SESSION 8B

BIOLOGICAL AND BIOTECHNOLOGICAL APPROACHES TO WEED CONTROL

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BIOCONTROL AND BIOTECHNOLOGY

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ABSTRACT

There is growing public interest in the development of alternatives to chemical pest control; in changing the kinds of chemicals employed and in reducing the amount of pesticides applied. There are two alternatives to chemical pesticides - one high tech and one low tech. The high technology of genetic engineering promises much, and despite massive scientific and legislative interest in recent years, is clearly still in its infancy. The low technology of biocontrol, meanwhile, has been bumbling along in the background and is not greatly favoured in modern high-input western agriculture. Long-term solutions to pest problems are much more likely to come through investment in biocontrol than through investment in genetic engineering. Long-term profits, on the other hand, may not be likely through either. Successful biocontrol spreads itself, and no individual vendor profits from it. Successful genetic engineering will make short term profits, but particular constructs are most unlikely to last, and individual engineered genotypes are unlikely to have commercial lives any longer than the products of conventional breeding techniques.

BIOCONTROL

A great many of the world's worst weeds are plants that have been introduced accidentally from other countries, where they left their pathogens and insect herbivores behind. The rationale behind biological weed control is that collection of herbivorous insects from the weed's native environment, followed by careful quarantine, should free the herbivorous insects from their own natural enemies. Then, following introduction to its new enemy-free environment, the biocontrol agent should be able to increase rapidly and destroy the weed (de Bach 1964; Julien, 1987). Sadly, things do not always turn out this way, and the history of biological weed control is marked by a small number of spectacular successes but a large number of disappointments. Even though the overall success rate of biological weed control is relatively low (about 1 project in 6 leads to satisfactory weed control), the successes are permanent, and highly cost effective. An additional benefit of biological weed control lies in its target specificity, and hence in its highly acceptable environment impact. Also, the safety record of weed biocontrol is good; there have been no disasters, and none of the introduced agents has become a nuisance in its own right, or switched hosts to become a pest of valuable plants in its new home.

Successes of biocontrol

It is difficult to define the success of a weed control project in absolute terms. It depends upon land values, the site that the land was in prior to the control attempt, and what the land was like before the weed became a problem. A weed density that was one tenth of its former level, might be regarded as high successful weed control by the farmer on whose land the infestation had been, but as a pestilential infestation by a farmer whose land was weed free.

A list of the weeds most frequently targeted for biological control would be dominated by two plant genera: the prickly pear cacti (Opuntia spp) and Lantana. Between them, they account for over half of all biocontrol attempts. It is striking that most weeds targeted for biological control are perennial plants of either badly-managed, semi-arid grazing lands (eg. Opuntia, Hypericum, Centaurea) or waterweeds of rivers, lakes and canals (eg. Eichhornia, Alternanthera, Salvinia). These two communities appear to be especially vulnerable to invasion by alien plants.

Virtually all kinds of herbivorous insects have been considered as biocontrol agents at some time or another. The most severe constraint on the choice of agents is their host-plant specificity, because unless the agent feeds only on the target weed, it is most unlikely to be released. Ease of handling, transport and rearing of the insect are other important practical considerations in the choice of agent species.

Because prickly pear cacti (Opuntia spp) have been the most frequent targets for bio-control, so cactus-feeding insects emerge as the most successful biocontrol agents. The world's most successful individual biocontrol agent is a chineal insect, Dactylopius ceylonicus, released in the late 18th Century to control Opuntia vulgaris over vast areas of India and Sri Lanka. Cochineals are tiny, wax-covered, sucking insects that belong to the bug family Dactylopiidae (one species is used in the production of the familiar red food colouring). The insects form dense colonies, eventually killing the cactus-pad allowing fungal and bacteria rot to set in. Overall, Dactylopius spp give successful weed control following 43% of introductions. Contrast this with the 24% success achieved by the 'text book' control agent employed against Opuntia, the pyralid moth Cactoblastis cactorum. The moth was introduced into Queensland, Australia from South America in the 1920's. The female lays her eggs in batches, and the caterpillars feed in dense aggregates, eventually causing the cactus to collapse under the weight of their numbers. The lower success of Cactoblastis is probably attributable to its higher risk of its being attacked by native natural enemies like predatory ants, insectivorous birds, and parasitic insects. It is an entomological curiosity that cochineal insects have no known parasitoids.

