MOLECULAR PERSPECTIVES IN CROP PROTECTION:INTEGRATION WITH ARABLE CROP PRODUCTION

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ABSTRACT

This paper reviews selected areas where molecular techniques are presently making an impact upon crop protection including biorational pesticide design and transgenic crops for improved resistance to diseases, pests and herbicides. The scientific, environmental, and commercial aspects surrounding the adoption of herbicide-resistant crops are described using examples from current Canadian cropping practices. These examples illustrate the need to bridge the field trial experience between regulatory/ research experimentation and farmer-orientated trials which incorporate an appreciation of crop rotational systems, the associated weed flora and herbicide use.

INTRODUCTION

Molecular biology has its origins in applied biology and chemistry progressing via the structure and function of DNA to our present knowledge of molecular genetics. It is from this evolving scientific base that molecular biology has been harnessed to hasten progress in a range of disciplines including crop protection. The impact of molecular biology on crop protection has been subject to several reviews including Marshall and Atkinson (1991), Gatehouse *et al.* (1992) and Marshall & Walters (1994). The object of this review is to consider the current status of the principle applications of molecular biology in crop protection: design of chemical crop protection agents, the development of crop cultivars resistant to herbicides, diseases and pests and assessing the environmental impact of genetically modified organisms. The practical integration of herbicide resistant crops into low input arable crop production systems will be examined by the use of case studies.

BIORATIONAL DESIGN OF NEW PESTICIDES

Crop protectionists continue to be required to apply all their ingenuity and skill to improve the food supply for arable agriculture given the ever increasing demands from world population growth. This requirement is now framed in a background where it is increasingly difficult and expensive to screen, identify and market a useful new pesticide. Furthermore, each year the loss of approved pesticides via unfavourable toxicological properties is rarely balanced by the gain of new products. New pesticides must be effective at low rates, provide crop safety, possess minimal environmental impact and favourable toxicological properties. While traditional methods of pesticide synthesis and subsequent screening are likely to remain the primary source of crop protection products, advances in our knowledge related to the mode of action of existing pesticides can be used to probe novel biochemical sites of action. Accordingly, new research based upon our biochemical knowledge can be described as biorational (reviewed by Pillmoor & Foster, 1994). Essentially, biorational design of pesticides can be viewed as a logical adjunct to traditional approaches for identifying and developing new pesticides.

In biorational design the starting point may be the identification of a new biochemical target. It may be possible to determine the effect of inhibition of that target site by reference to the use of a traditional mutant organism which has previously been characterised e.g. *Arabidopsis*. A molecular approach might also be applied where the gene has been isolated which is responsible for a specific enzyme in a plant or fungus. In plants, the gene may be nullified in its effect by using anti sense RNA technology, best known for the delayed ripening tomato. The effect of the enzyme system on physiological and metabolic plant processes can then be studied. In selected fungi the analogous process is known as gene disruption and it has application in the study of mutation vs pathogenicity (Stahl & Schafer, 1992).

Thus the design of new chemical inhibitors relies upon our understanding of a particular enzyme. Knowledge about known inhibitors is often the starting point for further investigations e.g. metabolism-based herbicide selectivity (Brown *et al.*, 1991). In practice the most fruitful approach to inhibitor design has been through a consideration of the chemical mechanism employed by the enzyme (Pillmoor *et* al., 1991). Still, biorational design of new pesticides is still developing and evolving since to date there are no commercial examples where this approach to new pesticide discovery has succeeded. However, in the pharmaceutical area this approach notably for antibacterial and anticancer treatments has become productive (Kuyper, 1990).

TRANSGENIC CROPS

It is a prerequisite of sustainable systems of arable agriculture that continuous improvements are made in the provision of new varieties. Traditional technologies employed by plant breeders have over the past two decades been supplemented by new biotechniques including the adoption of genetic engineering. While the general breeding objectives in crop cultivars have seen trends towards a greater emphasis upon crop quality and resistance to pests and diseases it is in these target areas that cell and molecular biology techniques can be exploited. It is now technically possible to identify, isolate, clone and transform single genes into a range of crop plants.

Transgenic crops are already a practical reality and have been released for controlled field experiments in a wide range of countries around the world. The dominant themes are resistance to virus diseases, insect pests and herbicides. Examining the published release permits for trials around the world (Table 1) provides a clear indication of the future opportunities (Beck & Ulrich, 1993).

