SESSION 3B

NEW TECHNOLOGIES AND NEW DIRECTIONS IN WEED CONTROL

CHAIRMAN DR E. J. EVANS

SESSION ORGANISER DR T. G. A. CLEMENCE

NOVEL APPROACHES TO WEED CONTROL: NEW TRICKS FOR THE OLDEST PROFESSION

R. J. FROUD-WILLIAMS

Department of Agricultural Botany, School of Plant Sciences, The University of Reading, TOB 2, Earley Gate, Whiteknights, Reading, Berks, RG6 2AU.

ABSTRACT

Recent developments in bio-technology are discussed in relation to selective weed control in intensive agriculture. Consideration is given to the use of biological control, natural phytotoxins and crop tolerance to herbicides. Each technology is discussed in relation to economic and practical limitations. Particular emphasis is given to the role of genetic manipulation involving recombinant DNA technology. It is concluded that, although environmentally desirable, microbial weed control is unlikely to receive commercial support until improvements in formulation and reliability of performance can be guaranteed. Conversely, the introduction of herbicide-tolerant crops, albeit attractive commercially, raises some undesirable implications.

INTRODUCTION

Prior to the discovery of selective herbicides, weed control was achieved by a combination of rotation, cultivations and use of clean seed. During the past fifty years temperate agriculture has enjoyed the privilege of selective weed control through the agencies of synthetic chemicals. However, recent concern as to toxicology, persistence and environmental safety have necessitated a reconsideration of their role. Furthermore, development of resistance and punitive costs associated with registration have contributed to this re-appraisal.

The purpose of this overview is to consider recent developments in biotechnology that have afforded new opportunities for weed management in intensive agriculture. Essentially this review considers opportunities for weed control afforded by recent developments in the areas of biocontrol, novel products and improved crop tolerance to herbicides.

BIOLOGICAL CONTROL

The classical approach

Biological control of weeds is achieved through the use of living organisms employing a range of agencies including arthropods and fungi. Various approaches to biological control have been adopted and have been reviewed recently (Wapshere *et al.*, 1989). The classical approach involves the control of alien species with exotic organisms from the host's native range. Typically, this has included phytophagous insects but the introduction of the fungal pathogen *Puccinia chondrillina* into S.E. Australia and the subsequent successful control of *Chondrillina juncea* in wheat/fallow rotations is worthy of mention.

The classical approach is most appropriate to areas of extensive

agriculture where the use of herbicides would be financially prohibitive. As yet, the introduction of exotic organisms into the U.K. for the control of indigenous species has not been sanctioned, although the proposed release of the South African moths *Conservula* sp. and *Panotima* sp. as bio-control agents of *Pteridium aquilinum* may set a precedent (Lawton *et al.*, 1988). Future prospects for the classical approach have been discussed elsewhere (Evans & Ellison, 1990) whilst the potential for weed control using fungi has also received considerable attention (Hasan & Ayres, 1990).

The inundative approach

The role of pathogens as biocontrol agents entered a new conceptual era following the development of the inundative approach. This involves the periodic release of native pathogens for the control of indigenous species. Indeed, biological control has "come of age" following the serendipitous observation of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* as a pathogen of *Aeschynomene virginica* and its subsequent development as the mycoherbicide COLLEGO (R) (Templeton *et al.*, 1986).

Hitherto, biological control had remained a function of the public sector, there being limited financial incentive, as a pre-requisite of the classical approach is that the organism should be self-perpetuating. Furthermore, once released, there would be little to prevent its spread to untreated areas. An advantage of mycoherbicides is that they should be restricted to the area of treatment, with limited capacity for dispersal, and that they should lack persistence, necessitating repeated application. Such attributes, together with the necessity for formulation, render their development appropriate to the agrochemical industry. However, as yet commercial developments have been restricted to COLLEGO(R) and DeVine(R), a liquid concentrate of chlamydospores of *Phytophthora palmivora* for the control of *Morrenia odorata* in citrus, but which failed to secure a commercial market owing to persistence.

Speculation as to the limited development of further products has been inferred, including restricted specificity, unacceptable levels of control, unpredictable reliability, production limitations and difficulties of formulation (Greaves & McQueen, 1990).

Specificity, although desirable from an environmental viewpoint, is of limited value to commercial development, for weeds rarely occur in pure stands. However, in practice, it is unlikely that complete host specificity would be achieved. Unacceptable levels of control may be augmented by the addition of herbicide (Wymore *et al.*, 1987). Indeed, for mycoherbicides to form an integral component of crop protection systems it is essential that they are compatible with other agrochemical use.

Recent developments in biotechnology could provide further opportunities for development of mycoherbicides, notably through genetic manipulation (Greaves *et al.*, 1989; Bailey, 1990). Current ignorance of factors that regulate fungal pathogenesis and host specificity necessitate a greater understanding of biochemical and genetic bases of these processes (Kistler, 1991). At present, little is known concerning the latter, for although the technology of gene isolation is developing rapidly, as yet few genes associated with pathogenesis have been described (Hargreaves & Turner, 1990). Processes necessary for infection include recognition of plant surfaces that enable development of appressoria and haustoria. However, attempts to improve pathogenicity must be consistent with natural infection processes,

for genes that improve pathogenicity in one organism may not necessarily be effective in another.

Despite a knowledge of enzymes involved in pathogenesis *viz* cutinases, pectinases and cellulases, little is known of genes encoding these enzymes. The introduction of the gene encoding cutinase from *Fusarium* sp. into *Mycosphaerella* sp. enabled this wound pathogen to breach intact cuticles (Dickman *et al.*, 1989). Considerable potential exists for the inhibition of phytoalexins. Transference of the gene encoding Pisatin dimethylase from *Nectria haematococca* into *Cochliobolus heterostrophus* enabled this pathogen, normally pathogenic on maize, to attack pea. Of particular note has been the observation that *Senecio vulgaris* infected by *Puccinia lagenophorae* suffered mortality following infection by *Botrytis cinerea*, suggesting a degree of synergism, possibly resulting from rust-induced accelerated senescence, enabling infection by the normally non-pathogenic *B. cinerea* (Hallett *et al.*, 1990).

Production limitations may result from the inability to sporulate on media. A possible reason for the widespread use of Deuteromycetes, in particular *Colletotrichum*, is their suitability for mass production, a characteristic as yet unrealised for obligate parasites. Much of the unreliability attributed to mycoherbicides may reflect minimum dew point requirements of between 6-8 hours, associated with the necessity for very high inoculum thresholds. Recent developments in formulation involving the use of invert emulsions may greatly improve their performance and avoid the necessity for inoculum thresholds (Amsellam *et al.*, 1991)

Novel Products

Limitations imposed on the commercial exploitation of mycoherbicides could be obviated through the use of microbial phytotoxins (Misato & Yamaguchi, 1984; Poole & Chrystal, 1985). The potential role of natural plant compounds and microbial phytotoxins as herbicides has been the subject of several recent reviews (Duke, 1986; Duke & Lydon, 1987; Cutler, 1988 and Kenfield *et al.*, 1988). Allelochemicals produced by higher plants are of limited phytotoxicity and generally non-specific, whilst the commercial production of secondary plant metabolites, e.g. terpenes and sesquiterpenes, is regarded as non-economic (Putnam, 1988). Conversely, microbial phytotoxins are of considerable phytotoxicity, show evidence of host and non-host specificity and are relatively inexpensive to produce by fermentation. In addition, relative to mycoherbicides they are easier to store, formulate and apply without risk of proliferation in the environment, active at low concentrations, independent of environmental factors and non-persistent. As such, microbial phytotoxins represent a considerably untapped resource (Mishra *et al.*, 1988; Jones *et al.*, 1988, Huang *et al.*, 1989). Furthermore they may provide the basis of directional synthesis of alternative herbicides. For example, bialaphos, a product of *Streptomyces viridiochromogenes*, the active ingredient of which is phosphono-thricin (PPT) provided the inspiration for the development of a synthetic analogue of PPT, namely glufosinate, a relatively non-selective herbicide (Fischer & Bellus, 1983). Tentoxin, produced by the fungus *Alternaria alternata*, shows potential for selective weed removal in maize, soyabean and cruciferous crops. Although difficult and costly to synthesise, current interest focuses on the production of an analogue (Lax *et al.*, 1988). Difficulties of plant penetration associated with mycoherbicides may be overcome by the use of surfactants, which is not possible with living organisms.

CROP TOLERANCE TO HERBICIDES

Intraspecific variation in response to herbicides has now been documented in at least 80 species of weed. Up until 10 years ago, the majority of instances were of as-triazine resistance, notably simazine and atrazine, the occurrence of which had been predicted, owing to their strong persistence and single-site mode of action. Since then resistances to a number of other herbicides have been recorded, including paraquat and the substituted phenyl ureas. With but few exceptions, these have occurred in situations of repeated mono-culture or mono-herbicide use.

The introduction of herbicide resistance into crops has some desirable benefits as it would extend the use of existing herbicides of shorter persistence and minimal environmental impact for use in a range of crops. However, this doesn't appear to have been the primary objective of chemical industry.

In order to confer resistance, it is vital to understand the mechanism(s) of resistance. At present our current state of knowledge concerning the precise mechanisms involved is incomplete, but the introduction of several herbicide groups of specific mode of action has greatly assisted in the quest to confer resistance into selected crop species.

At least five potential mechanisms of resistance have been identified *viz*:- morphological barriers, differential uptake/translocation, altered receptor sites, amplification of target enzymes and metabolic detoxification. Conferment of resistance could involve any one of these mechanisms, but modification of morphological barriers, e.g. cuticle thickness and altered compartmentalisation following herbicide uptake appear least likely. Until recently, it was considered that altered receptor sites, e.g. enzymes and thylakoid membranes, offered the greatest opportunity for manipulation, but remarkable advances in our knowledge of detoxification mechanisms have provided a new directional impetus.

Currently, there are four approaches to conveying herbicide resistance within crops, each of which has a number of advantages and disadvantages.

Classical plant breeding

Classical plant breeding has achieved some limited success but suffers the disadvantages of being time-consuming, laborious and involves a considerable space requirement. A limited number of generations may be selected per annum. However, the main limitation is that of incompatibility barriers between genetically dissimilar species. Nonetheless, some commercial varieties of atrazine resistant *Canola* have been achieved following backcrosses with birdsrape (Beversdorf & Kott, 1987). A yield penalty may be exacted if grown under conditions of environmental stress. This lack of ecological fitness is often typified by herbicide resistant weeds, accounting for their apparent infrequency in the absence of selection pressure imposed by herbicide use. This yield penalty is an acceptable cost enabling selective removal of *Sinapis arvensis*, which would otherwise result in crop rejection.

Somatic hybridization

Somatic hybridization avoids problems of incompatibility associated with

classical plant breeding through protoplast fusion, albeit that this has been restricted to closely related genera. Notably, it has involved conferment of atrazine resistance from weed species into closely related crops. Limited success has been achieved owing to hybrids retaining several weedy attributes (Gressel *et al.*, 1984).

In vitro mutant selection

In vitro mutant selection through the use of tissue culture has the advantage of selecting cell lines from a large number of individuals in a comparatively short time, using minimal space and at relatively low cost. However, not all species are amenable to regeneration from callus, the successes most cited involving members of the Solanaceae, in particular, tobacco.

Despite the difficulty of regenerating crops such as maize and soybean from tissue culture, an imidazolinone-resistant line of maize has been identified that will tolerate levels of imidazolinones 30 x greater than that capable of inhibiting the wild type (Shaner *et al.*, 1985). The apparent ease of selection by *in vitro* techniques belies the necessity for subsequent crossing with commercial germplasm prior to marketing (Newhouse *et al.*, 1990). Unlike the previous examples of classical plant breeding and somatic hybridization there does not appear to be an agronomic yield penalty.

However, tissue culture is not suited to selection of photosynthetic inhibitors since isolated cells are usually non-photosynthetic. The lack of differentiation may result in callus not performing as whole plants and consequently, selection may not reflect actual resistance. The greater sensitivity of individual cells requires a stepwise selection against herbicide concentration, the amplification of which may be lost after the selection pressure has been removed.

Transgenic engineering

Progress in the production of transgenic crops during the past decade has surpassed most expectations and the expression of herbicide resistance has been reviewed extensively (Fraley *et al.*, 1987; Botterman & Leemans, 1988; Botterman, 1989; Oxtoby & Hughes, 1990). These achievements now extend beyond laboratory testing to field evaluation and ultimate commercial release (De Greef *et al.*, 1989).

Essentially three approaches may be employed for the expression of herbicide resistance through genetic engineering. They involve structural modification of receptor sites, gene amplification and over expression of the target enzyme and detoxification (Hatzios, 1987). Of the various options, herbicide detoxification was initially considered the least appropriate because of insufficient knowledge of herbicide metabolism within plants.

