been studied and genes encoding these proteins have been cloned from a variety of sources. The herbicides for which tolerance has been achieved are discussed in detail below:

Glyphosate tolerance

The shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), involved in aromatic amino-acid biosynthesis has been identified to be the specific target of this herbicide in bacteria (Steinrucken & Amrhein 1980). Subsequent studies have shown that glyphosate also inhibits this enzyme in higher plants (Mousdale & Coggins 1984, Rubin et al. 1984, Nafziger et al. 1984).

The first attempts to engineer glyphosate tolerance in transgenic plants have taken advantage of a bacterial gene encoding a glyphosate-tolerant EPSPS. A mutant gene encoding a resistant enzyme was isolated from S. typhimurium and was shown to contain a single base pair change resulting in a proline to serine amino-acid substitution at position 101 of the protein (Comai et al. 1983, Stalker et al. 1985). Chimeric genes were constructed in which the mutant EPSPS coding sequence was driven by either the octopine synthase promoter or the mannopine synthase promoter (Comai et al. 1985). The chimeric genes were introduced into tobacco cells using Agrobacterium vectors and plants were regenerated from the transformed cells. The transformed plants carrying the chimeric genes were two to three times more tolerant to glyphosate than the control plants. Recently, the chimeric EPSPS gene has also been introduced into transgenic tomato plants (Fillatti et al. 1987). The transgenic tomato plants expressing the gene were tolerant to glyphosate at a rate of 0.84 kg a.i./ha. The plants, however, were reduced in growth after spraying relative to unsprayed control plants. Calgene Inc. expects to commercialize glyphosate-tolerant crops in the early 1990s.

In a second set of experiments, Monsanto researchers (Shah et al. 1986) utilized high-level expression of a plant EPSPS gene to engineer glyphosate-tolerant plants. A full-length cDNA clone for EPSPS was isolated from a glyphosate-tolerant suspension cell line of Petunia hybrida. The amino-acid sequence predicted from the nucleotide sequence indicated that the enzyme was synthesized as a precursor polypeptide with an amino-terminal 'transit peptide' sequence of 72 amino-acids. The transit peptide is responsible for post-translational targeting of the precursor enzyme to the chloroplast (della-Cioppa et al. 1986). The wild-type petunia EPSPS cDNA was placed under control of the promoter for the 35S transcript of cauliflower mosaic virus (CaMV) and the resulting chimeric gene was transferred to a binary vector. Introduction of the chimeric EPSPS gene into petinia cells led to the growth of callus at concentrations of glyphosate sufficient to inhibit completely the proliferation of wild-type callus. Transformed petunia plants were regenerated and these plants were tolerant to application of formulated glyphosate at 0.9 kg a.i./ha, approximately four times the quantity necessary to kill the control plants. However the growth of these

plants was reduced relative to unsprayed controls.

More recently, experiments have been carried out with a mutant EPSPS gene which encodes an enzyme that is 1000-fold less sensitive to glyphosate inhibition than the wild-type petunia enzyme. Transformed tobacco plants expressing the glyphosate-resistant EPSPS gene were found to be significantly more tolerant to glyphosate than plants over-expressing the wild-type EPSPS gene. Tobacco plants expressing the mutant gene displayed no visible injury when sprayed with 0.9 kg a.i./ha of glyphosate; the treated plants flowered normally and set seed at levels identical to unsprayed controls.

Phosphinothricin tolerance

Phosphinothricin is a potent competitive inhibitor of glutamine synthetase (GS) from E. coli and higher plants (Bayer et al. 1972, Leason et al. 1982, Colanduoni & Villafranca 1986, Manderscheid & Wild 1986). Inhibition of GS by phosphinothricin causes a rapid accumulation of ammonia which is toxic to plant cells (Tachibana et al 1986). Initial attempts to confer tolerance to this herbicide in plants focussed on the isolation of a resistance gene from herbicide-resistant plant cells. Donn et al. (1984) selected alfalfa suspension cell lines that were 20 to 100-fold more tolerant to the herbicide than the wild-type cells; GS activity was elevated 3 to 7-fold in the tolerant cell lines. Increased enzyme synthesis was apparently sufficient to overcome the toxic effects of the inhibitor. To date, there is evidence by these researchers that expression of the GS gene confers low-level tolerance to phosphinothricin in transgenic plants. Since the plant GS complements the glnA defect in E. coli (DasSarma et al. 1986), it may be possible to select for resistant forms of plant GS in E. coli to increase tolerance levels.

A second approach to confer tolerance to phosphinothricin in transgenic plants has been recently described which involves expressing an enzyme that detoxifies the herbicide (De Block, personal communication). A gene that encodes the enzyme phosphinothricin acetyl transferase in S. hygroscopicus has been cloned and characterized. This enzyme acetylates the free amino group of phosphinothricin and protects the bacterium from the autotoxic effects of bialaphos. The gene was placed under the control of the cauliflower mosaic virus 35S promoter and introduced into tobacco cells; the resulting calli were resistant to high levels of the herbicide (500 mg/l). A number of plants were regenerated from the transformed calli and sprayed with 4 to 10 times the dose of herbicide required effectively to kill the control plants. All transgenic plants assayed were completely resistant to the herbicide. The expression of the detoxifying enzyme at a level as low as 0.001% of the total extractable protein was sufficient to protect plants against field level applications of the herbicide. The expression of this gene in transgenic tomato, oilseed rape and potato plants also conferred complete resistance to the herbicide. There are no data available on the fate of the acetylated metabolite in transgenic plants.

Sulfonylurea tolerance

The biochemical site of action of sulfonylurea herbicides has been recently elucidated. LaRossa & Schloss (1984) first showed that sulfometuron-methyl inhibited the growth of certain strains of E. coli and S. typhimurium. The sulfometuron-methyl inhibition of bacterial growth could be reversed by the inclusion of branched-chain amino-acids in the

culture medium. It was subsequently determined that the enzyme acetolactate synthase (ALS) which is required for the synthesis of leucine, isoleucine, and valine was the target of sulfometuron-methyl in S. typhimurium. Analogous studies have also been carried out in higher plants by selection for sulfonylurea-tolerant mutants of haploid tobacco cells (Chaleff & Ray 1984). The diploid tobacco plants which were regenerated from the mutant cells retained the herbicide-resistant phenotype under field test conditions. It was established by genetic crosses that the herbicide-resistant phenotype was due to a single dominant or semi-dominant nuclear mutation at one of two unlinked loci which co-segregated with herbicide-resistant ALS activity. Sulfonylurea-tolerant mutants of Arabidopsis thaliana have also been isolated by screening for growth of seedlings in the presence of the herbicide (Haughn & Somerville 1986). The tolerance was due to a single dominant nuclear mutation at the locus designated csr. Recently sulfonylurea-tolerant soybean mutants have also been isolated (Chaleff, personal communication). Like the glyphosate target enzyme EPSPS, ALS is a nuclear-encoded chloroplast-localized enzyme in higher plants (Chaleff & Ray 1984, Jones et al. 1985). The genes encoding the wild-type and mutant ALS have been isolated from tobacco and Arabidopsis and their nucleotide sequences have been determined (Mazur, personal communication). The amino-acid sequences deduced from the nucleotide sequences predict the presence of a presumptive chloroplast transit peptide at the amino-terminal ends of these two polypeptides. Recently, transgenic tobacco plants containing the chimeric ALS genes have been shown to demonstrate high level tolerance to sulfonylurea herbicides. A program is in progress with Northrup King to evaluate and commercialize sulfonylurea tolerance in tobacco.

Imidazolinone tolerance

The mode of action of these herbicides is similar to that of sulfonylurea herbicides in that they interfere with the biosynthesis of the branched-chain amino-acids valine, leucine, and isoleucine and also inhibit the enzyme ALS (Shaner et al. 1984). Maize cell cultures that are tolerant of imidazolinones have been developed (Anderson & Georgeson 1986) and several mutant maize cell lines having greater than 100-fold tolerance have been isolated. Some of these cell lines have been characterized as having altered ALS activity. Plants were regenerated from one of these cell lines and the tolerance was shown to be inherited as a single dominant nuclear gene. Plants homozygous for the resistance gene were 300-fold more tolerant to a number of imidazolinone herbicides. Field studies have found no detrimental effect of the mutant gene on the growth and development of maize plants. A back-crossing program is in progress with Pioneer to introduce this gene into commercial germplasm - the first tolerant corn seed could be marketed in the early 1990s.