Table 1. Number of approvals granted by crop and trait to 1993*

	Traits							
Crop	Resistance Herbicides	to: Viruses	Fungal diseases	Insects	Crop quality	Crop fertility	Stress resistance	% Total
Rapeseed	242	2	2	1	21	27	2	37.2
Potato	14	46	19	29	17		2	16.0
Maize	42	8	1	13	3	5		9.1
Tomato	18	14	5	13	19			8.7
Flax	45							5.7
Cotton	26			15				5.2
Soybean	35		1		3			4.9
Sugar beet	21	8						3.7
Alfalfa	11	6				1	4	2.8
Others	8	34		5	4	4		7.0

* After Beck and Ulrich (1993)

Resistance to diseases and pests

It is apparent that our understanding of host-pathogen interactions is far from complete. Therefore only after very detailed studies with plant-virus interactions has it been possible to engineer pathogen-derived resistance in plants (reviewed by Ward *et al.*, 1994). In this resistance strategy the functions of the viral genomes are transferred to the host plant in order to interfere with the normal life cycle of the virus. Thus host expression of pathogen-derived genes is responsible for the protection against the specific virus disease.

Although the plant-virus interaction system has been well characterised it is clear that understanding resistance to fungal pathogens involves several extra levels of complexity. Therefore defining the genes of critical importance in the host response will not be a simple matter but rather will require an increased understanding of the biochemistry and molecular genetics of the host-pathogen response (Ward *et al.*, 1994). For the forseeable future it is likely that crop cultivars will rely for fungal disease resistance upon traditional breeding technologies.

Protection against insect pests which cause crop damage and may transmit viruses has conventionally been achieved by applying pesticides. Now however the use of pesticides can be reduced where insect-resistant crops are adopted. In general terms there are three strategies currently available (Gatehouse & Hilder 1994). First, the use of plant derived insecticidal genes such as proteolytic enzymes; second, insect-resistant transgenic plants expressing plant derived genes e.g. cowpea trypsin inhibitors (CpTI)- see Hilder *et al.* (1993); third, insect-resistant transgenic plants expressing the insecticidal toxin normally produced by *Bacillus thuringiensis* (*B.t.*), reviewed by Peferoen (1992) and Barton & Miller (1993).

One of the emerging problems in relation to field studies with this strategy for insect resistance is the development of resistance by insects to the *B.t.* crystal proteins (Tabashnik *et al.*, 1990). Gatehouse & Hilder (1994) concluded that insect resistant transgenic plants are a viable means of producing crops with significantly enhanced levels of resistance and the adoption of the technology was not limited by suitable genes but rather regulatory barriers and consumer acceptability.

Herbicide resistant crops: principles and current practices

The use of herbicides in arable crop production has revolutionised our ability to manipulate the availability of water, minerals, light and space in favour of crops while weeds are controlled. In addition, selective herbicides have evolved by the efforts of industry to possess low toxicity to non-target organisms and dissipate rapidly in the environment. Our knowledge of the biochemical, physiological and genetic basis of herbicide mode of action is now advanced to the extent that many of the world's major crops can now be transformed to confer herbicide resistance. Ambitions to produce herbicide-resistant crops are driven via two principal mechanisms. First, herbicides are frequently well characterised in terms of the biochemical basis of gene function. Therefore gene isolation, cloning and transformation of plants together with a readily-selectable morphological marker provide a challenging academic system to investigate. Clearly, where single genes can be manipulated in this fashion an excellent model system is established to provide a guide for other gene acquisitions in plant improvement. Second, the vast majority of this research has been funded by the agrochemical industry and its plant breeding or biotechnology-related partners. The private sector have essentially used modern environmentally benign herbicides which are not off patent to produce an opportunity to maximise the return on their research investment in both the herbicide and novel plant varieties. The practical consequences of this approach are outlined in a later section.

The scientific background, techniques and current state of the art in herbicide resistant crops are reviewed by Gressel (1993) and Cole (1994). A summary of the anticipated launch years for a selection of world crops is presented in Table 2. Clearly the current emphasis is on the development of crop resistance to two non-selective herbicides, glyphosate with its renowned translocation ability and glufosinate (phosphinotricin) for its contact and limited translocation properties. Within the next few years farmers in the UK are likely to have the opportunity to grow herbicide resistant rapeseed and perhaps sugar beet. As Canadian farmers are planting herbicide resistant rapeseed this year it is a useful case study to consider with a view to examining the integration of this development in arable agriculture.