Exploitation of transgenic engineering is dependent on the identification of genes conferring tolerance or resistance to herbicides. The discovery of several classes of structurally unrelated herbicides of single site mode of action has been particularly fortuitous to the development of this technology. In particular, the introduction of sulfonylureas and imidazolinones, inhibitors of acetolactate synthase (ALS) the first enzyme in the synthesis of the branched chain amino acids leucine, isoleucine and valine has been particularly instrumental in this respect.

Essential to the success of transgenic engineering is the availability of suitable vectors that permit efficient delivery of appropriate genes for transformation. Typically the T_i plasmid of *Agrobacterium tumefaciens* provides the vector but suffers a number of disadvantages including limited host range and lack of target specificity. Other potential vectors include viruses, including cauliflower mosaic virus (CaMV) which offers a single genetic system for transfer of foreign DNA and provides high levels of expression. Alternatively, vectors may be combined to facilitate greater host penetration.

SELECTED CASE HISTORIES

Sulfonylureas

Initially, tobacco mutants resistant to sulfonylureas were identified by means of tissue culture (Chaleff & Ray, 1984). Subsequently, mutant genes encoding for ALS resistance to sulfonylureas have been detected in a range of microorganisms. These are characterised by single amino acid substitutions coded for by the ilvG and ILV2 loci in bacteria and yeast respectively. However, attempts to introduce resistance from microbial genes are impaired by the fact that although nuclear encoded, ALS is localised within the chloroplast. Until comparatively recently, difficulties were experienced with chloroplast engineering. Furthermore, bacterial ALS is composed of two different sub-units, the expression of which may prove difficult (Fraley *et al.*, 1986).

Alternatively, sulfonylurea resistant mutants of *Arabidopsis thaliana* involving a single amino acid substitution have been identified (Haughn & Somerville, 1986) and the gene introduced into tobacco (Haughn *et al.*, 1988).

Tobacco mutants were demonstrated to contain two distinct ALS genes. One, designated the C3 mutant results from a single amino acid substitution of proline to glycine encoded by a single gene. The other, referred to as the S_4-Hra mutant, involves two substitutions, proline to alanine and tryptophan to leucine (Lee *et al.*, 1988). Transformation of tobacco involving these latter substitutions confirmed cross-resistance to both sulfonylureas and imidazolinones. These findings have been extended by *in vitro* mutagenesis and subsequent introduction into sensitive plants (Hartnett *et al.*, 1990).

Glyphosate

The transformation of glyphosate tolerance into crop plants has been pioneered by Comai *et al.*, (1985). They successfully conferred altered sensitivity to glyphosate in tobacco through the introduction of a mutant allele of the aroA gene from the bacterium *Salmonella typhimurium* which shows reduced affinity for glyphosate. In *S. typhimurium* the aroA gene encodes for the enzyme 5-enolpyruvylshikimate-3 phosphate synthase (EPSP synthase) essential to the formation of specific aromatic amino acids. Tolerance was subsequently conferred to tomato using a binary vector (Fillatti *et al.*, 1987). Although tolerant of glyphosate, transgenic plants of both species were stunted following herbicide application relative to the untreated control.

In plants, EPSP synthase is predominantly localised in the chloroplast whereas the bacterial aroA gene, which lacks a chloroplast transit-peptide

sequence gives rise to cytoplasmic forms of the enzyme only. This obstacle was elegantly addressed by fusion of a mutant EPSP synthase gene from *Escherichia coli* with a portion of cDNA that encodes for the transit-peptide sequence of the plant enzyme (Della-Cioppa *et al.*, 1987). This is of considerable significance to transgenic studies in that it illustrates that a plant enzyme may target a fully herbicide tolerant bacterial EPSP synthase to the chloroplast.

Alternative strategies for the conferment of resistance to glyphosate have involved gene amplification for overproduction of the target enzyme in *Petunia hybrida* and *Arabidopsis thaliana* respectively (Shah *et al.*, 1986; Klee *et al.*, 1987). In the former instance a cell line was selected for overproduction of EPSP synthase and a chimeric gene constructed with CaMV 35S promoter to attain high level expression and rapid import of precursor EPSP synthase into the chloroplasts. Transformed plants showed a four-fold level of resistance.

Phosphinothricin

Phosphinothricin, an analogue of glutamine, acts as an irreversible inhibitor of glutamine synthase (GS) resulting in lethal accumulation of ammonia in plant cells. Initial attempts to confer resistance involved gene amplification of the target enzyme (Donn *et al.*, 1984). An alternative approach involving detoxification has been successfully employed (Leemans *et al.*, 1987). This involved characterisation of the *bar* gene from *Streptomyces hygroscopicus* that encodes for phosphinothricin acetyl transferase which converts PPT to the non-toxic acetylated form (Thompson *et al.*, 1987). This gene has been introduced into tobacco, tomato and potato (De Block *et al.*, 1987).

Engineering for detoxification of herbicides has lagged behind other technologies because of insufficient knowledge of plant detoxification mechanisms. Such an approach is particularly desirable as attempts to modify target sites may incur loss of desirable agronomic characters. The appreciation that soil microorganisms degrade herbicides naturally has provided a new dimension to transgenic engineering. Thus a gene *bxn* encoding for a specific nitrilase that converts bromoxynil to its primary metabolite was cloned from the soil bacterium *Klebsiella ozaenae* and expressed in tobacoo and tomato plants (Stalker *et al.*, 1988). Likewise the *tfdA* gene of *Alcaligenes eutrophus* which encodes the enzyme 2,4-dichlorophenoxyacetate monoxygenase (DPAM) capable of the degradation of 2,4-D has been introduced into tobacco (Streber & Willmitzer, 1989).

Implications of herbicide resistant crops

Implications of engineering herbicide resistance has been discussed elsewhere (Goodman, 1987; Marshall, 1987; Van Oorschot, 1988 and Keeler, 1989). The latter author concludes that the risks of genetically engineered crops becoming weeds are minimal. Nonetheless, consequences of engineering resistance include not only the development of intransigent volunteers but also the possibility of introgression with wild and weedy relatives. Pollen is the most likely source of gene exchange and risks may be minimised by the use of cytoplasmically inherited resistance. Other potential vectors include viruses and nematodes.

However, a number of advantages may accrue such as extending the use of existing herbicides, including those off-patent, thus reducing further

development costs. Conferring resistance to more environmentally acceptable herbicides could allay public anxiety concerning toxicological issues.

Ideally, engineered tolerance should avoid the use of persistent herbicides of single site mode of action in favour of less persistent ones of greater environmental safety. Already resistance to sulfonylureas has been documented, together with cross-resistance to imidazolinones (Thill *et al.*, 1991). Conferring tolerance to foliar acting non-persistent herbicides such as glufosinate, 2,4-D and glyphosate would appear to be of particular merit. However, a note of caution should be offered, for naturally occurring resistance to both glyphosate and MCPA has already been reported (Duncan & Weller, 1987; Bourdot *et al.*, 1990).

CROP SAFENERS

Initial attempts at safening crops against herbicide injury involved the use of activated charcoal as a physical barrier, but subsequently more sophisticated approaches have been sought (Hatzios, 1989). Safeners have the advantage of extending the use of existing herbicides into new markets at favourable costs of development. They do, however, offer the prospect of allowing the use of more potent herbicides and higher rates of application.

Currently few crops are protected by safeners, e.g. maize, grain sorghum and rice against chloroacetanilides and thiocarbamates. As yet safeners for use in wheat, barley and oats have not been commercially exploited whilst difficulties have been experienced in safening of broad-leaved crops. Despite limited success of safeners against photosynthetic inhibitors and broad-spectrum herbicides such as glyphosate, a number of recent developments have been reported for use against the sulfonylureas and imidazolinones.

Future developments in the discovery of chemical safeners are likely to be hindered by a lack of understanding regarding their mode of action. However, considerable potential exists for the development of microbial safeners. The realisation that herbicides are subject to microbial degradation could permit their use as safeners. Although unlikely to be effective against foliar-applied treatments, they are likely to find use against pre-emergence soil-acting herbicides. Furthermore, such root-colonising organisms are potentially amenable to genetic manipulation, thus conferring additional crop tolerance (Karns, 1989).

CONCLUSIONS

The commercial improvement of crop tolerance to herbicides is currently being realised through recent advances in biotechnology including transgenic engineering. Likewise, mycoherbicides offer an alternative viable option but their commercial development presents a conflict of interest for chemical industry. The recent acquisition of plant breeding and seed development rights renders the exploitation of herbicide tolerant crops a more attractive proposition. Until mycoherbicides can attain reliable performance, they are unlikely to receive commercial support. This may be achieved through improved formulation, although their role may be superceded by the use of microbial phytotoxins. Nonetheless, mycoherbicides offer potential as adjuncts to chemical weed control or as "stand alone" products for intransigent species including herbicide resistant weeds.

Production costs associated with microbial phytotoxins may limit further expansion while the chemical complexity of secondary metabolites may constrain attempts at producing synthetic analogues. The release of herbicide tolerant crops and development of microbial safeners could conceivably undermine current political initiatives designed to reduce commodity surpluses through the adoption of less-intensive agriculture involving reduced herbicide use. It would be somewhat ironic if transgenic crops resulted in increased problems of volunteers, necessitating their removal with mycoherbicides.

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MICROBIAL COMPOUNDS WITH THE POTENTIAL FOR HERBICIDAL USE

STEPHEN O. DUKE, HAMED K. ABBAS, and C. DOUGLAS BOYETTE

U.S.D.A., Agricultural Research Service, Southern Weed Science Laboratory, P. O. Box 350, Stoneville, Mississippi 38776, U.S.A.

MASATOSHI GOHBARA

Mitsui Toatsu Chemicals, Inc., Life Science Laboratory, 1144 Togo, Mobara-City, Chiba-Pref., 297 Japan

ABSTRACT

Bacterial and fungal microbes produce a wide array of phytotoxic compounds with the potential for direct use as herbicides or as models for new structural classes and/or new sites of action for herbicides. Bialophos and glufosinate are the only microbial products that have been commercialized without modification. Industry has generally screened large numbers of non-pathogenic microbes for new phytotoxins; however, screening smaller numbers of plant pathogens that infect weeds for phytotoxins may be equally rewarding. Two examples of toxins from plant pathogens, colletotrichin and fumonisin B_1, are discussed in detail. Microbial toxins also offer potential new sites of action for biorational discovery of herbicides. Different strategies of herbicide discovery and development from microbial products are discussed with specific examples.

INTRODUCTION

With diminishing returns from traditional herbicide discovery strategies, new approaches with better probabilities of success are sought. One of these strategies is to screen secondary natural products for herbicidal activity. Many secondary products of plants and microorgansims are bioactive because they are the evolutionary result of eons of intra- or interspecies interactions. Therefore, one might assume that there is a higher probability of biological activity than with synthetic compounds, provided the targets (both the organism and the site within the organism) are properly identified. Some of the interest in microbial phytotoxins as pesticides is due to the popular, although questionable, notion that natural products are generally more toxicologically benign than synthetic compounds. Although plants have been good sources of antimicrobial and insecticidal compounds, they are perhaps less likely to generate potent phytotoxins because of autotoxicity (Duke, 1991; Lydon and Duke, 1990). Microbes, however, have proven to be a rich source of highly phytotoxic compounds. Thus, the herbicide industry has a generally increasing interest in this source of new compounds as older discovery strategies have become less productive.

The commercial pest control industry has more interest in microbially-produced compounds as herbicides or as leads for new herbicides than it has for commercializing the microbes themselves for biocontrol of weeds. A compound has the commercial advantages over the living organism of a longer shelf life, a requirement for yearly reapplication (it does not

renew itself in the field), generally more predictable and uniform results, and no possibility of spreading to non-target organisms. The requirements for successful development of biological control products have been reviewed by Reinecke (1990).

The topic of microbial products as potential herbicides has been reviewed several times and from various perspectives (Cutler, 1988; Duke, 1986; Fischer and Bellus, 1983; Hoagland, 1990; Kenfield *et al.*, 1988; Misato and Yamaguchi, 1984; Poole and Chrystal, 1985; Sekizawa and Takematsu, 1983). This brief review will update this topic with more recent literature and utilize several examples from the authors' own laboratories.

STRATEGIES

Several strategies, each with particular strengths and weaknesses, can be employed in utilizing microbial products as new herbicides or leads for new herbicides. One might decide to screen only compounds from non-pathogenic microbes such as soil microflora (*e.g.*, many of the the actinomycetes). This is the source of natural compounds that has been most commonly utilized by industry in antibiotic and pesticide discovery. There are two major advantages of these organisms. First, they are relatively easily cultured compared to pathogens. Second, they produce a multitude of bioactive products. Some of these compounds are relatively simple in structure and, therefore, might be economically synthesized. Unfortunately, many of these compounds have already been discovered, and a major problem with this approach is eliminating known phytotoxins such as cycloheximide or gabaculine early in the screening process (Ayer *et al.*, 1989; Heisey *et al.*, 1988). Much effort can be spent in discovering that new microbes produce known compounds. A screen which eliminates already discovered compounds early in the discovery process can save much wasted effort. In one company's microbial phytotoxin discovery program, 28% of all identified compounds proved to be new structures, and a further 16% were known compounds not previously reported to be phytotoxic (Ayer *et al.*, 1989). Most of these compounds were amenable to generation of synthetic analogues.