Atrazine tolerance

Atrazine and other triazine herbicides interfere with photosynthetic electron transport by interacting with the 32 kd chloroplast protein (Gardner 1981, Erickson et al. 1984). Back-crossing of atrazine tolerance from related wild species has been carried out successfully, but many of the atrazine-tolerant commercial lines fail to perform agronomically at levels comparable to non-tolerant lines (Souza Machado 1982). Although attempts are underway to develop chloroplast transformation systems using mutant 32 kd genes as selectable markers, there are currently no data available supporting the success of this approach. Similarly, attempts have been made

to deliver the 32 kd protein from the cytosol to chloroplasts using chloroplast transit peptide sequences; however little or no tolerance appears to have been derived from this method.

It is well known that atrazine-tolerant plants such as corn contain elevated levels of glutathione-S-transferase (GST) (Shimabukuro et al. 1971, Guddewar & Dauterman 1979). GSTs are 27,000-32,000 kd proteins which catalyze the conjugation of glutathione with a large number of hydrophobic, electrophilic compounds including atrazine (Mozer et al. 1983). In corn, GSTs are encoded by a small multigene family (Shah et al. 1986). Recently researchers at Ciba-Geigy have reported introducing an atrazine-metabolizing GST gene into transgenic tobacco plants (Helmer 1986). The plants demonstrate increased tolerance to atrazine; however, Ciba-Geigy has indicated it is only pursuing atrazine tolerance as a model research project in plant biology.

Bromoxynil tolerance

The herbicide bromoxynil is another known inhibitor of photosynthetic electron transport (Friend & Olsson 1967). Calgene scientists (Stalker & McBride 1987) have recently reported the isolation of a bacterium, Klebsiella ozaene, which contains a plasmid encoding a nitrilase enzyme specific for the hydrolysis of bromoxynil. The products of the reaction appear to be 3,5-dibromo-4-hydroxybenzoic acid and ammonia - these comprise some of the same metabolites as found in naturally-resistant grass species. It is not clear if the same enzyme also metabolizes ioxynil, a related herbicide. A 2.6 kb DNA fragment was identified which when cloned into E. coli conferred bromoxynil degradation capability. It has recently been reported that expression of the nitrilase gene in transgenic tobacco and tomato plants confers tolerance to bromoxynil.

COMMERCIALIZATION OF HERBICIDE-TOLERANT CROP PLANTS

Agrochemical company perspective

The research and development necessary to generate herbicide-tolerant plants has in most cases evolved from studies to understand herbicide mode-of-action and metabolism. Such programs, which have been carried out by most major companies, have primarily been aimed at designing new herbicides or generating information to support registration. The rapid development of gene transfer and cell selection techniques has now provided a new option and decision point for agrochemical companies - whether or not to pursue selective resistance mechanisms for new and existing products. In many ways this decision follows the same rationale as that used to justify traditional safener or antidote programs, to pursue crop selectivity through chemical synthesis, or to extend product range through package mix combinations. The key question is whether the particular chemistry has the performance and cost benefits and the environmental compatibility to be competitive against other existing and developmental herbicides in a given cropping and weed control situation. In addition to normal environmental impact considerations such as toxicity, soil leaching, ground-water contamination, metabolism, resistant weeds, etc., engineering herbicide tolerance in plants raises several additional issues (Hauptli et al. 1985). These include the potential for transferring herbicide tolerance to weed species and the introduction of undesirable agronomic characteristics associated with the tolerance trait itself. The mechanism for evaluating these impacts is in place within the framework of the USDA, EPA and FDA registration processes.

Given the vulnerability that agrochemical companies face in introducing and maintaining herbicide products, there is little doubt that all possible environmental impacts of engineered tolerance will be thoroughly evaluated prior to commercialization.

While the cost of registering an existing herbicide for use on tolerant crops will be substantially less than for discovery and development of a new product for that crop, many other factors enter into the decision-making process. Since it will take 4-7 years to introduce a tolerance gene into a significant percentage of the germplasm for a given crop, it is critical to understand thoroughly the economic and performance characteristics of the tolerant crop-herbicide combination compared to other competitive products. Such an effort is certainly not justified unless the tolerant crop-herbicide combination solves an uncontrolled weed problem or brings additional value-added benefits (reduced weed control cost or greater environmental acceptability) to the cropping situation. Given the lengthy time-lines for introduction of tolerant crops, another factor will be patent life and post-patent pricing strategies for a particular herbicide.

The cost of registering a herbicide on tolerant crops will depend largely on the particular crop, herbicide and resistance mechanism employed. For example, the cost of registering an established product on an engineered non-food crop would be minimal since the EPA has no residue metabolism requirements for these types of crops. However to extend the registration to a food crop could cost in the neighbourhood of $250,000-500,000. This would assume that all other existing data for that crop are deemed adequate to support the new uses. Registration in new crops or crop groupings or in new geographic areas could trigger additional studies on environmental fate, metabolism, etc. which could run into several millions of dollars.

The availability of selective crop tolerance to broad-spectrum herbicides such as glyphosate, sulfonylureas, imidazolinones and phosphinothricin promises to exert a dramatic influence on weed control practices. Such technology will accelerate a maturing herbicide industry's trend towards commercializing fewer, but more effective, less costly and environmentally more acceptable products. As the methods for gene transfer and for trait incorporation into commercial germplasm become routine, the engineering of selective herbicide tolerance will become an accepted and essential strategy for development of weed control systems.

Seed company perspective

The commercialization vehicle for engineered tolerant crop plants will be seed companies which are either owned by or are collaborating with agrochemical companies to introduce the tolerance genes into commercial lines. Where transformations or cell culture selections can be performed directly with commercial germplasm, such programs will involve routine agronomic evaluation and scale-up. If the herbicide tolerance genes are in an unacceptable genetic background, the breeding effort to back-cross herbicide tolerance into commercial lines will be extensive. For some crops (trees, vines, etc.) the long time-frames for back-crossing would make this strategy unattractive.

Seed companies have historically selected lines that demonstrate the highest level of tolerance to widely-used commercial herbicides in a given crop, but there has traditionally been little interest in working

exclusively with a particular company's product. Rather, the intent has been to make sure that newly-released lines are compatible with leading weed control products on the market. With the ability to engineer selective resistance to particular herbicides which may be more effective, less expensive or environmentally more acceptable than existing products, there is greater interest by seed companies in obtaining and introducing tolerance genes into their commercial germplasm. Herbicide tolerance provides a unique 'branding' for a company's germplasm and may provide a marketable value-added advantage in particular cropping situations. In some countries, patents on engineered herbicide tolerance genes, vectors or plants may provide a form of protection for other agronomic traits in a given cultivar. In such situations, the seed company's interest in branding their own germplasm and capturing some of the value-added benefits from the agrochemical's novel performance would be counter to the chemical company's desire to maximize value by introducing herbicide tolerance in all major sources of commercial germplasm.

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BREEDING HERBICIDE-TOLERANT CULTIVARS - A CANADIAN EXPERIENCE

V. SOUZA MACHADO, D.J. HUME

Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada

ABSTRACT

The breeding and physiological research programs which led to the development of triazine-tolerant cultivars of brassica crops in Canada are described. The agronomic advantages and drawbacks of the cultivars produced to date are discussed, together with their impact on crop rotational practice and their effect on weed control strategies. Spin-off benefits to other areas of basic and applied research, to extension services and to education are outlined. However the major benefit has been the opportunity to design and test through to commercial cropping the techniques required to utilise genetics and plant breeding to solve intractable weed control problems.

INTRODUCTION

The development of herbicide-tolerant cultivars in Canada occurred as an after-thought, following the initial detection and chemical control of chloro-triazine resistance in broadleaved weeds like Chenopodium album (Bandeen & McLaren 1976) and Brassica rapa (Maltais & Bouchard 1978) in maize. Basic research to unravel the resistance phenomenon led to the discovery of the mechanism of resistance located at the chloroplast level (Radosevich 1977, Souza Machado et al. 1977), the cytoplasmic inheritance of this trait (Souza Machado et al. 1978) and the potential to transfer triazine tolerance into economic crops like canola, rutabaga (swede) and cole crops (Souza Machado et al. 1980). The trait was transferred to cultivated spring rapeseed Brassica napus (Beversdorf et al. 1979) and broccoli B. oleracea (Ayotte et al. 1987).