Herbicide-resistant crops in low-input production systems

Rapeseed is grown on some 3.0 million ha in Canada. The production system uses spring-sown cultivars only and by comparison with rapeseed production in the UK can be considered low-input. The only significant crop protection chemical applied is

Table 2. Anticipated commercial availability of herbicide-resistant crops

Crop	Country	Phosphinothricin	Glyphosate	Imazethapyr	Chlorimuron
Rapeseed	Canada	1995	1995	1995	-
	Europe	1997-98	1999-2000	-	-
Soybeans	USA	1997	1996	1995	1993
Maize	USA	1997-98	2000	1991	-
Cotton	USA	1998	1998	-	-
Sugar Beet	USA	2000	2000	-	-
	Europe	2001	1998	-	-
Wheat	USA/Europe	>2000	>2000	-	-

herbicide. Although rapeseed is a competitive crop, uncontrolled cruciferous weeds, wild oats, *Setaria* species and cereal volunteers can reduce the crop yield and quality significantly. Traditional weed control programmes relied upon trifluralin for broad/grass weeds with a follow-up post-emergence graminicide application. In 1990 a new selective sulfonylurea herbicide (ethametsulfuron) was approved for use specifically to control the ubiquitous *Sinapis arvense* (wild mustard). Recently Canadian farmers have become aware of widespread resistance of grass weeds to the popular graminicides (acetolactate synthase or ALS inhibitors) and the introduction of herbicide-resistance rapeseed will provide a new management option.

For 1995 the Canadian farmer has three options with herbicide resistant rapeseed. This concept is not revolutionary since triazine-tolerant rapeseed varieties were used in Canada during 1985-90 (Marshall, 1987). The first option open to selected farmers is the Roundup Ready® Canola (rapeseed) to be 'trial-grown' on 800 ha. Monsanto will oversee the crop production, harvest and seed crushing. This introductory field production will serve to create awareness of the product and will undoubtedly generate subsequent demand for seed from farmers, assuming the variable costs are in line with maintaining the gross margin for the crop.

Glyphosate will be recommended for application at the 0-6 leaf stage of crop growth with use rate of *ca*. 356 g a.i./ha. Repeat applications may be required to control late weed growth especially since glyphosate's spectrum of activity favours grass weeds rather than broad leaved species at these low rates of application. The level of resistance to glyphosate is moderate only therefore transient crop yellowing may be noted. In addition only one Roundup Ready® canola cultivar is presently available based upon the previously popular cultivar Westar. Thus the agronomic performance of this transgenic cultivar (ignoring herbicide-resistance) will be generally inferior to currently available non-transgenics. To counter this initial lack of choice for farmers, Monsanto hope by 1997 to have 8 other cultivars available all with glyphosate resistance.

The second option is the use of the AgrEvo herbicide/cultivar package which is based upon the canola cultivar Innovator and Liberty Link® (glufosinate resistance). Again, the agronomic performance of the cultivar is not on a level with existing non-transformed genotypes but 4-5 new cultivars are awaited for 1997. Seed is available to treat some 16,300 ha in 1995. The resultant canola must be segregated from other rapeseed and sold only into the North American market. Crop safety following the use of glufosinate (at any stage of crop growth) is excellent although at the rates of use proposed (300 g a.i. ha) repeat applications will probably be required in one growing season especially since volunteer cereals and small perennial weeds may prove difficult to control.

The third option is a non-transgenically produced herbicide resistant canola, cv. Pursuit Smart® released via collaboration by American Cyanamid and Pioneer Hi-Bred companies. The cultivar is resistant to the imidazolinone herbicide inazethapyr (post emergence, selective only in legume crops, residual and translocated activity). Over 57,000 ha of Pursuit Smart canola could be planted in 1995. This herbicide has the advantages of requiring only one application per season and crop tolerance is good. Imazethapyr is however relatively weak on volunteer cereals and will not control ALS resistant weed biotypes which are already part of Canadian prairie agriculture. The agronomic performance of this cultivar appears to significantly better than Westar upon which most of the transgenic canolas are based.

The integration and adoption of these herbicide-resistant cultivars will depend on both economic and agronomic factors. At the moment a traditional herbicide programme (trifluralin or ethylfluralin or ethametsulfuron or clopyralid followed by a graminicide) would cost about $45-75/ha plus seed costs $11-50 /ha (mean cost $62-80 /ha). By contrast estimates for Pursuit Smart® are $45/ha for seed and $45/ha for herbicide, for Innovator/Liberty Link® $42/ha seed and $45-90/ha for herbicide and finally Roundup Ready® canola $87 for seed and $5-10/ha for herbicide. Overall, the extra cost of adopting the new herbicide resistant canola will be some $25-50/ha plus the disadvantage that some of the present herbicide-resistant cultivars may not show the same yield, quality and disease resistance as recent non-herbicide resistant cultivars.

Therefore it is evident that rather than becoming an overnight success and relegating traditional production systems to a more minor role, herbicide resistant rapeseed cultivars will occupy a specialist niche in Canadian agriculture. It may indeed appear ironic to those who considered new herbicide resistant crops would increase the risk of spreading resistance genes in the environment to discover their utility in weed control programmes designed to reduce the impact of existing herbicide resistant weeds. Certainly these remarks apply for the non-selective glyphosate and glufosinate-resistant rapeseed varieties. However, the use of imidazolinone-resistant rapeseed in areas where ALS-resistant weeds were present could not be recommended. The opportunity to shift the emphasis of soil-applied herbicides such as trifluralin towards post-emergence herbicides made possible by the herbicide-resistant rapeseed will be welcome as a means of minimising unnecessary tillage thus preventing soil erosion and enhancing moisture conservation. Similarly, herbicide carryover from one season to the next will not be a problem for either glyphosate or glufosinate.