Plant pathogens often produce highly active phytotoxins as virulence factors. In fact, many of these microbes are actually saprophytes, killing the tissue with one or several toxins before invading it. Plant pathogens commonly produce several phytotoxins. Thus, the probability of a plant pathogen producing at least one phytotoxin is good. However, plant pathogens are generally more difficult to culture and to produce toxins in culture than non-pathogens. Furthermore, the phytotoxins produced by these organisms are often structurally complex, making structural elucidation and synthesis difficult. Some of the compounds are too specific in their selectivity to have commercial appeal as a herbicide. For these reasons, the herbicide industry has committed relatively few resources to plant pathogens as sources of herbicides. Most of the activity in this area has been the testing of compounds discovered and characterized by plant pathologists and natural product chemists working with plant pathologists.

Many microbial phytotoxins are too structurally complicated to be synthesized economically for agricultural use. For example, these compounds commonly have several chiral centers. Only two strategies are available to overcome this problem. Structure-activity studies might

result in discovery of a simpler molecule with a commercially viable ratio of synthesis cost to herbicidal activity. An example of a synthesized herbicide that was developed from a more complex microbial product is methoxyphenone which was derived from anisomycin, a *Streptomyces* metabolite. Alternatively, the molecule might be produced by fermentation. The only successful example of this is bialophos.

Even if the microbial product can be synthesized economically, there are other reasons that an analog might be marketed rather than the natural product. First, if the phytotoxic nature of the natural phytotoxin or a related natural compound has been previously reported, the patent may be less defensible than that of the synthetic analogue, with no mention of its source. Structurally, methoxyphenone is sufficiently different from anisomycin that it could probably have been patented with no mention of its origin. It is not known what presently patented herbicides may have begun with a structural clue from a microbial source.

In addition to new compounds and chemical classes, microbial phytotoxins are sources of new sites of action (Duke, 1986). Considering the relatively few potential sites of action of the herbicides derived by more traditional discovery strategies (Casida, 1990; Duke, 1990), this aspect of microbial toxins may become very important. There is little overlap between the known sites of action of microbial phytotoxins and commercial herbicides (Table 1). In fact, microbial phytotoxins demonstrate that there are numerous potential sites of action that have not been exploited by the herbicide industry. One reason for this discrepancy may be that the pesticide industry has focused almost all of its attention on halogenated organic compounds that are insoluble or poorly soluble in water. Many of the phytotoxins produced by microbes are small peptides or other molecules that are water soluble. We know the molecular mechanism of action of very few microbial phytotoxins (Table 1). Further research promises to reveal a greater array of target sites.

Phytotoxins from pathogens

Most phytotoxins known to be produced by plant pathogens were discovered by plant pathologists studying pathogens that infect crops or ornamental plants. Comparatively little effort has been expended in studying pathogens that infect weeds (Kenfield *et al.*, 1988). However, some of the pathogens that infect crops or ornamentals also produce phytotoxins to which some weed species are sensitive, and some of the non-host-specific toxins generated by weed-specific pathogens are identical to toxins produced by some crop- and ornamental-specific pathogens. In most cases, there is no published record on the effect of known phytotoxins on a range of weed species. We will briefly discuss a few examples of phytotoxins from plant pathogens.

Host-specific toxins

Some phytotoxins appear to selectively affect only those plant species which are infected by the producing pathogens. These phytotoxins are termed host-specific. All known host-specific phytotoxins except one are produced by pathogens that infect crop species. These compounds tend to be structurally complex and, in some cases, are phytotoxic to only a subspecies or a single variety. Maculosin (Fig. 1), a relatively simple cyclic diketopiperazine analogue of cyclic L-tyrosine-L-proline, is a host-specific phytotoxin from *Alternaria alternata* that appears to affect only spotted knapweed (*Centaurea maculosa*) (Strobel *et al.*,

TABLE 1. Summary of the known molecular modes of action of herbicides and microbial phytotoxins (From Duke, 1990 and Devine *et al.*, 1992).

Physiological site	Molecular site	Herbicide or Microbial Toxin
Amino acid synthesis	EPSP synthase	glyphosate
	acetolactate synthase	sulfonylureas imidizolinones
	glutamine synthetase	*glufosinate, tabtoxin* *oxetin, phosalacine*[a]
	aspartate amino transferase	*gostatin*
	many transaminases	*gabaculine*
	ornithine carbamoyl transferase	*phaseolotoxin*
	β-cystathionase	*rhizobitoxin*
Photosynthesis	D-1 quinone-binding protein	triazines, biscarbamates, anilides, hydroxynitriles, substituted ureas, benzimidazoles, uracils *stigamatellin, aurachins, cyanobacterin*
	CF_1 ATPase	*tentoxin*
Photobleaching	photosystem I diverters	bipyridyliums heteropentalenes
	protoporphyrinogen oxidase	diphenyl ethers oxadiazoles *N*-phenyl imides
	photodynamic compound	*cercosporin*
Lipid synthesis	acetyl-CoA carboxylase	aryloxyphenoxypropanoates cyclohexanediones
	acetyl-CoA transacylase	*thiolactomycin*
	3-oxoacyl-ACP synthase	*cerulenin*
Photosynthetic pigment synthesis	phytoene desaturase	substituted pyridazinones fluridone *m*-phenoxybenzamides 4-hydroxypyridines
	lycopene cyclase	aminotriazole
	ζ-carotene desaturase	dichlormate
	IPP isomerase and/or prenyl transferase	isoxazolidinones
	ALA synthase	*gabaculine*
Cell division	β-tubulin	dinitroanilines phosphoric amides
Cellulose synthesis	cellulose synthase?	dichlobenil
Folate synthesis	dihydropteroate synthase	asulam
Pyrimidine synthesis	aspartate carbamyl-transfersase	*AAL-toxin*
Plasmalemma function	membrane ATPase	*fusicoccin*
Plastid nucleic acid synthesis		*tagetitoxin*

[a]Microbial products are in italics

1990). The interest of the herbicide industry in host-specific toxins is not great because they are too selective.

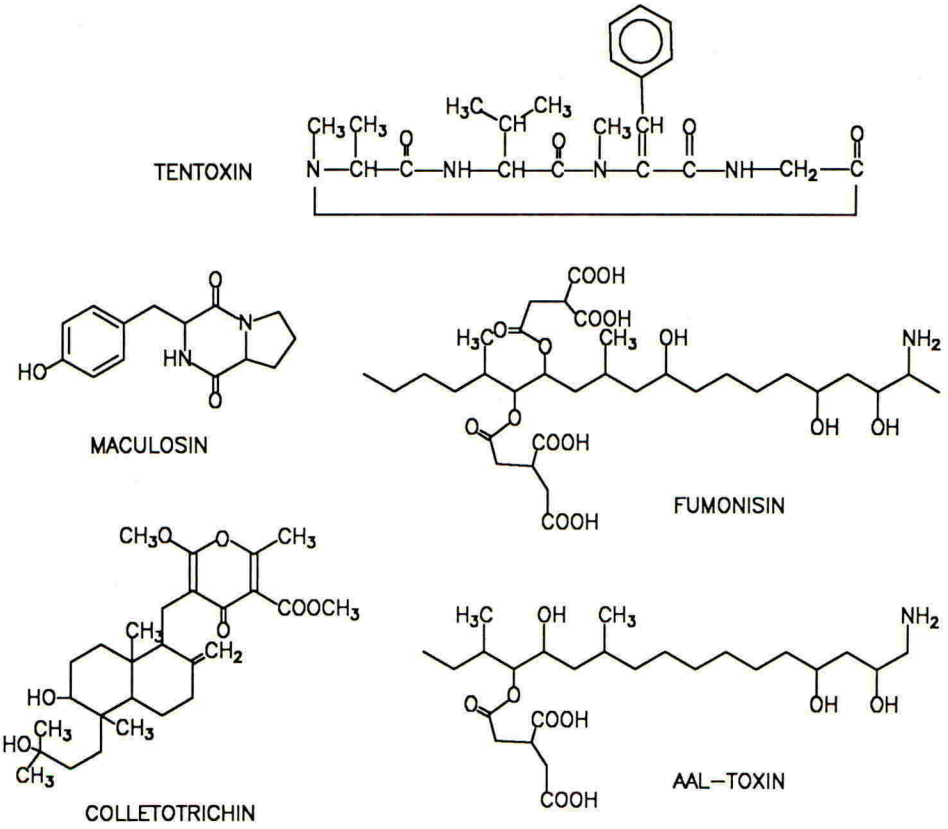

Fig. 1. Chemical structures of several phytotoxins mentioned in the text.

Non-host specific toxins

Non-host specific toxins are of considerably more interest because they often have the potential for killing a range of weeds in a particular crop without phytotoxicity to the crop. An example of such a phytotoxin is tentoxin (Fig. 1), a cyclic tetrapeptide produced by several *Alternaria* species. It causes severe chlorosis in many of the problem weed species associated with soybeans and maize without affecting either crop (Duke, 1986; Duke and Lydon, 1987). Tentoxin has two unique mechanisms of action. It inhibits choloroplast development by preventing movement of certain nuclear-encoded proteins into the plastid (Vaughn and Duke, 1984), and it inhibits photophosphorylation in green chloroplasts by inhibiting CF_1 ATPase (Steele *et al.*, 1976). Both synthesis and production of tentoxin by fermentation are currently too expensive for commercialization. Extensive structure-activity research has not produced a simpler, less expensive compound with similar activity (Edwards *et al.*, 1988). However, efforts have been made to understand the genetics of tentoxin biosynthesis so that the cost of its production by fermentation technology can be reduced (Lax and Shepard, 1988).

We have recently examined colletotrichin (Fig. 1), one of several related terpenoid products of *Colletotrichum tabacum*, a pathogen that infects tobacco and other solanaceous plants. It is toxic to tobacco, other solanaceous species (Duke *et al.*, 1992), as well as to cucumber (Fig. 2). It causes rapid peroxidative loss of plasmalemma integrity by an unknown mechanism that is not light-dependent. Unfortunately, the structural complexity of colletotrichin might limit interest in it as a potential herbicide. However, it is possible that structure-activity studies could identify simpler related molecules with equal or enhanced phytotoxicity.

Fumonisin (Fig. 1), a metabolite of *Fusarium moniliforme* that is structurally related to AAL-toxin (Fig. 1), causes symptomology similar to that of colletotrichin (Abbas *et al.*, 1991). It also causes loss of plasmalemma and tonoplast integrity, resulting in cellular leakage (Fig. 2) and eventual cell death. In most species, such as cucumber, the effect is light-dependent (Fig. 2), and, in others, it is enhanced by light. Fumonisin has a longer lag period prior to visual injury symptoms than does colletotrichin (Fig. 2).

Symptoms of fumonisin-induced injury are similar for a number of weed species, including prickly sida (*Sida spinosa*), spurred anoda (*Anoda cristata*), and jimsonweed (*Datura stramonium*) (Figs. 3 and 4), as well as sicklepod (*Cassia obtusifolia*) and hemp sesbania (*Sesbania exaltata*) (Abbas *et al.*, 1991). Monocot crop and weed species, as

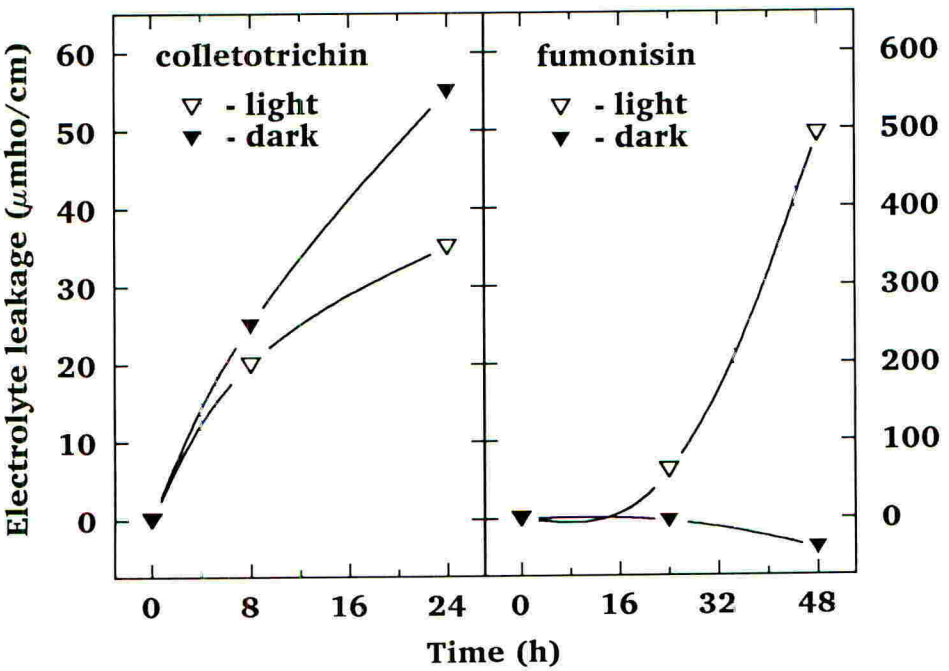

Fig. 2. Comparison of the effects of 30 μM colletotrichin and 33 μM fumonisin B_1 on cellular leakage of cucumber cotyledon disks in light (0.5 mE/m²/s PAR) at 25°C. Values are treated minus untreated values.