For insect taxa employed against other kinds of weeds, beetles emerge as easily the most successful. Weevils bring about successful weed control following 26% of releases, and the leaf beetles in 23% of cases. Compare this with only 14% for moths, and 15% for flies. Exactly why these particular insects should be so much more successful than others has yet to be understood, although a high reproductive rate,

long-lived adults, many generations per year, relatively small individual body-size and freedom from attack by native natural enemies, all correlate broadly with the degree of success achieved.

Some insect herbivores have never brought about successful weed control, despite repeated attempts (eg. leaf-mining flies, leaf-rolling moths and cerambycid beetles). While some successful weed control agents were local and rare in their native habitats, most of the really successful insects were both common and widespread as natives. There is much still to be learned about why certain insect species are abundant as natives, and why this should make them good invaders.

Rather few weeds have been controlled by seed-feeding insects, presumably because the abundance of many weeds is not determined primarily by the availability of seed. A spectacular exception is provided by the weevil Rhinocyllus conicus that was introduced from France for the control of nodding thistle Carduus nutans in Canada. The insect lays its eggs on the outside of the thistle head, and the larvae bore into the head and consume the seeds. Thirteen years after release, thistle densities had been reduced to 1/500th of their former abundance over large areas.

The most spectacular and repeatable success in modern weed biocontrol is brought about by the weevil Cyrtobagus salviniae against the floating fern Salvinia molesta in Australasia(Thomas & Room 1986).

Failures of biocontrol

A great many failures of biocontrol are caused simply by bad luck, bad timing or bad management. Some failures, however, appear to be associated with certain weed and insect traits. Most failures happen right at the outset, because the introduced consignment of insects fails to become established following field release. The main causes of failure are bad weather, attack by resident natural enemies (often ants) and, interestingly, bad taxonomy. A great many failures have subsequently been found to be due to so-called 'host-plant incompatibility' (ie. the insect could not eat the plant), resulting from misidentification of the weed, the insect, or both!

Of the introduced insect species that do become established, less than half bring about appreciable reductions in weed abundance. This is usually for one of two reasons. First, many plants have tremendous powers of regrowth following attack by herbivores. This is most likely to be important when a univoltine agents feeds on a plant with a long growing-season (eg. ragwort attacked by cinnabar moth caterpillars; Crawley 1989). Second, an established insect may fail because of attack by resident natural enemies. Insect parasitoids (insects that spend their larval stages inside other insects, eventually killing them before emerging as adults), viral and bacterial diseases, and generalist predators like spiders, ants, wasps and birds, may keep the introduced agents so scarce that they have no appreciable impact on weed abundance. This effect will be exacerbated if the weed provides a low quality diet for the control agents (eg. as a result of low nitrogen availability in the soil), because the insects may not be able to reproduce at a sufficiently high rate to escape control by the resident natural enemies, so that they became sufficiently abundant to wipe out the weed.

Some kinds of plants appear to be especially difficult to control using insects. For example, no annual weed of arable agriculture has ever succumbed to biocontrol. The short individual life-span, crop rotation, and the impatience of farmers, all conspire to reduce the likelihood of successful control by insects (note, however, that there may be scope for the use of plant pathogens for weed biocontrol in arable crops). Amongst perennial plants, conspicuous failures have come from such families as grasses, sedges and other herbs that have large, underground rhizome systems, and relatively low leaf nitrogen contents. These plants combine high powers of regrowth with low diet quality for the insects.