The transfer of chloro-triazine resistance into Brassica crops was justified, because of the absence of weed control recommendations involving registered herbicides at the pre-emergence and post-emergence stage of cultivation. The presence of cruciferous weeds like Thlaspi arvense and Sinapis arvensis (formerly Brassica kaber) in canola, the latter crop having been specifically bred for very low erucic and glucosinolate content, would lead to poor harvest seed grades due to adulteration by T. arvense and S. arvensis if these weeds were not controlled during the growing phase. The first chloro-triazine-resistant canola cultivar to be released was OAC Triton (Beversdorf & Hume 1984) followed by Tribute in 1986 and OAC Triumph in 1987.

BREEDING OBJECTIVES

With canola the objectives were to breed a chloro-triazine-resistant cultivar with as high a yield as was possible and of a quality to meet existing standards for canola, namely less than 5% erucic acid and less than 30 micromoles of glucosinolates per gram of oil-free meal. The cultivar was intended for areas with serious infestations of cruciferous weeds, which was one of the limiting factors restricting the expansion of the canola hectarage.

The breeding program with rutabaga was aimed at producing a 'Laurentian' type of cultivar with chloro-triazine resistance, purple top, globe shape and yellow flesh. The objectives with the cole group of B. oleracea crops was initially to produce a stable genotype with 2n=18 chromosomes of the CC genome and a chloro-triazine-resistant cytoplasm.

RESOURCES FOR RESEARCH

The initial grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to investigate the phenomenon of triazine tolerance and its economic potential amounted to about $92,000 Cdn. These were followed by assistance towards a breeding program to develop commercial cultivars, also from NSERC in the range of $200,000 Cdn. Subsequent support came from the Canola Council of Canada, Canada Packers Inc. and the New Crop Development Fund of Agriculture Canada. The breeding program has also been supported financially by the Ontario Ministry of Agriculture and Food (OMAF). Management and quality studies were also included in these assisted programs. Support for the spring canola breeding program up until the 1984 release of OAC Triton was about $300,000 Cdn. Funding to support the rutabaga and cole crops program was mainly from NSERC amounting to $140,000 Cdn. with assistance from provincial sources including OMAF.

THE GROWERS' SCENARIO

Initial problems with canola were that OAC Triton lodged badly and recorded poor yields. Emergence was slower in the spring than with triazine-susceptible cultivars and therefore the crop was more prone to damage by flea beetle (Phyllotreta spp.). The oilseed crushers did not like the fact that it was about 2% lower in oil content than comparable triazine-susceptible cultivars. Early test crushing experience with OAC Triton indicated that there was a heat-stable red pigment which was difficult to remove. In Western Canada OAC Triton was too late in maturity for much of the main canola-growing area and the harvested seed was often high in chlorophyll content. Attempts have been made to overcome these problems by breeding. The recent cultivar Tribute has earlier maturity and lower seed chlorophyll and OAC Triumph has better lodging resistance, about 1% higher oil content and slightly higher yields.

Better chloro-triazine-resistant cultivars continue to appear in the registration trials, some of which have been bred by a private plant breeding company. Following the release of OAC Triton in 1984, the area planted in Ontario increased to about 15,000 has in 1986. However, low canola prices in 1987 reduced the area planted. In Ontario OAC Triton occupies about 50% of the total spring canola area. It has been grown in fields where closely-related cruciferous weeds, which were not controlled by pre-plant incorporated trifluralin, were subsequently controlled by pre-emergence and post-emergence applications of triazines. The use of OAC Triton has also helped growers to diversify out of maize, where there were high atrazine residues in the soil and to permit the control of quackgrass or common couch (Elymus repens) with atrazine, while growing a rotational crop of canola. In Western Canada, the chloro-triazine-resistant cultivars OAC Triton and Tribute have constituted only about 3% of the total hectarage, or about 80,000 ha. These cultivars were grown in locations where weeds were difficult or expensive to control by other means.

Grower problems with rutabaga and the chloro-triazine-resistant line CTR/Laur mainly involved seed distribution, after the disbanding of the Rutabaga Growers Marketing Board which was originally to handle the seed sales. Trials at Research Stations indicated that when CTR/Laur and the chloro-triazine-susceptible cultivar Laurentian were planted early in May, no significant differences were noted between the two. However, delayed plantings up to late June resulted in significant depression of yield as compared to Laurentian. Susceptibility to Turnip Mosaic Virus was more severe in CTR/Laur at the later planting dates. Emergence and seedling development were initially slower with CTR/Laur than Laurentian, but if planted early in May, no yield differences were noted between CTR/Laur and Laurentian. Control of cruciferous weeds was possible within and between the rows, using cyanazine as a pre-emergence treatment.

SPIN-OFFS FOR RESEARCH

The research program has resulted in a considerable number of side benefits to basic and applied research. Collaboration with Dr C. Arntzen in the USA during the initial stages of the physiology research at Guelph (Souza Machado et al. 1978) had led to numerous refereed publications on photosynthesis involving chlorophyll fluorescence and chloroplast membrane alterations (Arntzen et al. 1979, Steinbach et al. 1981, McIntosh & Hirschberg 1983). The role of organelle involvement in cytoplasmic inheritance has been studied using chloro-triazine-resistant and susceptible biotypes of B. campestris and Amaranthus spp. (Vaughn 1985). Other areas of basic research involve protoplast fusion to combine the chloro-triazine resistance trait with mitochondrial male sterility, its use as a genetic marker and the creation and testing of chloro-triazine-resistant spring canola hybrids (Grant & Beversdorf 1985), as well as anther and microspore culture methods for producing doubled haploids.

Spring canola was not grown prior to the release of OAC Triton, therefore a whole testing program had to be developed to obtain data to support registration for this and other cultivars. Extensive trials in Eastern and Western Canada involving herbicide testing and registration have been completed, as well as research on the reasons for the depressed yields and photosynthetic rates of the chloro-triazine-resistant canola lines. Field trials have also been carried out to determine the extent to which they could become a volunteer weed problem. Several graduate students were involved in the breeding program at the Masterate and Doctorate level and many are now working in plant breeding professionally.

At the applied extension level, rapid techniques to detect chloro-triazine resistance or susceptibility in weeds have been worked out based on measuring chlorophyll fluorescence (Ali & Souza Machado 1981, Ahrens et al. 1981). This technique has helped extension and chemical company personnel to solve field problems associated with weed escapes or faulty herbicide application. Bioassays using chloro-triazine-resistant and susceptible seeds can be used to monitor atrazine or urea herbicide residues in top-soil used for gardens. Teaching experiments in plant physiology and weed science to undergraduates have involved these biotypes to demonstrate the Hill reaction with isolated chloroplasts and the role of photosynthetic inhibitors and pre-emergence and post-emergence herbicides.

CONCLUSIONS

The breeding potential of transferring chloro-triazine resistance into economic crops is a goal that has yet to be fully achieved, particularly with respect to yield levels. An encouraging aspect so far with the canola and rutabaga programs has been the stability of resistance maintained over several generations. Although it has not proven to be the panacea to all our cropping problems, it has served to test a novel approach to weed control, involving use of genetic and plant breeding techniques to adapt crops to existing inexpensive registered herbicides and has encouraged rotation of field crops to cope with volunteer crop problems, rather than sustain a monoculture system of cultivation.

The development of new selective chemical compounds like the DuPont product DPX A7881 to control wild mustard in Brassica crops, the re-registration toxicology requirements of cyanazine because of the Industrial Biotest Laboratory issue, and increasing pressure on atrazine registration because of alleged well-water contamination, make the future status of chloro-triazine-resistant crop cultivars unclear. However, the research and development work in this area to date has proven to be challenging, productive and an experience that has benefitted agricultural and plant science.

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APPEARANCE OF SINGLE AND MULTI-GROUP HERBICIDE RESISTANCES AND STRATEGIES FOR THEIR PREVENTION

J. GRESSEL

Department of Plant Genetics, Weizmann Institute of Science, Rehovot, Israel

ABSTRACT

The first resistances to appear were to single herbicide types. Sequential multiple resistances have evolved to photosystem II herbicides, and broad multiple resistance among oxidant-generating herbicides (paraquat, nitrodiphenylethers, PSII-inhibitors), as well as to herbicides degraded by monoxygenases (diclofop-type diphenylethers, and some phenylurea, dinitroaniline and sulfonylurea herbicides). The necessary strategies to delay and allay these resistances include herbicide rotations, mixtures, increased use of less persistent compounds and synergists that lower selection pressure. In the cases of triazine and dinitroaniline resistances, herbicide rotations have proven to be better ploys than the early models had predicted. Resistance has not evolved anywhere where these strategies have been used, either fortuitously or by design.