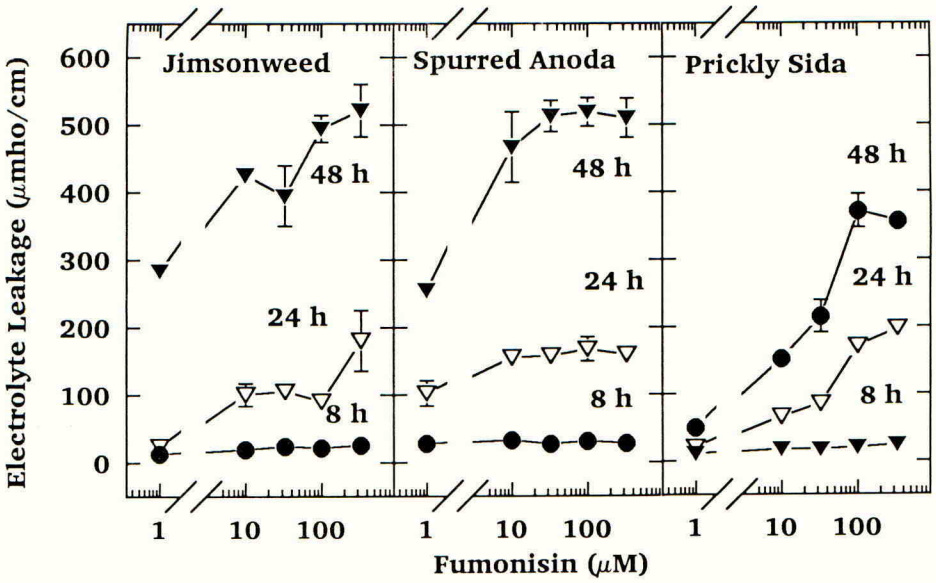

Fig. 3. Effects of different concentrations of fumonisin on electrolyte leakage of leaf disks of three weed species after exposure to the phytotoxin for 8, 24, or 48 h at 25°C under continuous light (0.5 mE/m²/s PAR).

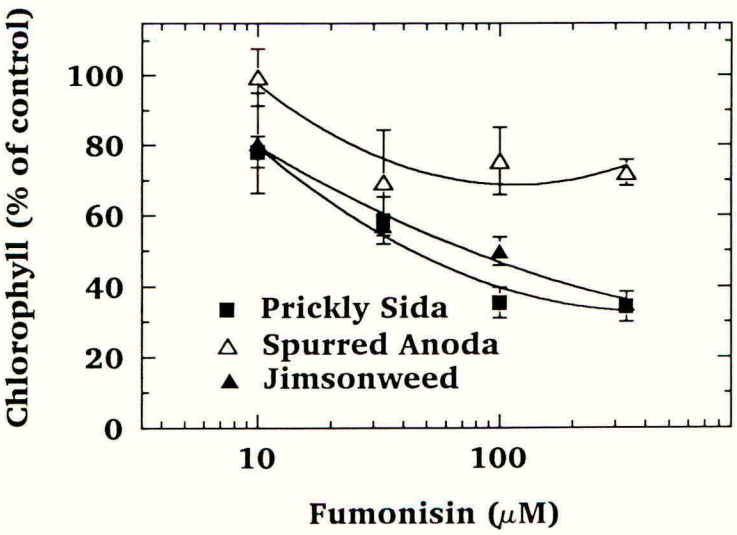

Fig. 4. Effects of different concentrations of fumonisin on chlorophyll content of leaf disks of three weed species after exposure to the phytotoxin for 48 h at 25°C under continuous light (0.5 mE/cm²/s PAR).

well as some dicot weeds like velvetleaf (*Abutilon theophrastii*), are generally resistant to fumonisin, even at high concentrations. This broad range of susceptible species was an unexpected result, since structurally similar AAL-toxin has been considered a host-selective toxin, affecting only certain varieties of tomato (Gilchrist and Grogan, 1975). However, AAL-toxin has recently been found to be phytotoxic to virtually the same spectrum of weeds as fumonisin (H. K. Abbas *et al.*, submitted for publication). Considering the similarities in symptomology and chemical structure, we suspect that these compounds have the same mechanism of action.

The molecular site of action of AAL-toxin has been reported to be aspartate carbamoyl transferase (Gilchrist, 1983), an enzyme involved in synthesis of uridine. However, this result has not been confirmed in other laboratories. No commercial herbicides are known to target this or a closely related molecular site of action. Orotic acid, a metabolic intermediate from aspartate to uridine, was reported by Gilchrist (1983) to prevent phytotoxic effects of ALL toxin. We found that orotic acid had no effect on the phytotoxicity of either AAL-toxin or fumonisin to jimsonweed. Thus, the mechanism of action of AAL-toxin on jimsonweed differs from that on tomato, or uridine synthesis may not be its primary site of action.

Compounds from non-pathogens

The only known commercial successes of herbicides from microbial products have been compounds from non-pathogens. Two of these compounds, bialophos and glufosinate, are related by the fact that bialophos must be metabolically degraded to glufosinate by the target plant in order to be active. Bialophos, glufosinate, and related microbial products such as phosalacine are all potent inhibitors of glutamine synthetase. This metabolic site is not targeted by any herbicide developed by other strategies.

A large proportion of the secondary products of microoganisms which are patented as herbicides are from non-pathogens. The majority of these patents are of Japanese origin. Most of those listed in Table 1 and the majority of those for which no mechanism of action is known are from non-pathogenic microbes. A complete list of these compounds would be too extensive for this limited review.

PROSPECTUS

Microbial products offer an array of novel phytotoxic compounds that, in many cases, have structures which are unlikely to be discovered by traditional pesticide discovery efforts base on synthesis. The interest in this rich source of new compounds is growing for several reasons. Traditional herbicide discovery methods are experiencing diminishing returns. Thus, the comparative cost-effectiveness of microbial metabolites as sources of new herbicides is an attractive alternative. Also, herbicide discovery teams have begun to place a high value on discovery of new molecular sites of action, and many microbially-produced phytotoxins have unique molecular targets. Furthermore, the methodologies for producing, isolating, and characterizing secondary products of microbes have improved. Advanced equipment and methods for culturing microbes and modern instrumentation for purification and identification of the compounds

produced have simplified and lowered the cost of this approach to herbicide discovery. Finally, although not justified on scientific grounds, natural products or derivatives of natural products presently have a certain level of public appeal over synthetic compounds. In coming years, we expect to see renewed efforts to exploit microbial products for control of weeds and other pests.

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GLYPHOSATE-TOLERANT CROPS FOR THE FUTURE: DEVELOPMENT, RISKS AND BENEFITS

S.WATERS

Monsanto Europe S.A., Belgium

ABSTRACT

A number of glyphosate tolerant crops are now being developed to provide farmers in the future with an additional tool for weed control management. The use of glyphosate tolerant crops can offer many benefits including decreased herbicide use, improved weed control efficiency, environmental and safety benefits and soil conservation. The potential risks of using such crops, including transfer of tolerance to related weeds, volunteers becoming weeds in rotation crops, and weeds becoming resistant to glyphosate are being evaluated, and should be weighed against the known benefits of using glyphosate-based herbicides.

INTRODUCTION

Weed control practices have evolved continually over the years from manual to mechanical to chemical weed control. Chemical weed control practices have also evolved towards the use of post emergence, selective herbicides, and more recently the focus has been on the use of highly active molecules with more favourable toxicological and environmental profiles.

Over the past decade a number of companies have been developing herbicide tolerant crops (OECD, 1990). While the development of such crops may be considered as a 'New Direction in Weed Control Technologies' it should perhaps be more appropriately considered as an extension of the more recent trends in weed control management. In all cases, the introduction of herbicide tolerant crops will provide farmers with valuable new tools for weed control management in the future.

DEVELOPMENT OF GLYPHOSATE-TOLERANT CROPS

Mode of action of glyphosate

The mode of action of glyphosate, the active ingredient of Roundup® herbicide, has been well characterised and documented (Steinruecken and Amrhein, 1980). The site of action of glyphosate is the shikimic acid pathway, which produces aromatic amino acids such as phenylanine and tyrosine, essential for protein synthesis. Glyphosate is a competitive inhibitor of the enzyme 5-enolpyruvylshikimic acid phosphate synthase (EPSPS), which catalyses the conversion of shikimate-3-phosphate and phosphoenol pyruvate to 5-enol pyruvryl-shikimate-3-phosphate.

Mechanism of glyphosate tolerance

The early genes coding for EPSP synthase were isolated from petunia (Padgette et al. 1987) and arabidopsis (Klee et al. 1987), however these wild-type genes resulted in only partial tolerance. More recently, marked improvements in glyphosate tolerance have been made possible through the use of genes modified to code for variants of EPSPS which have considerably reduced affinities for glyphosate, and hence markedly improved tolerance to glyphosate. Other improvements in glyphosate

tolerance have been obtained through the use of better promoters (Kay et al. 1987; Koncz and Schell, 1986) to enhance gene expression and tolerance in specific plant tissues such as the flowers. Secondly improvements in transformation techniques have permitted an expansion in the number of transformed plants produced and tested, enabling a wider choice of genotypes from which to select the most performant lines.

Production of glyphosate-tolerant plants

The transfer of glyphosate-tolerance genes to crops has been achieved using various methods, depending on the target crop. The most frequently used technique for transformation is that using *Agrobacterium tumefaciens*. There are many reviews available describing this technique (Fraley et al. 1986, Rogers and Klee 1987, Klee and Rogers 1989). The glyphosate tolerance gene is inserted into an *Agrobacterium* plasmid which, upon infection, transfers the glyphosate-tolerance gene into the plant cell where it is stably incorporated into the plant genome. This technique of transformation has been used to produce glyphosate-tolerant oilseed rape and sugar beet, among others.

Since *Agrobacterium* does not work effectively in monocotyledonous crops such as corn, nor in some important dicotyledonous crops such as soybeans, other techniques have been developed to transform these other crops, including electroporation, direct injection, and the the particle gun. Of these techniques the particle gun has probably been most successful in the transformation of corn and soybeans (McCabe et al. 1988, Klein et al. 1988). This technique has now been successfully employed to produce glyphosate tolerant corn and soybean plants.

Field testing of glyphosate tolerant crops

Experimental field testing is an essential step in the commercial development of any genetically modified crop, not only to assess the performance of the introduced gene, but also to verify that the genetic modification has had no secondary effects on varietal phenotype and quality. Variability in expression of the introduced gene between experimental lines is to be expected, depending on the position of gene insertion, so breeders must apply the classical selection procedures, that they now use routinely, in order to identify those lines meeting the dual requirements of acceptable variety performance coupled with adequate expression of the introduced gene.

More than 60 field tests of glyphosate-tolerant crops have been conducted since 1987 (Table 1). The first field tests were conducted with tomato in the U.S.A. In 1991 23 field tests with glyphosate-tolerant crops were approved, involving six countries (U.S.A, Canada, France, Belgium, U.K, and Denmark) and four crops (spring and winter oilseed rape, soybeans and sugar beet).

Commercialisation of glyphosate-tolerant crops

All EEC countries have strict seed certification procedures, requiring at least two years of official national trials to assess the performance of experimetal lines proposed for inscription in national catalogues. This assessement includes evaluations of distinctness, uniformity and stability, as well as agronomic value, considering such traits as yield, quality and pest resistance. Commercialisation of any herbicide-tolerant variety will necessitate passing through this type of varietal registration procedure, perhaps with some modifications. Under such registration procedures, if a herbicide-tolerant line is not superior to the best existing commercial varieties in agronomic value then it will be legally excluded from registration.

TABLE 1. Approved field tests with glyphosate-tolerant crops, 1987-1991.