Just as nothing succeeds like success, so nothing is guaranteed to produce more failures, than the knowledge that a particular weed has been successfully controlled somewhere else. Thus, the two weeds most frequently failed against the same species that have been controlled successfully most often (the thorny shrub Lantana camara, and the St John's Wort, or Klamath weed, Hypericum perforatum. Because practitioners know that Telenemia or Chrysolina has brought about successful control somehwere else, they persist with repeated introductions, even though the ecological circumstances may be completely different, and the attempts doomed to failure.

Overview of weed biocontrol

Some biocontrol projects have been developed to the point where success can be virtually guaranteed. The best example involves the release of the beetle Cyrtobagous salviniae against infestations of the floating fern Salvinia molesta on tropical rivers, lakes and reservoirs. At the other extreme, there are combinations of weeds and agents that are desperately unpredictable; control is achieved at some times but not at other times, or in some places not in others. The best example here is Lantana camara, with is sometimes controlled by different agents in different microhabitats, but rarely controlled by a single agent over a side geographic area. The most obvious reason for the difference between these two extreme cases lies in the genetic make up of the plant populations. In the case of the floating fern, it appears that the species known as S. molesta is a sterile polyploid, and the entire population, world-wide, consists of a single genotype (it was cloned widely and sold as an aquarium plant). Having discovered a strain of Crytobagous salviniae in Brazil that is effective in reducing Salvinia molesta density, it was relatively straightforward to repeat the success. Salvinia molesta is particularly vulnerable to biocontrol because it lacks any means of persisting through unfavourable conditions (e.g. it produces no spores), and it is killed out-right by desiccation.

In contrast, Lantana camara is highly polymorphic, with numerous morphological and chemical phenotypes. Therefore, the insect strains that inflict substantial damage on one plant phenotype, may not have any marked impact on the abundance of other phenotypes, due to differences in plant chemistry, feeding stimulants, defensive compounds, nutritional aspects, or plant morphology. Thus, in any single infestation of Lantana, only a small proportion of the plants may be susceptible to a given strain of insects. Lantana also possesses considerable powers of

regrowth following defoliation, and can persist through unfavourable periods both as seeds and as vegetative individuals. Success is likely to be guaranteed only when a specialist herbivore is released against a genetically uniform weed.

BIOTECHNOLOGY

Genes have been manipulated by man, first unconsciously and then by deliberate breeding, for many centuries and much is known about the consequences of recombining and selecting genes in this way. Millions of novel plant genotypes created by conventional breeding have passed through evaluation, and those with desirable growth characteristics and acceptable products have been selected for commercial use.

Genetic manipulation and the insertion of genes by transformation in the laboratory is now an additional means by which breeders and researchers can create new combinations of genes; the source of DNA can, in principle, be any organism, simple or complex, plant or animal. The DNA might even be chemically synthesised in the laboratory.

In 1982 the bacterium Pseudomonas carrying the ice-minus gene was set to be the first genetically engineered organisms deliberately introduced to the field. The object was to displace forms of Pseudomonas syringae carrying genes specifying an ice-nucleation protein, and which caused frost damage to susceptible plants like strawberries. Such was the public outcry, however, that legal action by environmental groups in the USA led to the trial being delayed by several years.

Engineered insect resistance

Genetic engineering offers the environmentally attractive prospect of reducing the use of chemical insecticides in agriculture by making the crops themselves insecticidal or repellant to the pests. Engineering traits such as plant surface hairiness or waxiness, the production of toxic secondary plant compounds, or the ability to produce inducible defences following insect attack, are all on the drawing board. There is a general problem, of course, in that the engineering must not make the plant unsafe for human consumption, or the product less attractive for sale.

Two main insect resistance traits are already well known, and have been incorporated into solanaceous plants and tested in the field. One is a bacterial gene encoding an insecticidal protein that comes from the common soil bacterium Bacillus thringiensis. This is lethal to a number of lepidopteran caterpillars, and causes substantial yield improvements under laboratory conditions. The same gene has been placed in Pseudomonas bacteria that inhabit the leaf surface of crops, in the hope this will render the plants inseciticdal, while reducing the UV sensitivity of the toxin.