INTRODUCTION

Resistance to herbicides has evolved throughout North America and Europe, in spots in the Middle and Far East, Australia and New Zealand. There is less propensity to publish new cases so that the extent of resistance becomes harder to ascertain. There has been a high level of complacency, as in almost all cases there have been suitable alternative herbicides to control the resistant weeds. Resistance has evolved very rapidly to some of the replacements. The cost of alternatives may be quite large; in certain cases, ten-fold greater (Ammon & Irla 1984). The most widespread resistances are to s-triazine herbicides but others have been appearing. The multiple resistances, i.e. resistances to herbicides of different chemistries or modes of action, are especially worriesome. One need only look at similar multi-resistances in insecticides to realize the dire implications. Still, there are simple strategies available to preclude, delay or alleviate the evolution of resistant populations. Except for a few understandable and explainable exceptions, all cases of resistance have occurred where there was repeated mono-culture/mono-herbicide use. A possibility exists that *Avena fatua* may evolve the same mechanism that cereals use for differential tolerance. Resistance has not appeared where strategies of rotation of herbicides have been used, despite predictions by earlier models (Gressel & Segel 1982) based on the data then available. The use of mixtures and the single annual applications of non-persistent herbicides have historically proven to be very effective strategies to prevent or delay resistance. The reasons why synergists should delay the appearance of resistance in certain weeds are discussed. Often, it will only be possible to design strategies after the first cases of resistance appear and genetics and the modes of action studies are performed. It is then necessary to overcome the emotional-geographic barrier which says that "what happened there won't happen here", and to apply preventative strategies.

THE EVOLUTION AND SPREAD OF RESISTANCES

Photosystem II inhibiting herbicides

Resistance has appeared world-wide to the s-triazine herbicides atrazine and simazine in about 50 weed species. In almost all cases these highly persistent herbicides were used alone, repeatedly for 5-10 years, in maize, orchards or along rights-of-way. Except in a recent case with *Abutilon theophrasti* (Andersen & Gronwald 1987), triazine resistance is maternally inherited, and has been traced to a single amino-acid transversion coded by a chloroplast gene which controls herbicide binding to its site of inhibition (e.g. Gressel 1985). Other sites are mutable in algae resistant to photosystem II herbicides, giving varying resistances to phenylurea and uracil type herbicides and the same may be true in weeds. There are many plastids per cell, each with DNA. The generation of oxygen radicals by the triazine-susceptible plastids would be phytotoxic, even if there were some resistant plastids. For this reason it is supposed that: (a) plants bearing recessive plastid mutant traits would be exceedingly rare - so rare that the frequency can only be guessed as being lower than 10^{-20}; (b) there is probably some stage of a plant's life cycle where there are fewer copies of plastid DNA per cell, or there is some sort of segregation, allowing plastids bearing recessive mutations to become predominant. If not, such mutant phenotypes would be even more rare. Peculiar leaf mosaic patterns in triazine-resistant *Solanum nigrum* plants were genetically traced to a a nucleus-coded gene which greatly enhanced the rate of chloroplast mutations (Arntzen & Duesing 1983). This "plastome mutator" gene may have increased mutations allowing triazine-resistant populations to evolve, despite the infinitesmally low natural frequency of the gene. The presence of other chloroplast mutations would cause the mutator gene to be bred out of resistant populations after repeated herbicide selection.

Sequential appearances of PSII herbicide resistance

Atrazine-resistant weeds are usually resistant to all s-and as-triazines, some phenylureas (but not diuron) and some uracils. They have usually remained sensitive to pyridazinone-type herbicides as well as to pyridate. A case of atrazine/chloridazon (pyrazon) co-resistance in *Chenopodium album* has been reported in fields with a crop rotation of maize (with atrazine) and sugar-beet with chloridazon (Solymosi *et al.* 1986). It should be presumed that each mutation was an independent event and the frequency of each different resistant chloroplast biotype should have been the same. Thus, if it took eight years to obtain populations of triazine-resistant biotypes, it should take another eight to obtain resistance to each of the PSII herbicides used as a replacement. Triazine resistance became a fact throughout the Hungarian monoculture maize growing areas within 8-12 years of use after maize and atrazine were co-introduced. Resistance to PSII herbicides that previously controlled atrazine-resistant *Chenopodium album* evolved, not in the expected 8-12 years but in 2-3 years after each was introduced. Thus, there are recently evolved *C. album* biotypes that are atrazine-resistant, atrazine and choridazon-resistant, chloridazon-resistant, atrazine and pyridate-resistant, and atrazine, chloridazon and pyridate-resistant (Solymosi *et al.* 1986 and Solymosi pers. comm.). In all cases resistance seems to be at the chloroplast level, as with atrazine resistance. The plastome mutator gene frequency should be much higher in triazine-resistant populations than in wild-type populations. This higher frequency of the mutator genes would facilitate a much more rapid sequential evolution of secondary and tertiary resistances to PSII herbicides, and has obvious implications in designing strategies to prevent resistance (Gressel 1986).

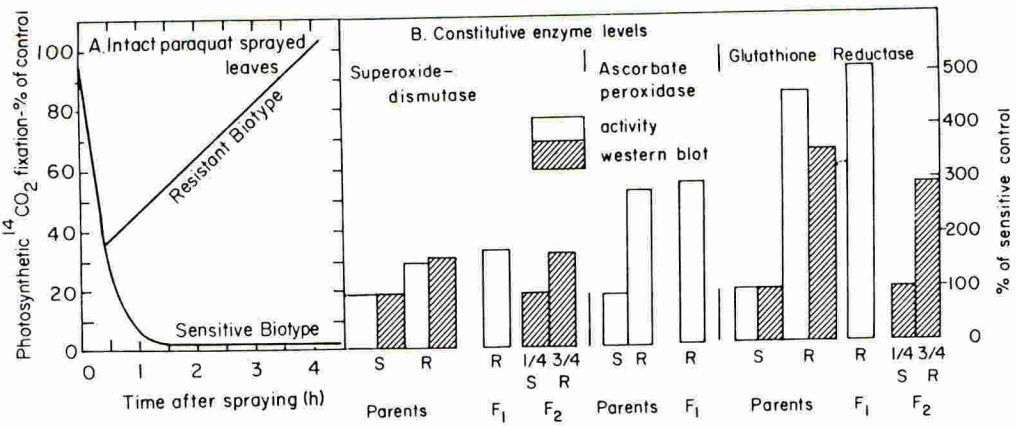

Fig. 1. Paraquat effects in resistant *Conyza bonariensis*. A, The transient effects on chloroplasts. B, The levels of Halliwell-Asada active-oxygen detoxification pathway in the chloroplasts. A, Resistant and susceptible plants of *Conyza bonariensis* were sprayed with 0.1 mM paraquat and photosynthesis was measured as an estimation of paraquat affecting chloroplasts. Source: Redrawn from Shaaltiel & Gressel (1987b). B, Enzyme levels without paraquat treatment in resistant and susceptible *Conyza bonariensis* and their crosses. The F_1 plants were all resistant. Source: Collated and drawn from data in Shaaltiel & Gressel (1986) and Shaaltiel (1987).

Oxidant-generating herbicides

Three *Conyza* spp. have evolved paraquat resistance in Egypt, Japan and Hungary (Gressel *et al.* 1982, Tanaka *et al.* 1982, Pölös *et al.* 1987). *Poa annua* and *Lolium perenne* in the UK (cf. Gressel *et al.* 1982), *Epilobium ciliatum* in Belgium (Bulcke *et al.* 1985) and *Hordeum glaucum* in Australia (Powles, 1986) have also evolved paraquat resistance. These cases seem inconsistent with predictions that resistance would appear only to persistent herbicides exerting season long selection pressure (Gressel & Segel 1982). However, the persistence of the herbicide was replaced by that of growers who persisted in applying paraquat 4-10 times per season for 6-14 years. In at least two cases, there is a modicum of cross-tolerance to the oxidant-generating herbicide such as atrazine in *C. canadensis* (Pölös *et al.* 1987), and atrazine, acifluorfen and SO_2 in *C. bonariensis* (Shaaltiel 1987).