Year	Crops	Locations	Tests approved
1987	Tomato	U.S.A	1
1988	Tomato, Spring OSR	U.S.A, Canada	3
1989	Cotton, Soybeans Spring OSR, Flax	U.S.A, Canada	9
1990	Spring OSR, Cotton, Sugar beet, Winter OSR	U.S.A, Canada, F, B, U.K, DK	28
1991	Soybeans, Spring OSR, Sugar beet, Winter OSR	U.S.A, Canada, F, B, U.K, DK	23

In parallel with varietal registration, the herbicide label must also be extended to include the new timing of application. In the case of glyphosate, post emergence applications will require documentation of crop residue levels, following the registration procedures applied to all post emergence herbicides. Such procedures have already been followed for the registration of glyphosate for pre-harvest applications (O'Keeffe, 1980) in small grain cereals, peas and oilseed rape. In other cases of herbicide tolerance, where tolerance is obtained by herbicide inactivation (Thompson et al. 1987), the metabolic pathway will need to be described and any significant metabolites characterised.

The approval of herbicide-tolerant crops for commercial use will therefore to a large extent follow regulatory procedures already in place. The additional dimension of assessing the environmental safety of genetically modified plants, including herbicide-tolerant plants, has been introduced to cover the experimetal field testing of genetically modified plants. These safety assessements are the responsibility of national committees, such as ACRE of the Health and Safety Executive in the U.K. It is anticipated that the results of the safety assessments conducted during the field research and development phase will also form the basis of the environmental safety assessment necessary for commercialisation.

BENEFITS OF GLYPHOSATE-TOLERANT CROPS

Decreased herbicide use

In Europe the major arable crops are treated at least once with herbicides, so the introduction of herbicide tolerant crops is unlikely to significantly increase the number of treated hectares. The introduction of glyphosate tolerant crops will result in an increase in the number of hectares treated with glyphosate, but with a corresponding reduction in the use of other herbicides. In cases where more than one herbicide is required for complete weed control, the number of herbicides applied will be reduced. In addition, for certain crops, the substitution of lower unit activity herbicides by glyphosate will result in an overall lower input of active ingredient into the environment.

Improved efficiency of weed control

The availability of glyphosate-tolerant crops will offer farmers a new option for weed control management. Because of its non-selective systemic mode of action, glyphosate is highly effective in controlling a broad spectrum of weeds, including

almost all annual and perennial broadleaf and grassy weeds (Bovey 1985). Using a combination of glyphosate tolerant crops and glyphosate, farmers will also have greater flexibility in the timing of applications, being largely independent of the stage of development of the crop and weeds. In some cases, where weed infestations are minor, farmers may opt to avoid herbicide sprays. The broad weed spectrum of glyphosate, combined with its relatively high unit activity will provide farmers with a very cost effective option for controlling weeds.

Environmental and safety

Glyphosate is well known for its favourable toxicological and environmental profile (Carlisle and Trevors, 1987). It is rapidly and tightly adsorbed onto soil particles, and is practically immobile, so the risks of groundwater contamination are minimal. Glyphosate does not persist in the environment, being completely biodegraded to natural molecules. Toxicology studies have demonstrated that it is non-toxic to mammals, birds and fish. Also the shikimic acid pathway, the primary target of glyphosate is only found in plants and microorganisms, and is not present in humans or other animals.

Soil conservation

Repeated tillage to control weeds is a major energy cost to farmers and is one of the leading causes of soil erosion. The combination of a non-selective and foliar applied herbicide, such as glyphosate, offers new possibilities in conservation tillage to reduce soil erosion and maintain soil fertility.

RISKS OF GLYPHOSATE-TOLERANT CROPS

Gene transfer by pollen

The possibility of the transfer of herbicide tolerance genes to related weed species has been raised as a potential risk of using herbicide tolerant crops. Before considering interspecific crosses as a real risk it must be demonstrated that a number of potential barriers can be overcome with considerable frequency. For interspecific hybridisation to be successful the crop and its related weed must be in close proximity, flowering must be coordinated, pollen must be mobile and remain viable, they must be sexually compatible, and the hybrids must be stable, vigorous and competitive. If such hybridisations can be demonstrated then gene transfer will have occurred, not only of the introduced gene, but also of any endogenous, naturally occurring genes in the crop in question. It should also be added that crops already demonstrate natural resistance to herbicides which are applied post emergence. The practical significance of any transfer of herbicide tolerance to a weed species is ultimately the key question, since gene transfer *per se* does not necessarily result in risk or major inconvenience. A number of studies are currently in progress, such as the PROSAMO project in the U.K. to assess the potential for certain crops, including oilseed rape and potato, to outcross with weedy relatives. The preliminary results of studies conducted in Canada with oilseed rape indicate that natural hybridisations with weedy relatives are unlikely to occur (Bing et al.), although clearly such risks should be evaluated on a crop by crop basis.

Crops becoming weeds in other crops

Volunteer growth of some crops, such as potato, oilseed rape and sugar beet can create weed problems in subsequent crops. Generally these volunteers can be controlled by the use of appropriate herbicides in the rotation crops. The fact that

volunteers are tolerant to glyphosate may or may not affect farmer management practices. In the case of oilseed rape, volunteers are largely controlled mechanically, or they are controlled chemically using herbicides other than glyphosate. If they were to be glyphosate tolerant, this would have little or no impact on current practices. Weed beet can be controlled in rotation crops by the use of specific dicotyledonous herbicides, but they are a significant problem in sugar beet, largely because they are sensitive to the same herbicides as sugar beet. Rather than creating new problems, the cultivation of glyphosate tolerant sugar beet would provide farmers with the opportunity to eliminate weed beet, provided that they combined this with the regular control of subsequent bolters.

When more than one glyphosate tolerant crop becomes available, for instance sugar beet and oilseed rape, growers would need to avoid multiple cropping with glyphosate tolerant crops in regions where these crops are grown in rotation.

Weeds becoming resistant to glyphosate

There are a number of examples of weeds becoming resistant to specific herbicides (Putwain and Mortimer, 1989). Glyphosate has been used widely for more than 15 years with no such reports of weed resistance. Based on the complexity of engineering glyphosate tolerance into crops, it is unlikely that high levels of tolerance can be obtained naturally. For such tolerance to occur would probably require multiple modifications to the EPSP synthase molecule. The added selection pressure from an increased use of glyphosate is not anticipated to increase the likelihood of resistance developing, since any increased use is likely to be marginal, rather than an order of magnitude. In addition, other herbicides will continue to be used in crops grown in rotation with glyphosate-tolerant crops.

CONCLUSION

The results of the safety assessment studies in progress will provide the basis of any risk assessments of genetically modified crops, including herbicide tolerant crops, conducted by national or EEC authorities. In addition commercial considerations should ensure that glyphosate-tolerant crops will be properly managed to prevent the spread of glyphosate tolerance genes to weed populations. Whether farmers elect to use glyphosate tolerant crops as tools for weed control management will clearly depend on the relative benefits, either economic, environmental or technical, compared to other weed control options available. The choice among these options may vary according to the crop, region, weed spectrum or other specific factors facing particular farmers.

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STIMULATION OF *BROMUS STERILIS* SEED GERMINATION BY 1-(3-CHLOROPHTHALIMIDO) CYCLOHEXANECARBOXAMIDE (AC94377) OR GIBBERELLIC ACID (GA_3)

N.C.B. PETERS, T.M. WEST

Department of Agricultural Sciences, University of Bristol,
AFRC Institute of Arable Crops Research, Long Ashton Research Station,
Long Ashton, Bristol, BS18 9AF.

ABSTRACT

Present, herbicidal and cultural methods for the control of *Bromus sterilis* (barren brome) are unreliable. Most biotypes of *B. sterilis* shed large numbers of seed of which a small but highly important number are innately dormant. A high percentage of the seed can also be enforced into dormancy by light. Both features allow infestations of this weed to occur in autumn sown crops in the year of shedding. We investigated whether the seed could be stimulated to germinate by treatment with 1-(3-chlorophthalimido) cyclohexanecarboxamide (AC94377) or gibberellic acid (GA_3). Weed control could then be achieved before crop emergence by seedling destruction. Five doses of AC94377, one of GA_3, a water and a surfactant control were separately applied to seed in pots by three methods: i) sprayed onto seed sown on the soil surface; ii) sprayed onto the soil surface with seed sown 25 mm deep; iii) sprayed onto seed sown on the soil surface and then after 24 h the seed incorporated into the soil. After 2 weeks, only AC94377 at the highest dose increased germination using method i). Although increases in germination were given by both chemicals using method ii), the largest effect was observed using method iii), in which doses between 0.5 and 2.0 kg AI/ha of AC94377 gave 91-92% germination compared with 26% in the surfactant control. It was concluded that stimulation of germination in *B. sterilis* seed by AC94377 application could be developed as a method of control for this species.

INTRODUCTION

One of the reasons why weeds continue to pose a threat to crop production is that many of their seeds remain dormant, but viable, in the soil for many years (Roberts, 1962). Germination of such seeds can therefore cause infestations of weeds in subsequent crops over many years. It has been long recognised, that if the dormant seeds could be stimulated to germinate in one season and then subsequently killed by herbicides or cultivation, this would represent a major step forward in weed control (Chancellor, 1981) and, in the long term, reduce herbicide inputs. A wide range of chemicals are known to stimulate weed seed germination in the laboratory. Some, such as ethylene, have already proved useful in the field (Eplee and Langston,1976) but, in other cases, treatment in the field has resulted in only partial success (Fay and Gorecki, 1978; Hurt and Taylorson, 1986; Bond and Burch, 1990).

More effective chemicals will probably be discovered, but there are many problems in getting sufficient chemical into seeds once they are

buried in soil. The seed is often buried at different depths, so that to get sufficient active ingredient near enough for the seed to absorb it, means that large, and possibly uneconomic and environmentally unacceptable, amounts of chemical need to be applied. Also, the soil can absorb/adsorb much of the chemical, thus making it unavailable to the seed. In addition, if the seed has a hard seed coat, or is already fully imbibed, or if the soil is very dry, this may also prevent uptake of any chemical applied. To overcome many of these problems, in the current research, it was decided to apply chemicals to the seeds placed on the soil surface, as well as to buried seed.

B. sterilis is a difficult weed to eradicate because as yet there are no reliable herbicidal or cultural control measures. This weed seems to be an ideal candidate to be controlled by stimulating its seed to germinate and then subsequently destroying the seedlings because, i) when most of the known biotypes shed seed only a small (but highly important) proportion of it is innately dormant, the dormancy being relatively short lived (max. approx. 6 weeks), so that breaking it should not be difficult; ii) no large, long term, seed banks are present, and iii) it is a patchy weed, the locations of patches are known prior to harvest via the conspicuous nature of the purple panicles. Application to the patches where the weed had occurred could then be made after harvest and this may be an economic strategy, even if large scale treatment were not.

In our laboratory at Long Ashton, gibberellic acid (GA_3) is routinely used to stimulate the germination of *B. sterilis* seed, including those biotypes capable of readily being enforced or induced into dormancy. Clearly, treatment of large field areas with GA_3 would be uneconomic, and so a comparable alternative was sought. Therefore, the plant growth regulator 1-(3-chlorophthalimido) cyclohexanecarboximide (AC94377) which has gibberellin-like properties (Suttle and Hulstrand, 1987) was tested.

MATERIALS AND METHODS

In an outdoor pot experiment, protected by a well-ventilated polythene tunnel, different doses of AC94377 or GA_3 were applied to seed of a *B. sterilis* biotype (LARS 34) which had been stored in dry outdoor conditions for one month after collection. This biotype is known to possess some innate dormancy and to have a strong capability for enforced or induced dormancy. Only viable seed with full caryopses was used. The treatments were as follows: water control; surfactant control (Agral 0.1%); 0.125, 0.25, 0.5, 1.0 and 2.0 kg AI/ha of AC94377 (SC); 0.435 kg AI/ha of GA_3 (water soluble powder). All treatments, apart from the water control, were made up in 0.1% Agral solution.

Seed was sown in 230 mm diameter pots containing a sandy clay loam. Three methods of chemical application were used: i) treatment sprayed onto seed sown on the soil surface; ii) treatment sprayed onto the soil surface with seed sown 25 mm deep; iii) treatment sprayed onto seed sown on the surface of a 25 mm layer of soil, and after 24 h the seed removed, placed on an untreated soil base, and covered with the sprayed 25mm layer of soil which was stirred before placement to incorporate the chemical treatment. In iii), the method ensured that all seed was buried into darkness, whereas if the seed had been sprayed on the soil surface and then incorporated by stirring, some seeds may have remained on the surface. Treatments were applied using a laboratory track sprayer fitted with a 80015E flat fan

nozzle delivering 435 l/ha at a pressure of 210 kPa. There were four replications per treatment arranged in completely randomised blocks. The soil and seed were initially dry, but were lightly watered 15 h before the treatments were applied to simulate more closely the moisture condition in the field. The pots were watered 24 h after application and kept moist thereafter.