A second Canadian example of the integration of a transgenic crop into a traditional cropping programme has recently been described by McHughen and Holm (1995). In this field study the concerns raised about the commercialisation of transgenic herbicide-resistant crops (increased useage of herbicides, non-sustainable practices, lack of gene expression in the field or agronomic penalties) were addressed in a three year field trial using sulfonylurea-resistant linseed cultivars. The results showed that at least one transgenic line was fully resistant to the field rates of herbicide, no agronomic penalties were shown in the presence or absence of herbicide and the adoption would lead to reduced chemical usage and more sustainable agronomic practices in commercial production.

Herbicide resistant crops: future issues

The above represents an interpretation of the immediate impact following release of these herbicide-resistant rapeseed cultivars. There remains however some longer-term issues which are not so easy to resolve or predict with certainty. Pricing policies of the vendors of the herbicide-resistant seeds and the associated herbicides will undoubtedly have a major influence on the adoption of these crops by farmers. Similarly unless the agronomic performance of these cultivars sold at 'premium' prices can more closely compete with the best of traditional cultivars they will remain as minor use or relegated to obscurity. It will also be interesting to see if the market and consumer loyalty for the high quality image of Canadian rapeseed (canola) will remain unmoved by the introduction of the transgenic herbicide resistant cultivars.

The remaining environmental issue which is presently incompletely resolved with universal satisfaction is that of the possible introgression of herbicide resistance genes from rapeseed into weedy relatives such *Sinapis arvensis*. Controlled and natural interspecific crosses were performed by Downey *et al.* (1991) among four Brassica species and *S. arvensis*. These authors concluded that gene transfer from the three major oilseed species to *S. arvensis* was not achieved under the most favourable conditions, and no hybrids were identified from natural crossing of these species when they were co-cultivated in field plots over a three year period. Still these authors acknowledged that although gene transfer among the oilseed-brassicas under natural conditions can and probably does occur, the natural barriers for such gene flow in the weedy species is formidable and would not occur. Similarly Darmency (1994) concluded that hybrids between rapeseed and *S. arvensis* set no seeds, however those between the crop and wild radish (*Raphanus raphanistrum*) set 0.3 viable seeds per hybrid in the first backcross generations. These results show that gene introgression in wild Brassica populations can occur at different rates in different species.

Clearly the opportunities for introgression of herbicide-resistance genes are going to depend upon the local associated vegetation, the flowering dates of the species and reproductive behaviour of the various plants. To date risk assessment field studies have been criticised for their lack of attention to the dynamics of pollen flow (Mellon and

Rissler, 1995) although the invasiveness of transgenic rapeseed in 12 different habitats in the UK has been reported by Crawley *et al.* (1993). While the transgenic rapeseeds included in this research proved no more invasive than non-transformed rapeseed the authors cautioned that risks for other transgenics must be assessed on a case by case basis.

Volunteer crops represent some of the potentially most serious weed control problems and herbicide resistant crops might potentially reduce the herbicide choice which farmers have in their control. With a glyphosate, glufosinate or imidazolinone-resistant rapeseed, volunteer control should be possible by the application of a phenoxyalkonoic herbicide similar to non-transgenic rapeseed. If however, glyphosate-resistant potato cultivars were introduced, volunteers would present a serious weed problem since glyphosate is presently a preferred method of volunteer potato control. It is obvious that the introduction of a herbicide-resistant crop cultivar must be carefully considered with respect to the existing cropping regimes and herbicide availabilities for a region or country.

CONCLUSIONS

Within the rapid evolution of techniques in molecular biology there can be no doubt that many aspects within the food and fibre production chain can benefit scientifically from their application. The residing uncertainties in terms of the benefits which will be accrued in practical crop production are principally concerned with the unchartered territory between laboratory or researcher trials and commercial production. Present world-wide trialling of transgenic crops has its focus on herbicide resistance conferred by single genes, but in years to come should the transformation of polygenes become a reality this present development will become eclipsed. As we adopt such high-technology crops into our traditional systems with all their heritage of regulated trialling and release, Dyer (1994) asks the prudent question will anyone monitor the use of herbicide resistant crops ? In the UK if we are to integrate the benefits which molecular technologies can bring to sustainable systems of crop production we need to consider which transgenic crops are most suitable, which transgenes should be used and which should be rejected as unsuitable for our cropping systems. Perhaps the real test for the products of molecular biology is just about to begin in earnest.

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