We have studied paraquat resistance in *C. bonariensis*. Paraquat rapidly penetrates into leaves and chloroplasts of the resistant leaves, transiently inhibiting photosynthesis in both *C. bonariensis* (Fig. 1A) and *C. canadensis* (Pölös *et al.* 1987). Resistance is due to a single dominant gene that pleiotropically controls elevated plastid levels of at least three enzymes that participate in the detoxification of the active oxygen generated by paraquat: superoxide-dismutase, ascorbate-reductase and glutathione-reductase (Fig. 1B). The oxygen-detoxifying enzymes probably preserve the plants from death while paraquat is dissipated or sequestered, a process which can be visualized only much later (cf. Fuerst *et al.* 1985). It is not clear if this is the sole mode of paraquat resistance that has evolved. The levels of the oxygen-detoxifying enzymes have not been measured in intact chloroplasts of other resistant (vs. susceptible) biotypes that have evolved.

Resistance to tubulin-binding dinitroaniline herbicides

Eleusine indica has evolved resistance to dinitroaniline herbicides. In all cases trifluralin was used as the sole herbicide in some U.S. monoculture cotton for more than 6 years (Mudge *et al.* 1984). This resistance has been traced to a modification in the tubulin of the resistant biotype which precludes herbicide binding (Vaughn 1986). Thus, there is no herbicide-inhibition of microtubule formation. This is analogous with resistance to benzimidazole fungicides by fungal pathogens. It would be quite informative to know the mode of inheritance of this resistance.

Resistance to herbicides degraded by monoxygenases

Wheat is naturally resistant to a large number of herbicides because it is capable of degrading them using monoxygenases (=mixed function oxidases = cytochrome P_{450}s) (e.g. Sweetser *et al.* 1982, Brown *et al.* 1987). This property of wheat is especially useful as some key weeds such as *Avena fatua*, *Alopecurus myosuroides* and *Lolium* spp. are normally incapable of degrading the same herbicides, allowing differential control. The recent widespread multi-resistances may be the unwanted swallows of spring. These include *Lolium rigidum* resistant to diclofop-methyl (and related diphenylethers) and sulfonylureas (Heap & Knight 1986), and *Alopecurus myosuroides* to chlortoluron, sulfonylureas, diclofop-methyl and pendimethalin (Moss & Cussans 1987). This is probably due to the evolution of greater monoxygenase activity (Cole & Owens 1987, Kemp & Caseley 1987). The spread of such multi-resistances, could abrogate most grass-selective wheat herbicides. It is imperative to ascertain the genetics and molecular biology of this resistance. As discussed below, the strategies are different for preventing single-gene resistances (usually controlling binding), and for polygenic or gene-dose resistances. *A. fatua* already possesses low levels of wheat type degrading enzymes but the activity is too low to allow resistance (e.g. Shimabukuro *et al.* 1987). Is it sufficient to modify an *Avena* control gene to obtain resistance? Would resistance be polygenic requiring combined gene doses or must there be gene amplifications as with insecticide resistance (e.g. Mouches *et al.* 1986)? Different modes of resistance can evolve to the same herbicide. Sulfonylurea resistance selection has led to tobacco (Chaleff & Ray 1984) and *Arabidopsis thaliana* (Haughn & Somerville 1986) with modified acetolactate synthase, the target enzyme of the herbicide. Chlorsulfuron-resistant alfalfa (lucerne) (Stannard *et al.* 1987) and soybean (Sebastian & Chaleff 1987) were also selected, and they have some other (unknown) mechanism of resistance. Are the latter cases due to monoxygenases, and are there multiple cross-resistances? Will plants that have a multiple resistance, including to sulfonylurea herbicides, also be resistant to the imidazolinone and to the new triazolo-pyrimidine herbicides that inhibit acetolactate-synthase (Shaner *et al.* 1984, Berwick *et al.* 1987)? It will also be interesting to know more about the mode(s) of resistance and further cross-resistances in *Stellaria media* that evolved resistance to mecoprop and other phenoxy herbicides (Lutman & Lovegrove 1985).

STRATEGIES TO DELAY AND CONTAIN RESISTANCE

Two major factors control the duration it takes for resistant populations to evolve: the selection pressure and the initial frequency of resistance genes. The buffering effect of the soil seed reservoir and the fitness of the resistant biotypes can considerably delay the evolution of resistance. The problem of

frequency of resistant individuals is paramount in deriving strategies: there are differences in genetics and frequencies that require diametrically different strategies. For single major-gene resistances, the initial frequency is usually a fixed number; it does not change with herbicide application rate (selection pressure). The frequency can vary considerably with application rate, especially when there is polygenic inheritance or gene amplification responsible for resistance. In the latter cases, if we treat with a low dose of herbicide, we may obtain populations with resistance due to any of the separate effects of any number of genes which together will be at a high frequency. We might also select for a duplication of any one of these genes. If the dose is later raised, we will rapidly get further resistance, due either to a combination of the pre-enriched polygenes or to further gene duplications. Had high dose been used from the beginning, resistance would have been much delayed because of the much greater rarity of individuals with two or more of the polygenes or with multiple gene amplifications. In these gene dose cases, high initial doses are clearly called for from the time the herbicide is released. The "Catch 22" situation for single genes is that the use of the lowest herbicide rates will lower the selection pressure for target site mutations for resistance, considerably delaying resistance. Thus, we rarely know how to delay resistance, until resistance occurs for the first time, can be studied, and strategies outlined. Another "Catch 22" situation - whenever a new resistance has appeared and has been confirmed, it has either been discounted as being a rare instance (true), and not being indicative of future evolution (untrue). Such instances have often been ignored or concealed. The necessary research effort has usually been initiated 4-6 years after the first confirmed reports... too late to delay or prevent co-evolution elsewhere. Spreading resistance by seed or pollen for distances of more than a few hundred metres is usually rare in agronomic situations, although vehicular spread is probably the norm along rights-of-way. Thus, most cases of resistance are due to separate instances of parallel evolution through selection.

Most of the studied significant cases of resistance seem to be traced to single genes (but we have no genetics for the dinitroaniline or monoxygenase resistances). We will thus assume in the following sections that a major ploy to delay resistance will be to decrease selection pressure by using lower herbicide rates or less persistent herbicides.

Thresholds/windows/persistence

Lowering the selection pressure allows more susceptible individuals to remain alive. Their seeds dilute the seed produced by any resistant individuals in the population. The susceptible individuals are usually more fit than the resistant ones, i.e. they have a greater reproductive output per plant. There are a variety of ways to lower selection pressure without losing effective weed control. The use of thresholds for deciding when to treat with herbicides is discussed in Sessions 9 and 10 of this conference. As innocuous levels of weeds are left, treatments are applied later, and not always over the whole field, selection pressure becomes greatly reduced.

Herbicides of the phenoxy type gave adequate economic weed control, but because of their low persistence, they allowed later non-competitive weed germination of susceptible seeds. This low selection pressure is probably a major reason why there are hardly any reported resistance problems from 2,4-D or MCPA use. If *s*-triazines with less persistence had been used in maize, resistance would probably have never evolved. New wheat herbicides (e.g. chlorsulfuron) have an effective persistence of 2 years or more. It is logical to predict that some weed species will rapidly evolve resistance to this phenomenal group of herbicides as well as to the similarly-acting imidazolinones and triazolo-pyrimidines.

Mixtures and synergists

Mixtures can and have prevented the appearance of resistance. Chloroacetamides such as alachlor and metalachlor have long been mixed with atrazine, mainly because they are superior to atrazine alone for grass control. They control *Amaranthus* and *Chenopodium* spp., which are also controlled by the lowest atrazine doses. The use of chloroacetamides allows using less atrazine, decreasing the selection pressure. It also requires that resistant *Amaranthus* and *Chenopodium* spp. would have to evolve co-resistance to chloroacetamides. If there is a gene for chloroacetamide resistance, it would have to be in the same plant as that having atrazine resistance. The frequency of doubly-resistant individuals would be the compounded frequencies of atrazine and chloroacetamide resistances, a very low number.

Herbicide synergists allow the use of lower herbicide doses. Tridiphane is an example of such a compound. It prevents grasses from metabolizing atrazine (Lamoureux & Rusness 1986), allowing grass control at lower atrazine rates (Ezra *et al.* 1985). These low rates are still sufficient to control broad-leaved plants... but with lower selection pressure. Tridiphane may not delay grasses from evolving triazine resistance but it could delay resistance in the broad-leaved species. The latter evolved triazine resistance much before the grasses. Grasses would have to co-evolve tridiphane and atrazine resistance.