After 16 days, assessment of seed germination was made in the soil surface treatments, and of seedling emergence in the buried treatments. Therefore, in the buried treatments a few seeds that had germinated but not emerged as seedlings will not have been recorded, but for the purposes of this paper, germination and emergence at the day 16 assessment are regarded as the same. After 51 days, seed/seedlings were dug up and the actual germination recorded in all treatments. Ungerminated seed was not tested for viability. The germination percentage data were analysed using an analysis of variance; data transformation was found to be unnecessary.

RESULTS

Seed on surface, treatment applied to soil surface

After 16 days, there was little germination in the untreated and surfactant controls (8.0% and 5.0% respectively), and there was no significant increase in germination at 0.125 to 0.25 kg AI/ha of AC94377 or with GA_3, (Fig.1a). However, at 1.0 and 2.0 kg AI/ha of AC94377, germination was significantly increased above the controls (23% and 30% total germination (T.G.), respectively). After 51 days, there was still little germination in the untreated and surfactant controls (22% and 20% T.G., respectively), but there were substantial increases for all AC94377 treatments, especially at the 2.0 kg AI/ha dose (85% T.G.), with a small increase from the GA_3 treatment, (39% T.G.).

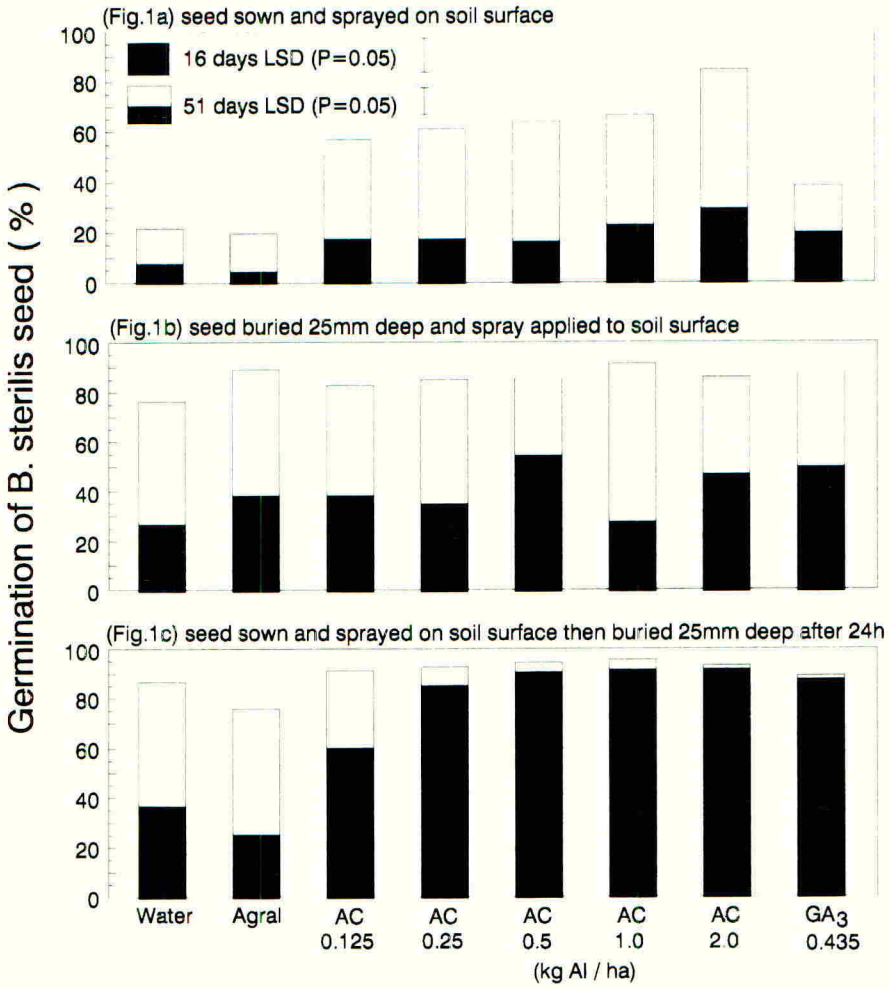
Seed buried 25 mm deep, treatment applied to soil surface

After 16 days, there was no significant difference between the untreated and surfactant controls, the total germination being 28% and 39%, respectively (Fig.1b). At 0.5 and 2.0 kg AI/ha of AC94377 and in the GA_3 treatment, germination was significantly increased above the controls (55%, 48% and 50% T.G., respectively). By 51 days, germination in the controls had increased to between 77% and 90% and similar levels of germination were recorded from the other treatments.

Seed treated on the soil surface then buried after 24 h in treated soil

Sixteen days after treatment, all the chemical treatments had significantly increased percentage germination above that of the controls, (Fig.1c). The highest germination (91% - 92%) was given by doses of AC94377 at 0.5 - 2.0 kg AI/ha. Germination in the controls (26% - 37%) was similar to that where the seed had been buried before treatment. By 51 days after treatment, the amount of germination in the controls had increased to almost the same level as that in all of the chemical treatments.

FIGURE 1. Effect of AC94377 (AC) and Gibberellic Acid (GA_3) on the germination of B. sterilis seed, (assessed 16 & 51 days after spraying).



DISCUSSION

When *B. sterilis* seeds were untreated, germination was much higher when the seeds were buried than when they were left on the soil surface, illustrating the strong inhibitory effect of diurnal light on the germination of this species, (Froud-Williams, 1981); even after 51 days, little further germination had occurred. However, AC94377 was able to overcome any inhibitory effect of light, especially at the 2.0 kg AI/ha dose. The relatively low germination from the GA_3 surface treatment suggests that either GA_3 could not overcome the inhibitory effect of light or that insufficient chemical was penetrating into the seed, possibly due to the more rapid breakdown of GA_3 compared with AC94377. In many respects, any germination stimulant applied to the soil in the autumn should not be too persistent, otherwise residue problems may arise with the

growth of an autumn-sown crop. If necessary, the land could be left unplanted until the following spring. However, this would to some extent negate the use of a chemical stimulant, for by spring, much of the seed if buried, would have germinated anyway, as evidenced here by the behaviour of seed in the untreated buried controls. However, ample time would be given for chemical treatments to work.

There was little difference in germination between seeds buried in the untreated controls after 15 h imbibition (controls for seed buried before treatment) and that buried after a further 24 h imbibition (controls for seed buried after treatment), so the slightly longer imbibition time in the latter treatment did not differentially affect the seed in the two treatments. The 24 h delay was introduced in an attempt to simulate field conditions.

Although there was some stimulation, by both AC94377 and GA_3, of the germination of seeds buried before treatment, the most rapid and greatest increases (up to 92% T.G.) were recorded where the chemicals were applied directly to the seed on the soil surface and then incorporated into the treated soil. In these treatments GA_3 worked as well as any of the AC94377 treatments. A slight dose response could be noted with AC94377, with doses above 0.5 kg AI/ha giving little additional increase in germination. Although remaining seed was not tested for viability, 92% germination probably represents almost the total viable seed in the pot, since some will have died through natural causes such as fungal attack. At the time of the last assessment, small seedlings were found in the untreated controls of the buried treatments, which indicates delayed germination/emergence, whereas, no seedlings were found in any of the chemical treatments, showing how much more rapid the germination had been in the treated pots. The rapidity with which the seed can be germinated and thus eliminated is of great importance, because where continuous winter cereals are grown, little time elapses between harvesting and replanting.

For germination to occur, moisture needs to be present. In the present experiment, the soil was kept moist throughout the experiment, but, in the field, lack of moisture might be a limiting factor. To help overcome this difficulty, cultivation of the seed to a depth where sufficient moisture was available would be necessary. In the present experiment, the most successful treatments were those where the seeds were exposed to the direct chemical spray and were then almost immediately immersed in treated soil. If the treatments were applied in the field, subsequent cultivation would have a similar effect. Cultivation seems a pre-requisite for success of the method in the field, particularly with *B. sterilis*, where light is a strong inhibitor of germination, for the slowness of the seed to respond to treatment on the soil surface may have been due both to a lack moisture and the presence of light.

One noticeable feature of the effect of AC94377, particularly at the higher doses, was the considerable extension growth of the young seedlings, which made them somewhat etiolated and prostrate. In field conditions, this would have made them very vulnerable to damage and would probably lead to seedling death. Minimal cultivation or minimal herbicide treatment would therefore be sufficient for seedling destruction.

Further research needs to be done to determine if AC94377 could successfully stimulate the germination of other important U.K. grass weeds such as *Alopecurus myosuroides* (black-grass) and *Avena fatua* (wild-oat).

If effective against these and/or other weed species, this would increase its commercial attractiveness. In addition, other surfactants need to be tested to see if there are some which would enhance the uptake of AC94377 by weed seeds.

There is no current recommendation for AC94377 to be used for weed seed stimulation. Having determined that the compound can be successfully used to stimulate the germination of a *B. sterilis* biotype known to possess a relatively high quota of dormancy features, the next step is to test the compound under field conditions. It would probably be necessary to use doses of 0.5 kg AI/ha and above as an incorporated treatment. A biotype with fewer dormancy features could be used, as this would be nearer the average for U.K.populations. Stimulation of such a biotype may be even greater than that reported here. With the banning of stubble burning, which was a major weapon used to destroy *B. sterilis* seeds, and the possible restriction in the use of some herbicides to control the weed, other ways of preventing the build up of its populations need to be found. The present research suggests, that stimulated seed germination coupled with subsequent mechanical or chemical seedling destruction, might provide the farmer with an alternative and efficient method of control.

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OPTIMISING THE INTENSITY OF HARROWING FOR MECHANICAL WEED CONTROL IN WINTER WHEAT

JESPER RASMUSSEN

Department of Weed Control, Flakkebjerg, DK-4200 Slagelse, Denmark

ABSTRACT

The effect of harrowing on weeds and crop yield in winter wheat was examined in 6 experiments carried out during 1989-90. Regression models were used to describe the relationship between the amount of weeds and the number of passes of a harrow carried out in the spring. A proposed model made it possible to predict the minimum number of harrowings required to achieve the maximum degree of weed control. Experiments carried out in 1989 with a flexible chain harrow showed that even 5 harrowings at one cultivation time in the early spring were not sufficient to achieve the maximum degree of weed control. Only 37 to 50% weed control was obtained after 5 harrowings. In 1990 a heavy finger weeder was used at two cultivation times in the late spring. Then maximum weed control was achieved ranging from 69 to 95% depending on fertilization and seed rate. The minimum number of harrowings required to achieve these maxima varied from 2.5 to 3.9 harrowings per cultivation time. Harrowings carried out under weed free conditions did not show any negative impact on yield. It is concluded that new demands on implements and tillage strategies have to be made to ensure sufficient weed control by one or two harrowings per growing season.

INTRODUCTION

In order to develop harrowing as a mechanical weed control method an important question is how strong the intensity of harrowing has to be to kill as many weeds as possible without associated crop damage (Rasmussen, 1991). Previous research concerning harrowing in winter cereals has not been adapted to solve this question. It has been dominated by experiments consisting of a few qualitative treatments examined without any modeling approach (Koch, 1959; Schmid & Steiner, 1987; Rasmussen, 1989; Bräutigam, 1990; Dierauer, 1990; Böhrnsen & Bräutigam 1990, Samuel & Guest, 1990). To find the optimum intensity of harrowing, experiments consisting of graded levels of a quantitative factor are appropriate and models corresponding to the well-known dose-response models in herbicide research (e.g. Streibig, 1988) are required.

The objectives of this paper are to examine two proposed regression models in order to describe the relationship between the amount of weeds and the intensity of harrowing and to examine the efficiency of harrowing to control annual weeds in winter wheat in the spring.

MODELS

The 2 models considered in this paper both describe the relationship between the intensity of harrowing and the amount of weeds. The intensity of harrowing is expressed as number of harrowings per cultivation time, and weed amount is expressed as dry matter or density.