A second important trait for insect resistance is the production of proteinase inhibitors, small protein molecules which are lethal to many lepidopteran cateripillars, causing them to starve to death (they interfere with the production of digestive proteinase enzymes like trypsin within the caterpillar's gut).

Engineering virus resistance

A number of approaches have been taken to improve crop resistance to viral disease. A particularly cunning approach is to engineer traits into the plant for the virus' own coat protein. Then, when the virus is introduced into the plant, the coat proteins produced by the cell immediately wrap-up the viral RNA, preventing its multiplication. The system has been successfully tested by making tobacco at least partially resistant to infection by tobacco mosaic virus.

Risk assessment for genetically engineered crops

The commercial introduction of genetically engineered crop plants is certain to be contingent upon a thorough assessment of the potential risks involved. Little detailed information is available to allow assessment of risks but research is currently underway to identify areas of risk and to develop assessment protocols that will provide such data as are likely to be required prior to introduction.

Realistic, small scale field tests are likely to be the only way potential risks from commercial-scale uses of genetically engineered organisms can be evaluated. However, the experiments must be sufficiently well replicated, and carried out in a sufficiently wide range of climates and habitats if they are to be taken seriously as risk assessments (Urban & Cook 1986). An assessment of risk (Johnson 1982) is encompassed by the following questions:

1. What is the extent of normal 'field containment'? What natural barriers are there to the spread of introduced genes through pollen, seeds and vegetative propagules to adjacent crops and natural habitats?

2. Does the introduced gene affect the persistence of the crop (ie. create weeds) or the invasiveness of the transgenic plant or its progeny in natural habitats?

3. Can the inserted DNA be transferred to other organisms of the same or different species, and if so, what would be the consequences (eg. is the gene product toxic, pathogenic, or able to modify pathogenicity of other organisms).

The aim of our current experiments, carried out under the PROSAMO project (funded jointly by DTI, AFRC and a consortium of industries), is to establish baselines of field containment, persistence and invasiveness for a selection of crop plant species, and to use available transgenics to develop risk assessment protocols that can be used in future to test whether the introduction of specific genes have influenced these characters. We aim also to determine how genetic engineering influences plant fitness and competitive ability both in natural habitats and in arable fields (Hedrick 1986; Loveless & Hamrick 1984), and to assess the probability of gene transfer to non-crop plants, and the risks associated with such a transfer, should it occur. We do not propose to attempt any generalised, detailed predictions of risk from currently available transgenics to all future products of genetic transformation. Given the current state of knowledge on the ecology of

crop plants, and on the consequences of genetic engineering for altering ecological performance, it is clear that each transgenic product will need to be tested on a case-by-case basis for the forseeable future.

Present evidence suggests that the release of genetically engineered organisms will be safe because: (1) almost all genetic changes reduce plant fitness (Bradshaw, 1984; Davies & Snaydon, 1976; Snaydon, 1978); (2) wild type genotypes are almost always competitively superior to introduced genotypes of the same species (Harlan & Martini, 1938; Clausen et al, 1940; Jennings & Jesus 1968); (3) trade-offs are universal in evolution, and this will lead to reduced fitness in the wild, where the engineered gene is more likely to be a liability than a benefit (eg. if there is no selection in favour of the inserted gene). Concern is often expressed, for example, about the transfer of herbicide resistance to weeds. The risks associated with this are less than might be anticipated because: (1) herbicide resistance is not universal, and a weed that is resistant to one specific herbicide is almost certainly susceptible to another; and (2) herbicide resistance would not be of any benefit to a potential weed living in natural habitats, because herbicides are not applied to these plant communities.

It is necessary to consider the fate of the genetically engineered plants (and their pollen), and the effects of the introduction on the environment (ie. on subsequent crops in the same fields, on adjacent crops, and in nearby natural habitats). We have tackled these issues under three headings:

(a) problems concerned with the persistence of the vegetative plant and its propagules in different kinds of environment;

(b) problems relating to the spread of the plant by vegetative growth and by seed, in both arable fields and natural habitats;

(c) problems involving the risks of lateral spread of the engineered genes, either by pollination of different plant species, or by other means.