We have recently shown that paraquat resistance (Fig. 1) can be suppressed by adding chelators which remove the copper or zinc from superoxide dismutase or the copper from ascorbate peroxidase (Shaaltiel & Gressel 1987a, Shaaltiel 1987). These allow the use of much lower paraquat rates to control some of the weeds which were harder to kill with this herbicide. The use of lower rates (lower selection pressure) should delay the evolution of other modes of paraquat resistance as well as suppress this type of resistance.

The multiple-resistance due to elevated monoxygenases can be suppressed by aminobenzotriazole (Kemp & Casely 1987) and presumably by more plant-compatible monoxygenase inhibitors such as tetcyclasis (Cole & Owens 1987). These compounds not only suppress the weeds' monoxygenases, they suppress those of wheat (Cabanne *et al.* 1985). It is unclear how these synergists could be used in wheat/weed selective systems, and it is thus not yet apparent how they will be useable in agriculture. One can try to predict their effect on the evolution of resistance when the uses are known. In summary, mixtures will probably delay resistance in weeds that are targets to both components... especially if the selection pressures are lowered. Synergists will delay resistance in the weeds which were not the direct reason for their use, again by lowering selection pressure.

Herbicide rotation

It was initially modelled that if it would take ten years for resistance to occur to a given herbicide used in mono-herbicide culture, it would take 15 years if that herbicide were used in 2 out of 3 years and 20 years if the herbicide were used every other year (Gressel & Segel 1982). There are large areas in the U.S. corn-belt with 15-20 such treatments with atrazine in maize in rotation, without the appearance of triazine resistance. Triazine resistance has repeatedly occurred on smaller total acreages worldwide after 7-12 consecutive usages in monoculture. It is clear that rotation in this case has proven to be a far better ploy in preventing resistance than the models predicted, for reasons

recently understood. Most triazine-resistant weeds that have evolved are very non-competitive with the wild types of the same species. This is best expressed when there is no triazine present, i.e. in the rotational years. Early measurements of this lack of fitness, while showing 2-5 times greater fitness of the wild-type (Conard & Radosevich 1979) were probably underestimates. Fitness was not measured throughout the life cycle (including to overwintering, tolerance to late frosts, early germination and the critical period of establishment), nor were measurements made at field seeding densities. In addition there is now evidence that cultivation and many herbicides such as ioxynil (Thiel & Boger 1984), DNOC (Lehoczki *et al.* 1984), and 2,4-D (Salhoff & Martin 1986) harm the resistant biotypes more than the wild type. Similarly, dinitroaniline-resistant *Eleusine* is supersensitive to some tubulin inhibitors (Vaughn *et al.* 1987). Thus, the totality of events during the rotational years when triazines are not used has the effect of pushing the triazine-enriched frequency of resistant types back towards their initial frequencies. These factors are being considered in newer models which show that herbicides like triazines and dinitroanilines can have very long usefulness when used in rotation.

Another aspect of these newer models is that predictions can be made about what might happen when resistance appears and practices perforce change. Unfortunately the agricultural ecologists have not followed the decrease in frequency of resistant individuals after triazine usage was stopped. It was predicted that the frequency of resistance would decay to *ca* 1% of the population in 6-7 years (Conard & Radosevich 1979). Our present model suggests that the decay will be much quicker and that the low levels of resistant material will allow triazines to be used in rotations a few years after resistant populations appeared.

The trifluralin-resistant *E. indica* is also unfit (Mudge *et al.* 1984), which probably explains why this resistance has occurred only in mono-herbicide mono-culture despite widespread dinitroaniline use. Still, this situation may not be universal. There have been no indications that the paraquat-resistant weeds are much less fit (our group's unpub. results). Crop plants selected for non-binding of sulfonylurea (Chaleff & Ray 1984) and imidazolinone (Anderson 1986) herbicides at the level of the target enzyme do not apparently lack much fitness. In such cases rotation may at best delay resistance for only the time the rotation is in place. At worst, because of the persistence of levels killing some weed species during the rotational year, selection pressures will be continued throughout this period. These are factors which must be measured.

CONCLUDING REMARKS

Learning from the mishaps of history is the best way to prevent the evolution of resistance. It is impossible to integrate in advance all knowledge about a new herbicide to predict when or if resistance will occur. The more information on the physiology, biochemistry and genetics of a compound's action and selectivity is known, the more can be predicted. When the first resistances appear in the lab or in the field, further, more knowledgeable predictions can be made. When there is widespread resistance, we can learn much on prevention and containment by studying resistance *and* by analyzing why resistance did not occur elsewhere. The worst thing we can do, whether as producers or users of herbicides, is to ignore confirmed resistance. There are many different strategies that can be applied to delay or prevent resistance.

These strategies may reduce the current levels of usage of that herbicide, dismaying both farmer and chemical-manufacturer. Still, such strategies will keep the herbicide in useage for longer, compensating for the initial loss of use or sales. It is this longer-term benefit that must be realised.

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IMPLICATIONS OF HERBICIDE-TOLERANT CULTIVARS AND HERBICIDE-RESISTANT WEEDS FOR WEED CONTROL MANAGEMENT

GEORGE MARSHALL

Department of Botany and Plant Pathology, West of Scotland Agricultural College, Auchincruive, Ayr KA6 5HW

ABSTRACT

This paper examines the rationale behind research which endeavours to produce herbicide-tolerant cultivars. The value and limitations of herbicide-tolerant cultivars are described for triazine-tolerant oilseed rape in Canada and imidazolinone-tolerant maize in the USA. Consideration is given to the possible problems which might arise in releasing herbicide-tolerant crops with respect to their agronomic performance, the risk of spreading the tolerance trait to related weeds, cross-resistance to other herbicides and volunteer crop control. The background to herbicide-resistant weeds is examined together with the principles of herbicide-resistant weed management. The competitive effects, biological fitness and long term consequences of herbicide-resistant weeds are reviewed. Suggestions are made as to areas for future research which would contribute to our understanding of the consequences of introducing herbicide-tolerant cultivars and the development of herbicide-resistant weeds.

INTRODUCTION

Herbicides may be regarded as essential tools in successful crop production. Increasing scrutiny is being placed on environmental, management and economic aspects of herbicide use therefore it is important not to forget the intrinsic value of these chemicals for weed control. The property of selectivity of action in a herbicide is often fundamentally desirable in the development of a compound. Selectivity is seldom absolute. The terms herbicide-tolerance and herbicide-resistance exemplify the variation in response to a herbicide that may occur. Tolerance may be described as a relatively minor or gradual difference in intraspecific variability (LeBaron & Gressel 1982a), whereas resistance is defined as a decreased response in a population of animal or plant species to a pesticide or control agent as a result of their application (Anonymous 1965). Thus, a resistant weed is one that survives and grows normally at the usual effective dose of a herbicide (LeBaron & Gressel 1982a).

The purpose of this paper is to consider the applied aspects of the development of herbicide-tolerant cultivars and herbicide-resistant weeds. The background, value and limitations of herbicide-tolerant cultivars will be reviewed together with the management of herbicide-resistant weeds.

GENERAL CONSIDERATIONS : HERBICIDE - TOLERANT CULTIVARS

The development of herbicide-tolerant cultivars can be pursued via three principal methods; (1) classical plant breeding, (2) tissue culture and (3) gene manipulation. Individual techniques are reviewed by Chaleff (1986) and Gressel (1987a). In each procedure the object is to produce herbicide-tolerance de novo in a crop species previously susceptible to the target herbicide. From the herbicide manufacturer's point of view herbicide-tolerant cultivars may provide an extension to the existing market and prolong the market share for herbicides that are off patent. Furthermore, the impending withdrawal from use of a range of hitherto valuable herbicides on the basis of environmental or toxicological grounds could leave some important gaps in the choice of chemical weed control. The production of herbicide-tolerant cultivars could help to alleviate any inadequacies in weed control. Current liaison between chemical manufacturers and seeds companies provides an adequate testimony to the idea that farmers may soon be offered an integrated crop production package including the compatible cultivar-herbicide combination. Commercial interest in such specialist crops is perhaps unlikely to provide speedy or significantly profitable returns in the short term.

The situations where herbicide-tolerant cultivars could receive consideration are varied. Generally, however, one would wish to see the use of a broad-spectrum, environmentally safe, low cost herbicide where herbicide tolerance had been specifically incorporated. Therefore, a poorly competitive crop where the existing expensive herbicides do not give enough crop safety or a sufficient weed control spectrum would stand to benefit from the introduction of a herbicide-tolerant cultivar. Crop rotation restrictions may be imposed due to the carry-over of herbicides in soil. Such cropping restrictions can be reduced where a crop has tolerance to the residual herbicide. More specialised situations where herbicide-tolerant cultivars might be used for weed control include companion crops (Faulkner 1982) and clean seed production (Faulkner 1976).