The first model implies that the logarithmic transformation of the amount of weeds, log(Y), plots linearly against the intensity of harrowing

$$\log (Y) = a + bx \qquad (1)$$

where Y denotes the amount of weeds (density or biomass) and x denotes the number of passes of a harrow per cultivation time. The intercept a, expresses the density or biomass of weeds (Y_0) in untreated plots

$$Y_0 = \exp(a) \qquad (2)$$

and the parameter b, is the relative effect of each harrowing. Equation (1) implies that the percentage of weed reduction obtained at each harrowing is constant

$$WC_{con} = 100(1-\exp(b)) \qquad (3)$$

The second model accounts for the fact that harrowings cannot always control weeds entirely. Particularly late harrowings, where only the inter-row spaces are cultivated, cannot be expected to control weeds rooted in the rows. A practical approach to solve this problem is to extend Equation (1), so log(Y) approaches a lower limit where further harrowings do not increase the weed control. This is done by a segmented model

$$\begin{aligned} \log (Y) &= a + bx \quad \text{if } x < x_m, \\ \log (Y) &= k \quad \text{if } x > x_m \end{aligned} \qquad (4)$$

where x_m denotes the minimum number of harrowings necessary to achieve the maximum weed reduction

$$x_m = (k-a)/b \qquad (5)$$

The maximum percentage of weed control (WC_{max}) can be derived from Equation (4)

$$WC_{max} = 100(1-\exp(bx_m)) \qquad (6)$$

and the minimum amount of surviving weeds

$$Y_{min} = \exp(a+bx_m) = \exp(k) \qquad (7)$$

MATERIALS AND METHODS

In order to examine the effects of harrowing on weeds as well as crop yield 6 field experiments were performed at The Department of Weed Control, Denmark, during 1989-90 on a loamy soil containing 15-25% clay (Table 1). Two experiments (Experiment 5, 6) were carried out under weed free conditions to examine crop yield effects, one experiment (Experiment 1) was sprayed with a herbicide four weeks after harrowings to examine the effects of harrowings on weed density and crop yield, and three experiments (Experiment 2,3,4) were carried out under natural weed conditions with *Stellaria media, Veronica arvensis, Lamium purpureum,* and *Myosotis arvensis* as dominant weed species.

Besides intensity of harrowing the experiments involved different levels of fertilization and different seed rates as factorial treatments according to Table 1. In all experiments a split-plot design with 4 replicates was used with intensity of harrowing as subplots. Plot size was 25 m^2 (2.5 m x 10 m).

All harrowings were carried out parallel to the sowing direction. In 1989 the flexible chain harrow manufactured by Schönberger was used (type: ES-EN-EL) and in 1990 a heavy spring tine harrow (manufactured by Rabe-Werk), a finger weeder, was used. All harrowings were carried out with a driving speed ranging from 6 km h^{-1} to 8 km h^{-1}. In 1989 harrowings were carried out in the early spring to control weeds when they were still small. In 1990 harrowings were carried out when the crop was 20-25 cm high in order to minimise damage on the above-ground parts of the crop. Late harrowings performed with finger weeders only cultivate the inter-row spaces due to the resistance offered by the crop against the tines of the harrow.

In all experiments, except for Experiment 1 (Table 1), weed populations were determined by subsampling plots at random using 4 x 0.25 m^2 quadrats.

In statistical analyses all data concerning weeds were log-transformed to stabilize variance and to fit Equations (1) and (4). Crop yields were not transformed. The segmented model, Equation (4), was fitted by PROC NLIN in SAS and the linear model, Equation (1), by PROC GLM. The influence of seed rate and fertilization on the weed response curves was examined by implementing the variables in the regression models as dummy variables (Weisberg, 1985). By consecutive runs non-significant parameters were omitted on the basis of F-tests in order to simplify models.

RESULTS

In both experiments from 1989 Equation (1) gave a good approximation of the weed response (Fig. 1). Lower limits in terms of surviving weeds were not attained, so Equation (4) was not considered. 5 harrowings only controlled 37-50% of the weeds in these experiments (Table 2). There was no influence of fertilization in Experiment 1. This was not expected because weed levels were assessed early in the growing season. In Experiment 2 the high seed rate reduced the level of weed dry matter ($p < 0.001$) but the seed rate did not influence the effects of harrowing in terms of percentage weed control.

TABLE 1. Experimental details concerning Experiments 1-6.

	Number of experiment					
	1	2	3	4	5	6
Variety	Kraka	Kraka	Slejpner	Slejpner	Slejpner	Slejpner
Date of drilling	22 Sept 88	27 Sept 88	28 Sept 89	28 Sept 89	21 Sept 89	21 Sept 89
Seed rate (kg ha^{-1})	1) 210	1) 200 2) 270	1) 110 2) 205 3) 265	1) 205	1) 110 2) 205 3) 265	1) 205
Date of fertilization	10 April 89	31 March 89	28 March 90	28 March 90	29 March 90	29 March 90
Fertilization (kg ha^{-1})	N P K	N P K	N P K	N P K	N P K	N P K
1)	50 10 24	140 27 67	180 34 86	60 11 29	180 27 67	60 11 29
2)	100 19 48			120 23 57		120 23 57
3)	150 29 71			180 34 86		180 34 86
Number of harrowings	0,1,2,3,4,5	0,1,2,3,4,5	0,1,2,3,5	0,1,2,3,5	0,1,2,3,4,5	0,1,2,3,4,5
Date of harrowings	28 March 89	29 March 89	23 April 90 15 May 90	23 April 90 15 May 90	25 April 90 15 May 90	25 April 90 15 May 90
Herbicide application	All plots 24 April 89	None	Single plots[1] 24 April 90	Single plots[1] 24 April 90	All plots 3 April 90	All plots 3 April 90
Herbicide (AI ha^{-1})	150 g Ioxynil 150 g Bromoxynil 45 g Clopyralid 1 kg Mechlorprop		114 g Ioxynil 69 g Bromoxynil 552 g Dichlorprop 705 g MCPA		75 g Clopyralid 1.4 kg Mechlorprop 700 g MCPA	
Assessment of weed amount	1 May 89	4 July 89	4 July 90	4 July 90	5 July 90	5 July 90

[1] = One plot in each main plot.

TABLE 2. Regression estimates of the amount of weeds on number of harrowings according to Equations (1) and (4), and predicted number of harrowings per cultivation time (X_m) required to achieve maximum percentage weed control (WC_{max}), percentage weed control after 5 harrowings (WC_5), percentrage weed control for each harrowing (WC_{con}) and the amount of weeds (g m^{-2}) in unweeded plots (Y_o). Predictions according to Equations (2), (3), (5) and (6).

Experiment	Treatment	Parameters a	b	k	X_m	WC_{max}	WC_5	WC_{con}	Y_o
1	All	4.75	-0.139	-	-	-	50	13	116.0[1)]
2	Seed rate								
	200 kg ha^{-1}	4.40	-0.0918	-	-	-	37	8.8	81.5
	270 -"-	4.08	-0.0918	-	-	-	37	8.8	59.1
3	Seed rate								
	110 kg ha^{-1}	4.56	-0.747	2.73	2.44	84	84	53	95.6
	205 -"-	4.13	-0.747	1.22	3.90	95	95	53	62.2
	265 -"-	3.43	-0.747	1.59	2.46	84	84	53	30.9
4	Fertilization (N)								
	60 kg ha^{-1}	4.12	-0.427	2.95	2.72	69	69	35	61.6
	120 -"-	4.12	-0.556	2.00	3.81	88	88	43	61.6
	180 -"-	4.12	-0.790	1.42	3.42	93	93	55	61.6

1) Weed density (plants m^{-2})

Crop yield was not influenced by harrowings in Experiment 1 but increased in Experiment 2 (Fig. 1). Fertilization increased crop yields in Experiment 1 whereas seed rate had no effect on crop yields in Experiment 2 (Fig. 1).

In 1990 higher weed control levels were achieved (Experiment 3 and 4) than in 1989 (Table 2). This was probably due to the application of a heavier harrow at two cultivation times. Equation (4) gave good descriptions of the relationship between intensity of harrowing and weed dry matter as shown in Fig. 2 where lower limits of surviving weeds (Y_{min}) were achieved. In Table 2 parameters characterizing the weed control are shown, and it is seen that the effects of harrowings were affected by seed rate and fertilization.

In Experiments 3 and 4, where natural weed infestations were present, the crop yields were not affected by harrowing and were comparable to yields in herbicide treated plots (Fig. 2). When harrowings were carried out under weed free conditions (Experiments 5 and 6) there was no significant effect on crop yield. The late harrowings therefore did not appear to damage the crop regardless of the intensity of harrowing. Increasing the seed rate increased yields in Experiments 3 and 5 whereas fertilization had no clear impact on yield in Experiments 4 and 6 (Figs. 2-3).

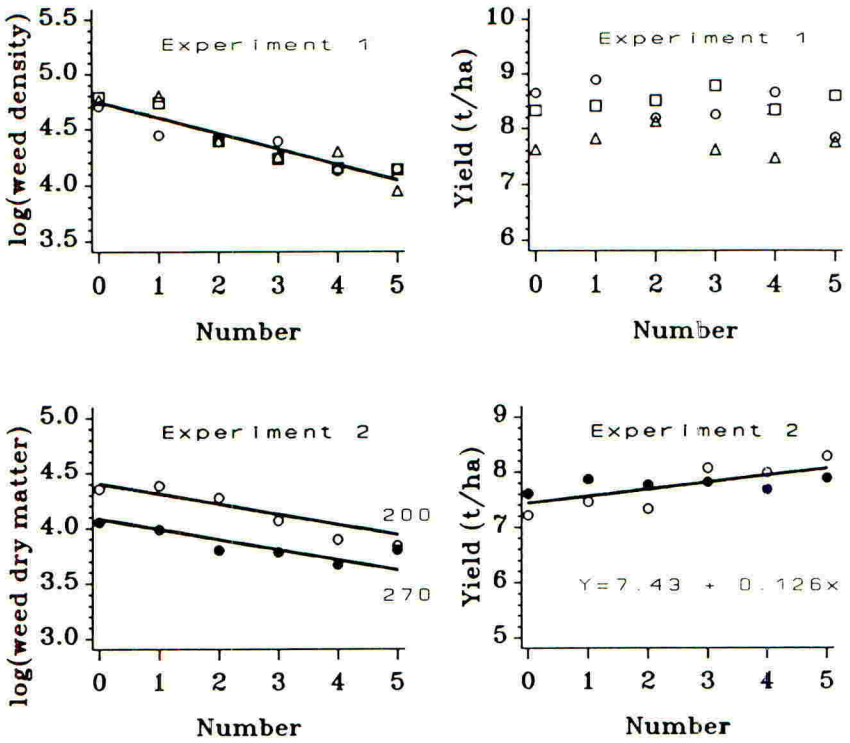

FIG. 1. The impact of harrowings on weed amount and crop yield in Experiments 1 and 2. Fitted weed response curves according to Equation (1). Parameters in Table 2. Symbols for fertilization in Experiment 1: ▵: 50 kg N ha^{-1}, ○: 100 kg N ha^{-1}, □: 150 kg N ha^{-1} and symbols for seed rate in Experiment 2: ○: 200 kg ha^{-1} and ●: 270 kg ha^{-1}.

DISCUSSION AND CONCLUSIONS

Experiments 1-4 show that the effect of harrowing on weeds is well described by the two proposed models (Equation (1) and (4)). From theoretical considerations it can be hypothesized that the most appropriate response curve should be smooth. Nevertheless, the segmented model, Equation (4), possesses some advantages which should not be ignored. The parameters are easy to interpret in an agricultural context and it is possible, even by eye, to get an idea if it is reasonable to assume a simple exponential decay function between the intensity of harrowing and the weed amount.

It is a common opinion that harrowings have to be carried out as early as possible in the spring to ensure that weed plants are still small (Schmid & Steiner, 1987; Bräutigam, 1990; Böhrnsen & Bräutigam, 1990). However, if it is possible to control weeds with harrowings in the late spring this strategy appears promising, because the risks of associated crop damage are small due to the inter-row performance of the finger weeders. Earlier harrowings are often associated with some degree of crop soil cover, which in general reduces the crop yield (Rasmussen, 1991)

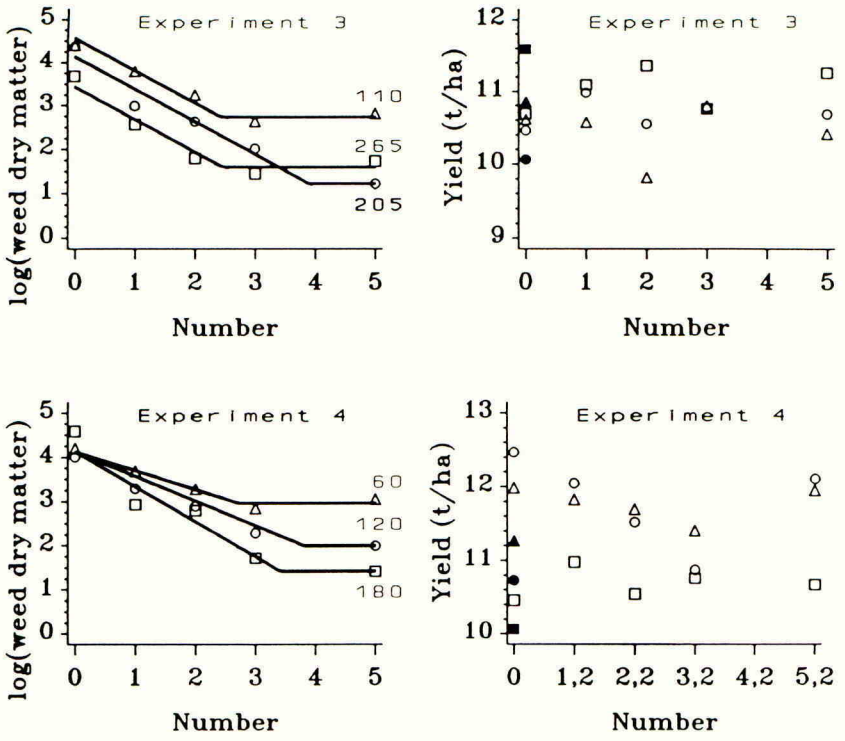

FIG. 2. The impact of harrowings on weed amount and crop yield in Experiments 3 and 4. Fitted weed response curves according to Equation (4). Parameters in Table 2. Symbols for seed rate in Experiment 3: ▵: 110 kg ha^{-1}, ○: 205 kg ha^{-1}, □: 265 kg ha^{-1} and symbols for fertilization in Experiment 2: ▵: 60 kg N ha^{-1}, ○: 120 kg N ha^{-1}, □: 180 kg N ha^{-1}. Filled symbols are herbicide treated treatment means.