The model we use for persistence and spread is shown in Box 1. The risk assessment aims to establish the value of the rate of increase; the risk assessment is satisfactory if the rate is negative or zero, whereas if the rate is positive, a judgement needs to be made (ie. that the benefits of introduction outweigh the costs of the risks being realised).

BOX 1. A model for invasion by transgenic species (see Crawley 1986, 1987).

The rate of increase of the transgenic plant in a given habitat =

plant development rate +

its seed production (and its timing and duration) +

survival of vegetative parts (discounted by their mortality rate)-

the effects of competition with other plants of the same kind -

the effects of competition with other plant species -

the effects of herbivores (molluscan, insect and vertebrate) -

the effecs of fungi and other plant diseases -

the effects of mutualists (if they are in short supply; pollinators, seed dispersal agents, mycorrhizal fungi, etc) +

immigration of transgenic seed from other sites +

establishment of transgenic plants from dormant seed bank material

DISCUSSION

Biological weed control has a bright future, and for an increasingly large number of problems, it represents the only practical solution (eg. control of invasive alien weeds in tropical nature reserves). The success rate of biocontrol may have been rather disappointing in the past (1 case in 6), but its cost-effectiveness is impressive (most biocontrol projects cost less than £100,000 in total). Again, the success rate per attempt is vastly higher than is ever achieved in searching for potentially useful agrochemicals.

The future of genetic engineering in agriculture is extremely difficult to predict. Many people argue that it holds great promise because of its potential environmental benefits (reduced pesticide use, clearing of oil spills, detoxification of wastes, and so on). Others, however, argue that these high-tech solutions to environmental problems will only benefit the biotechnology companies, and that more fundamental changes in our approach to crop management are required. What is already quite clear, however, is that genetic engineering is no panacea. The products of genetic engineering, once commercially released, are subject to natural selection just like conventionally bred organisms. If they are introduced sufficiently widely, then the products will, themselves, act as potent agents of natural selection on the pests and diseases attacking them, selecting for increased resistance to the engineered traits (just as happened with the evolution of resistance to chemical pesticides).

On balance, the long term future of biocontrol looks brightest from the environmental viewpoint. Equally, it looks bleakest from the commercial side (eg. how will investment in biocontrol research and development be repaid, since one released, a biocontrol agent will work for all and sundry).

There are clearly vast profits to be made from genetic engineering in medical, pharmaceutical and industrial production. But profits from engineered crops are likely to be less dramatic; pest-resistant transgenic crops, for example, are likely to be similar to crop varieties bred to be pest-resistant by conventional means, and to have a short commercial life-span. There is also the currently uncertain cost of pre-commercial-release testing that may be imposed under future legislation.

In summary, it is clear that if only a fraction of the resources were put into biocontrol research and development, as are invested currently in genetic engineering, then some major pest control successes would be achieved. Given the way in which the benefits of biocontrol are distributed, however, it is probably more appropriate that this research and development should be funded by governments and international agencies, rather than by individual companies. Perhaps in the future we might augment biocontrol using genetic engineering (eg. to confer host specificity on an otherwise suitable agents). In any event, we should recognise that biocontrol has enormous potential; no other form of weed management offers such lasting control, with so little deletrious environmental impact, at so little cost.

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ADVANCES IN ENGINEERING HERBICIDE RESISTANCE IN PLANTS

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ABSTRACT

Modifying plants to become tolerant to herbicides would allow a selective use of these chemicals for crop protection. Genetic engineering of plants has demonstrated its significant potential to achieve this goal. Several strategies have been followed to isolate genetic traits which encode herbicide resistance. Recently, success with resistance engineered towards a number of commercially important herbicides has been reported. Several of these transgenic crops have already entered a next phase of development and are presently evaluated in open field conditions.

INTRODUCTION

In the last decade, a remarkably fast progress in plant molecular biology has taken place. The transformation and regeneration of more than 20 different plant species including several important field crops has been achieved. At the same time, dramatic progress has been made in the identification and improvement of genes encoding valuable agronomic traits (for review, see Gasser & Fraley, 1989).