The engineering of herbicide-tolerant cultivars does raise important issues as far as potential problems are concerned. Will herbicide use increase ? While pesticide inputs are designed to be reduced with disease and pest-resistant cultivars, this situation is unlikely to arise with respect to herbicide-tolerant cultivars. Will herbicide-tolerant cultivars pose problems as volunteer weeds ? Is it possible that cross resistance will spread to related weed species and be extended to include herbicides which have a similar mode of action ? These questions and others can perhaps be addressed by taking practical observations of the use of herbicide-tolerant cultivars outside Europe.

CASE STUDIES : HERBICIDE-TOLERANT CULTIVARS

Triazine-tolerant oilseed rape

Triazine tolerance as a trait was obtained from a weed biotype of B.campestris and transferred by Beversdorf et al. (1980) to both Polish rape (B. campestris) and Argentine oilseed rape (B.napus). In the species described triazine tolerance is a trait which shows maternal inheritance

(Souza Machado 1982) based on genes coded for by the chloroplast genome. The important point about understanding the nature of inheritance is that a cytoplasmically-controlled trait of this type cannot be inherited via the pollen (Tilney-Bassett 1975). Thus the spread of triazine tolerance or resistance from a cultivated resistant oilseed rape plant to closely related weeds would not be a major threat (Souza Machado 1982).

Spring sown oilseed rape (canola) is grown annually on 2.8 million ha in Canada, making this the most important oilseed crop. Farmers are encouraged to produce canola since it fits well with rotational practices and provides a sound financial return. There are two principal restrictions to the adoption of canola production. First, in regions where continuous maize production occurs, the residues of triazine herbicides in soil preclude canola production due to the crop's sensitivity to triazine carryover. Second, the widespread infestations (400,000 ha) of wild mustard (B. kaber) and stinkweed (Thlapsi arvense) (Grant & Beversdorf 1985) cannot be controlled by trifluralin, the most popular herbicide used for the control of green foxtail (Setaria viridis) and a range of broad leaved weeds. The competitive effects of stinkweed and wild mustard are severe, reducing yield by up to 25 %. In addition the weed seeds contaminate the grain, reducing the oil quality.

The introduction of the triazine-tolerant canola (TTC) cultivar OAC Triton has provided an attractive option to farmers who wish to grow canola in triazine-burdened land or where stinkweed and wild mustard are problems. The herbicides cyanazine (post-emergence) or metribuzin (pre or post-emergence) provide the selective broad spectrum weed control together with Cruciferous weed control in TTC. Unexpectedly, one of the dramatic improvements in weed control which followed the introduction of TTC in Eastern Canada took place in the maize crop. In the move away from continuous maize growing the previously uncontrolled wild prosso millet (Panicum dichotomiflorum) could now be effectively controlled in TTC using the graminicide sethoxydim. This compound also gave important control of wild oat (Avena fatua) and Setaria species. Volunteer TTC does not present a weed problem since the phenoxyacetic acid-based compounds used in the following wheat crop provide the necessary control.

The incorporation of the triazine tolerance trait causes significant reductions in several areas of agronomic importance. OAC Triton shows poor early seedling vigour, lower oil yield and oil content together with delayed maturity. The triazine-tolerant genotype also reduces the competitive fitness of the crop compared to near-isogenic lines of triazine-susceptible genotypes (Gressel and Ben-Sinai 1985). Triazine tolerance has been found to result from a gene mutation. A chloroplast membrane-located polypeptide changes in one amino acid (serine to glycine) (Hirschberg and McIntosh 1983). This mutation causes various chemical and structural changes in the chloroplasts of tolerant plants (Fuerst et al. 1986), thus reducing the affinity for triazine binding at the site of action and thereby promoting triazine tolerance. Associated with these changes are slower CO_2 fixation rates in the resistant biotypes (Ahrens & Stoller 1983, Holt et al. 1981). This physiological disability is reflected in the agronomic performance of present cultivars of TTC. Grant & Beversdorf (1985) used the heterosis from F1 hybrid forms of B. napus to examine the feasibility of improving the yield and quality of a TTC genotype Atr-Regent. Hybrids produced yields which were significantly

greater than Atr-Regent but in most cases the hybrids were intermediate to the parents in the economically important traits.

The adoption of TTC cultivars by Canadian farmers is presently restricted by the agronomic performance of the cultivars. Faulkner (1982) suggested that unless the weed problems of the crop are exceptionally severe, it would be pointless to breed tolerant cultivars unless they were as good as existing cultivars in other characters. The order of importance of TTC can be judged by the fact that in 1986 3.2 % of the canola area grown in Western Canada (84,000 ha) was sown to OAC Triton. Recently the future of TTC has been questioned with the forthcoming introduction of a selective sulfonylurea herbicide, DPX A7881 for the control of stinkweed and wild mustard in canola.

Imidazolinone-tolerant maize

The imidazolinones are a new class of herbicide which include the non-selective compound imazapyr (Shaner & Anderson 1985) and the compounds imazaquin and imazethapyr, providing broad- spectrum weed control in leguminous crops (Peoples et al. 1985). The biochemical mechanism of action of the imidazolinones is quite specific (Shaner et al. 1984), they kill plants by inhibiting amino acid biosynthesis via an interference with the enzyme acetohydroxyacid synthase (AHAS) (Shaner et al. 1984, Shaner & Anderson 1985). Imidazolinone-tolerant maize cell lines have been selected in cell culture and regenerated into whole plants which possess imidazolinone tolerance (Shaner & Anderson 1985). The tolerance is due to an altered AHAS enzyme and the trait is believed to be inherited as a single dominant gene. Recently, Anderson & Georgeson (1985) have shown that imidazolinone-resistant maize cells also showed cross-resistance to sulfonylurea herbicides. This is not unexpected since the primary mechanism of action of both herbicide families is similar (Shaner et al. 1984).

The development of imidazolinone-tolerant maize cultivars could provide farmers with the option to use a new generation herbicide which may be environmentally more desirable than the existing triazines. Broad spectrum weed control could be particularly valuable with respect to the troublesome graminaceous weeds, shatter cane (Sorghum bicolor) and wild proso millet (Panicum dichotomiflorum). Indeed, farmers could usefully integrate imidazolinones with the existing range of herbicides used in the traditional maize-soyabean crop rotation. Volunteer imidazolinone-tolerant maize plants could be controlled selectively in soyabeans using graminicides such as sethoxydim or fluazifop-butyl. Rather than restrict these crops to only a few herbicides, imidazolinone-tolerant maize cultivars would allow an extra choice of herbicide. It will become apparent in later discussions that it is desirable to have access to a wide range of herbicides to minimise the risk of developing herbicide-resistant weed species. Future adoption of herbicide-tolerant cultivars may again depend upon the agronomic performance of the cultivars available. To date, there is no evidence to suggest that imidazolinone-tolerant maize lines will be at any yield disadvantage over their imidazolinone-susceptible counterparts (Shaner 1987, Personal communication).

FUTURE CONSIDERATIONS

Present knowledge suggests that herbicide-tolerant cultivars will not cause a major revolution in cropping practices, rather they will fulfil a specialist niche where particularly troublesome weeds cannot be controlled by existing herbicides. In Europe it would seem imprudent to invest in herbicide-tolerant cultivars for compounds whose long-term futures are in doubt on the basis of their environmental fate or toxicology eg. triazines or bipyridilliums. Similarly, it must be acertained that the volunteers from herbicide-tolerant cultivars could be easily controlled in mixed cropping situations. The cost of glyphosate will probably preclude the introduction of crop tolerance to this herbicide from all but high value specialist crops. Sulfonylureas and imidazolinones are herbicides which may be in the greatest demand in pursuits to generate herbicide-tolerant cultivars. These herbicides combine a specific mechanism of action with low mammalian toxicity and weed control efficacy. Increased use of the imidazolinones and sulfonylureas in general crop production would be undesirable since due to their similar mode of action , both herbicide families would exert a heavy selection pressure on a weed flora which is likely to produce resistant biotypes.