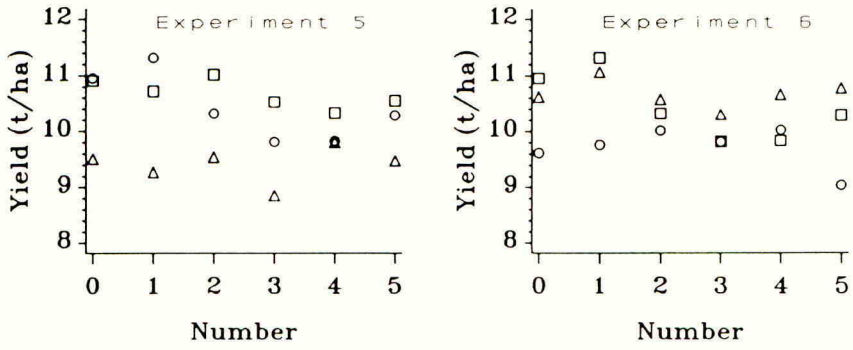

FIG. 3. The impact of harrowings on crop yield in Experiments 5 and 6. Symbols as in Fig. 2.

At the present stage of research aimed at developing mechanical weed control methods, the most interesting result of the experiments is, that it has been possible to achieve high degrees of weed control without associated crop damage. 90% weed control is exceptional with respect to the use of harrowing in winter cereals (Koch, 1959; Schmid & Steiner, 1987; Rasmussen, 1989; Bräutigam, 1990; Dierauer, 1990; Böhrnsen & Bräutigam 1990, Samuel & Guest, 1990), but it is also exceptional to apply more than 4 passes per year. High degrees of weed control might have appeared in the previous studies too, if the intensity of harrowing had been increased. Only late harrowings, however, are expected not to reduce crop yield when high intensities are applied.

From a farming point of view, it is, impractical carry out up to 8 passes per year with a harrow, so new demands on implements have to be made. Besides the technical aspects, an important objective of further research aimed at developing mechanical weed control is to adjust timing and intensity of treatments to the given conditions. The weed species composition might play an important role in this respect.

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WEED CONTROL IN ORGANIC FARMING SYSTEMS.

C. STOPES, S. MILLINGTON

Elm Farm Research Centre, Hamstead Marshall, Newbury, Berkshire, RG15 0HR

ABSTRACT

The successful performance of organic farming systems relies upon a viable crop rotation, defined as a sequence of crops which can maintain fertility and contribute to the control of weeds, pests and diseases. In organic systems, synthetic agro-chemical herbicides are prohibited, and the rotational approach to weed control is coupled with other husbandry and management techniques. This paper reviews the options available to the organic farmer, and the potential for alternative husbandry and management practices.

INTRODUCTION

Organic farmers cite weed control as the most significant production problem they encounter. Organic systems of farming rely primarily upon a viable crop rotation, defined as one which can maintain fertility and contribute to the control of weeds, pests and diseases (Millington *et al*, 1990). The soil type and climate, as well as the balance of enterprises on the farm, will determine the sequence of crops chosen by the farmer. Both species and variety will need to be considered since varietal characteristics may be significant in determining the success of a crop sequence. Since there are no permitted herbicides in organic agriculture (see production standards: Soil Association, 1989; UKROFS, 1991), optimal weed control strategies must be an integral part of rotation design and crop husbandry.

A balance between the supply and use of nitrogen in a viable crop rotation is expected to be achieved by including a sufficient proportion of leguminous crops (to fix atmospheric nitrogen) which can support the nitrogen requirements of a phase of non-leguminous arable cropping. Standards for organic agriculture prohibit the use of soluble "synthetic" fertiliser inputs and restrict the quantity of manure which may be brought onto the holding. The constraint imposed by these restrictions will ensure that important weed control objectives are met through the rotation selected by the farmer. Typically this may include several years of legume based pasture, either conserved or grazed, supporting a livestock enterprise.

WEED CONTROL OBJECTIVES

Weed eradication is not a central objective of organic farming systems. The greater diversity of non-crop plant species found on organic as compared to conventionally managed farms (Herrmann, 1989; Elsen, 1989 and Plakholm, 1989) allows for a wide range of animal species including predators of crop pests. They may also act as 'trap crops' for pests (Kloen & Altieri, 1990). Higher populations of birds on organic as compared to conventional farms have been observed and have been considered to be due to the greater feed availability from non crop plants (Hald, 1990).

The authors of the studies cited above considered that although there were a wider range of plant species on the organic farms, a similar limited range of species were dominant in both organic and conventional crops. It was observed that the use of herbicides on the conventional farms tended to increase the extent to which the dominant species became problem weeds, whilst at the same time destroying those with little economic significance but greater ecological importance.

Although a number of beneficial effects of weeds can be identified, their detrimental consequences require appropriate and acceptable control measures to be adopted. In the context of organic farming systems the problems resulting from unacceptable weed populations may be readily identified:

1. Reduced yields due to increased competition.
2. Harvesting difficulties and crop contamination.
3. Host plants for pests and diseases.
4. Reduced productivity of grassland.
5. Increased weed seed bank in the soil.

These demand that an adequate level of weed control is an objective of organic farmers in order to minimise these problems. Although the potential increase in the weed seed bank may be a very important long term consequence of inadequate weed control, the competitive effect on the crop and the consequent reduction in yield has been of greater concern.

COMPETITIVE EFFECTS OF WEEDS IN CROPS

Research in conventional systems is allowing the calculation of 'economic threshold' levels of weed density above which it is considered to be worth spraying. Crop equivalent values (Wilson, 1987) have been calculated although Wilson and Wright (1990) have shown that these may not relate well to the impact of a certain weed density on yield of, for example, winter wheat.

Since no comparable studies have been conducted in organically managed crops, it is not known whether the patterns of crop responses to weeds will be similar to those observed in conventional crops. Browning & Unwin (1986) reported no benefit of weed removal (by hand) in late April in a first winter wheat on two sites, and suggested that the weeds were not competitive, despite accounting for over 10% of the total nitrogen accumulated above ground. No yield benefit was observed by Stiefel (1990) from spring hoeing of organic winter wheat. Samuel and Guest (1990) reported that, although spring harrowing in organic winter wheat reduced weed populations, weed biomass at harvest was similar irrespective of weed control operations.

A trial which involved maintaining experimental plots weed free during the entire growing season did, however, lead to a 20% increase in wheat yield over unweeded plots, but no such effect was observed in beans (Bulson, 1991). In winter beans, Patriquin *et al* (1986) reported a slightly higher yield in weeded plots compared with unweeded, but concluded that in most cases, weeds did not inhibit crop growth, filling in spaces between crop plants rather than competing directly.

WEED CONTROL STRATEGIES

Crop sequence and variety choice

The sequence of crops chosen offers a significant opportunity for satisfying weed control objectives. Different crop species will compete with weeds to a variable degree. Oats and rye are noted as having a higher competitive ability against weeds than is the case for other cereal species, and thus these are particularly appropriate towards the end of the phase of arable cropping when populations of weeds may become greater.

The most obvious example of the rotation contributing to weed control is the reduction in weed populations during the ley period within a ley/arable rotation. This is due to many factors, including competitive exclusion by the more vigorous sown pasture species, prevention of seeding by the repeated cutting or grazing and depletion of the soil weed seed bank by seed death over the 3-5 year period.

The choice of autumn or spring sown crops within the rotation will allow specific opportunities for control of spring or autumn germinating weeds, whilst the use of over-winter green manures may provide additional opportunities for weed suppression. Specific crop varieties may be more effective at competing or suppressing weeds than others. Currently little information on such varietal attributes is available, although on-farm experience can indicate the most suitable varieties.

The use of mixtures of species, whether intercropped (both components harvested) or not, can contribute to the control of weeds. For example, clover/grass mixtures undersown in cereal crops as a method of ley establishment can limit weed growth (Williams, 1972; Hartl, 1989), whilst weed populations have been reduced in intercropped mixtures of beans and wheat (Bulson *et al.*, 1990).

During the arable phase of a mixed rotation, each crop chosen will provide different opportunities for mechanical cultivation or thermal treatment of weeds allowing for weed removal before or during crop growth. Furthermore, allelopathic interactions may contribute to the suppression of weed growth in some sequences more than in others. These options are considered below.

Mechanical cultivation

Physical disturbance of weeds by cultivation can effectively and economically contribute to weed control. This may be achieved either by burial of weed seeds below germination depth (for example by ploughing) or by killing emerging weed seedlings prior to establishment of the crop. Burial will be effective during primary cultivations, whilst killing emerging seedlings may be effective during seed bed preparation or within the growing crop.

Equipment suitable for pre-drilling or in-crop cultivation is readily available. In organic cereal husbandry, emphasis is placed upon pre-drilling methods using stale seed bed techniques. The opportunity for in-crop cultivation methods is exploited in row crops using hoe, harrow or brush type weeders and these are particularly important in field scale vegetable and horticultural crops. However, there is unexploited potential for the use of this equipment in cereal and other arable crops. Some research and development is underway in resolving the problems, particularly those of machine guidance.

Thermal techniques

Thermal (or flame) weeding is an integral part of the weed control strategy for many organically produced row crops. It involves the use of flaming equipment where there is direct contact of the flame and plant, or infrared equipment where the effect is from radiated heat.

Thermal techniques are used in higher value crops and/or in crops where slow germination can lead to weed seeds emerging prior to the crop. Extensive development work has led to sophisticated equipment which can be successfully used both pre- and post-emergence of the crop (Parish, 1991).

Allelopathy

Rice (1974) has defined allelopathy as 'the direct or indirect influence of one plant upon another through the production of chemical compounds that escape into the environment'. Although it is difficult to ascribe weed suppressive effects of certain crops to allelopathic interactions (rather than other competitive effects) there is evidence that the effect does occur with a large body of research having been undertaken (Rice, 1974). Altieri & Doll (1978) review the potential for this approach to weed management. It is very likely that with appropriate systems developed this method could be usefully employed in organic production systems.

FUTURE DIRECTIONS

Many approaches to weed management which are currently available to the organic farmer combined with future technological developments and increases in scientific understanding may yield new techniques. Already, approaches based upon allelopathic interactions may be practicable (see above), whilst "photocontrol" has also been explored (Hartmann & Nezedal, 1990). This approach involves exclusion of light during cultivation to exploit the phytochrome mediated initiation of germination of weed seeds.

However, these new techniques will share a common problem with those already available. Weed growth may cause yield reductions or contribute to the seed bank in organic systems if acceptable control techniques are not successfully applied. It is the 'successful application' of these more subtle systems of weed management, rather than the use of herbicides, which poses the greatest challenge to the researcher, advisor and farmer. Although there has been some attention paid to methods of weed control, little has been given to improving the ability to predict the optimum timing of weed destruction, nor to identifying the critical period of competition where weed destruction is necessary to achieve yield benefits.

Both of these requirements must be met in order to improve the efficacy of weed control currently achieved by organic farmers. Information on periodicity of emergence and competition in a range of crop/weed agro-ecosystems is essential and will be site specific to some extent. When this site specificisity is coupled with the requirement for the farmer to apply the necessary skills of observation and analysis to be able to operate these approaches, it is clear that substantial progress needs to be made.

The exclusion of all 'synthetic' herbicides in organic standards raises the question of the acceptability, at some future time, of a natural or nature-identical compound which has herbicidal action. There are already examples of myco-herbicide technologies which could be applied, whilst allelochemicals offer a new range of potential active ingredients derived from natural systems. However, the acceptability of such approaches to the organic standards authorities are unclear at present.

It is clear that optimal weed control strategies must be a fundamental component of rotation design and crop husbandry practices in organic farming systems in order to maintain long-term sustainability. There is a diverse range of strategies currently available and widely used, although few have been subjected to thorough scientific investigation. A significant research commitment into the area of weed control in organic systems is required in order to evaluate the effects of weeds in organic crops and to examine the potential for new methods of weed control.

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