The introduction of herbicide-tolerant cultivars may be accompanied by a risk of spreading this trait to closely-related weed species and the development of cross-resistance to more than one herbicide. The potential for inter and intraspecific transfer of herbicide tolerance should be evaluated with the same thoroughness which biological control agents receive with respect to host specificity. Presently, research on this topic is notably scarce. In the genus Avena it is possible to produce fertile progeny from inter-specific crosses between cultivated oats, Avena sativa L. and the wild oat Avena fatua (Luby & Stuthman 1983). Therefore the value of achieving selective wild oat control in the diclofop-methyl tolerant oat cultivar, Savena 1 (Warkentin et al. 1987) must be carefully considered. The diclofop-methyl tolerance trait in Savena 1 appears to be inherited by two genes in a simple Mendelian manner and can be incorporated into oat cultivars by a series of backcrosses. However, the risk of passing the diclofop-methyl tolerant genes into wild oat populations would make commercial exploitation of this trait unacceptable. By contrast, the triazine tolerance found in Setaria viridis has been shown to be of potential value in inter specific crosses with Setaria italica, foxtail millet, (Darmency & Pernes 1985). Weed control in foxtail millet is presently difficult to achieve and if satisfactory agronomic performance of a triazine-tolerant cultivar could be produced, this would be very useful.

LeBaron & Gressel (1982b) recognised that cross-resistance to different members of the triazine family could be expected due to their similarity of mode of action. However, evidence of cross-resistance in plants from members of different herbicide families has not been documented. A comprehensive study by Fuerst et al. (1986) showed that triazine-resistant biotypes of smooth pigweed (Amaranthus hybridus), common lambsquarters (Chenopodium album), common groundsel (Senecio vulgaris) and canola (Brassica napus) showed resistance to atrazine, bromacil and pyrazon. The triazine-tolerant biotype showed a greater sensitivity to dinoseb. Similarly, triazine- resistant biotypes of awned canary-grass (Phalaris paradoxa) and black-grass (Alopecurus myosuroides) showed

resistance to methabenzthiazuron, a urea herbicide, and also increased tolerance to diclofop-methyl (Rubin et al. 1985). The competitiveness and the biological fitness of the resistant biotype of P. paradoxa was not disadvantaged over the susceptible biotype, indeed emergence rate, plant height and main spike weight were greatest in the resistant biotype. Herbicide cross resistance was also found in a population of annual ryegrass, Lolium rigidum resistant to diclofop-methyl. The resistance was extended to fluazifop-butyl, chlorsulfuron, metsulfuron-methyl and CGA 82725 (Heap & Knight, 1986).

These studies should alert researchers to the need to examine the specificity of the herbicide tolerance trait which has been included in the new cultivar. Crops can pose serious problems as volunteer weeds and it is important to establish the spectrum of activity of a wide range of herbicides on the herbicide-tolerant cultivar before it is released. In the case of increased sensitivity to a herbicide compared to the normal herbicide-susceptible cultivars, this herbicide could provide a useful means of volunteer control. The corollary is that where the herbicide-tolerant cultivar is found to show decreased sensitivity to a herbicide which is non selective in the herbicide-susceptible cultivars, this herbicide may also be used for selective weed control in the herbicide-tolerant cultivar.

GENERAL CONSIDERATIONS : HERBICIDE-RESISTANT WEEDS

The title herbicide-resistant weeds is immediately alarmist. The implications of herbicide-resistant weeds similar to herbicide-tolerant cultivars require explanation. To achieve this one can review historically the discovery, distribution and practical consequences arising from the occurrence of herbicide-resistant weeds in North America (Bandeen et al. 1982) and outside North America (Gressel et al. 1982). In summary, herbicide- resistant weeds have been recorded in monoculture situations where one persistent herbicide has been applied annually to provide weed control. Cross-resistance of weeds to unrelated groups of herbicides may also appear as a consequence of the initial resistant population (Gressel 1986).

CONSEQUENCES : HERBICIDE-RESISTANT WEEDS

In Europe, a wide range of species are reported to show herbicide resistance. These include Senecio vulgaris, Amaranthus retroflexus, Solanum nigrum, Poa annua, Chenopodium album and Alopecurus myosuroides. Herbicide-resistant weeds must be correctly identified before corrective action can be taken. Immediately this may present a problem, since the number of weed scientists who would have the expertise and the facilities to diagnose a herbicide-resistant weed biotype may be very limited.

Parochetti et al. (1982) described the specific alternative control methods available for herbicide-resistant weeds. The paucity of documented case histories in Europe was noted. However, these authors suggested some general principles. Essentially the following criteria might be examined

in selecting a control programme for herbicide-resistant weeds:

(1) Containment or voluntary quarantine of the location where the resistant weeds are found, to prevent the spread of the weed to other locations.
(2) The use of alternative or additional herbicides; in both cases the mechanism of action should be different to that of the original herbicide.
(3) Alteration of the existing cropping system especially if a monoculture can be avoided.

These principles have been used to varying degrees of success in the control of herbicide-resistant weeds. Ammon (1984) reported that in Switzerland atrazine-resistant plants of Chenopodium album were observed in the sixth year of continuous maize cultivation in the early 1970's. A crop rotation including maize (2 years), cereals (2 years) and sugar beet (1 year) was introduced. The herbicides used included atrazine plus contact foliar herbicides, phenoxy compounds and soil-applied herbicides. However, these measures did not appear to limit the increase of atrazine-resistant biotypes of C. album. Also in Switzerland, Beuret & Neury (1983) found that triazine-resistant weed species were present in maize, vineyards, fruit crops and non-crop areas. While a change towards the use of substituted urea herbicides gave satisfactory control of the triazine-resistant weeds, the risk of building up a new form of resistance was emphasised.

In the event that herbicide-resistant weeds cannot be controlled, competition with the crop will follow. There may be however, a significant difference between the competitive abilities of the resistant and susceptible biotypes. Putwain et al. (1984) showed that in the presence of interspecific competition, triazine-susceptible Senecio vulgaris was at a substantial advantage in terms of ecological fitness over the triazine-resistant biotype. Under non-competitive conditions Holt (1983) reported greater dry matter production and plant height in the triazine-susceptible biotype of S. vulgaris than in the resistant biotype. In competitive plantings with resistant and susceptible biotypes, the susceptible biotype had a greater biomass production of stems, roots, leaves and reproductive structures than predicted from non-competitive studies, while the resistant biotype had less. The physiological basis for the reduced growth vigour associated with the triazine-resistant genotype has been discussed previously.

The population dynamics of the triazine-resistant and susceptible biotypes of S. vulgaris have been examined in conjunction with observations of the phenology (Putwain et al. 1982). The long-term objective of the study was to formulate a model which could be used to predict the equilibrium of resistant and susceptible genotypes. Putwain et al. (1982) concluded that research should be initiated to determine the ecological fitness of resistant and susceptible biotypes in a crop environment, the comparative longevity and dynamics of seeds of these genotypes in soil and the impact of other environmental factors.

CONCLUSIONS

History has shown that weeds respond to the annual application of persistent, broad spectrum herbicides by developing herbicide-resistant biotypes. It is therefore naive to expect that herbicides can continue to be used simply as a means of providing efficient, cost-effective weed control. Instead, the longer term effects of herbicides on the abundance and diversity of the weed flora should receive consideration. Presently, one may attempt to model or predict the influence of herbicides on the development of resistant biotypes. Such a model may consider : (1) herbicide effects on selection pressure, (2) herbicide persistence and (3) herbicide effects on the seed bank for herbicide-resistant biotypes (Gressel & Segel 1982). Research is needed to provide data from field experiments upon which one can test the validity of such models and therefore assess their value as forecasting tools.

In the meantime there is clearly merit in making farmers and growers aware of the herbicide-resistant weed situation. Informed advice should also be given on the most satisfactory ways in which to implement weed control programmes which encompass the use of herbicide rotations, mixtures, increased use of less persistent compounds (Gressel 1987b) and appropriate cultural control and crop rotation practices. Basic research should continue in both crop and weed species to determine the mechanism of action of herbicides, the inheritance of herbicide tolerance, the ecological and environmental consequences of deliberately engineered herbicide-tolerant cultivars and the accidental spread of herbicide-resistant weed biotypes. It is only by improved understanding in these areas that we can hope to manage herbicide-resistant cultivars and herbicide-resistant weeds. Careful consideration should be given to local appraisal of the legislative, rotational, environmental, volunteer crop and resistant weed implications of herbicide-tolerant cultivars. The challenge is available now for weed scientists, crop breeders and agronomists to exchange expertise and collaborate in the research efforts required for the continued safe use of herbicides.

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