Engineering crops resistant to herbicides has been one of the first issues targeted (for review, see Botterman & Leemans, 1988). Indeed, the engineering of plants resistant to herbicides would allow their use for post-emergence applications in more effective and flexible weed control programs. Research has largely concentrated on these herbicides with properties such as high unit activity, low toxicity, low soil mobility and rapid biodegradation and which have a broad spectrum against various weeds.

In this paper, the different approaches followed in engineering herbicide tolerance are described and illustrated with the results and progress of some important examples. Several of these achievements have already entered a next phase of development. At present, crops engineered for herbicide tolerance are already evaluated under open field conditions. These field tests allow to compare qualitative and quantitative characteristics of the transgenic crops and to analyze the potential risks coupled with the deliberate release of transgenic plants in the environment.

APPROACHES TO ENGINEERING HERBICIDE TOLERANCE

Two general approaches have been taken in engineering herbicide tolerance: the first consists of altering the level and sensitivity of the target enzyme for the herbicide, while in the second a gene that detoxifies the herbicide is incorporated in the plant genome.

Modification of the target of the herbicide action

Many biochemical sites of action of herbicides in plant cells have been identified in amino acid biosynthesis pathways and in photosynthesis. Genes encoding herbicide-sensitive or -insensitive target proteins have been isolated from both plants and microorganisms and have been used to engineer tolerance to a number of herbicides. Examples of these are the engineering of resistance to glyphosate and the sulfonylureas.

Glyphosate acts by specifically inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP), a key enzyme in the biosynthesis of aromatic amino acids. EPSP synthase is a chloroplast enzyme that is encoded in the nuclear genome. Glyphosate is a systemic herbicide that accumulates in plant apices and affects more severely cells in growing parts of the plant than in fully developed tissues. The strategies followed to engineer tolerance to glyphosate have been focused on the introduction of gene constructs for 1) the overproduction of a wild type EPSP synthase or of glyphosate-tolerant variant EPSP synthase enzymes, 2) the delivery of EPSP synthase to the chloroplast and 3) to optimize the expression of the EPSP synthase gene by the use of promoters that confer high-level expression in the shoot and root regions, where glyphosate accumulates. The gene encoding EPSP synthase from Petunia hybrida or the EPSP synthase encoded by the aroA gene isolated from Salmonella typhimurium have been used in chimeric gene constructs following one or a combination of these strategies (Comai et al., 1985; Shah et al., 1986). For example, by crystallization and biochemical analysis, more tolerant EPSP synthases have been designed by site directed mutagenesis. A promoter fragment has been isolated from cauliflower, which is specifically active in meristematic tissues and used to drive the expression of a mutant EPSP synthase. This yielded oilseed rape with normal flower phenotype (Fraley, personal communication). The EPSP synthase gene constructs have thusfar been introduced in several crops such as cotton, soybean and oilseed rape.

The sulfonylurea herbicides inhibit acetolactate synthase (ALS), an enzyme involved in the biosynthesis of the branched chain amino acids leucine, isoleucine and valine. An unrelated class of herbicides, the imidazolinones also inhibit ALS. Dominant mutations that confer resistance to sulfonylureas have been found in Salmonella typhimurium, Saccharomyces cerevisiae and Escherichia coli (Falco et al., 1987). Characterized mutants have single amino acid substitutions in their ALS proteins. Mutants have also been isolated in plants by selection for resistance. The ALS genes are nuclear encoded and include a sequence coding for a chloroplast transit peptide. In two isolated ALS genes from resistant tobacco, three sites were mutations occured have been identified and a single amino acid substitution has been observed in the mutant Arabidopsis gene (Haughn et al., 1988; Lee et al., 1988). The mutant genes do not generally confer cross resistance to the imidazolinone herbicides (Falco, personal communication). Resistance to sulfonylurea compounds, has been produced by the introduction of gene constructs containing mutant acetolactate synthase (ALS) genes in transgenic plants. Transgenic tobacco plants expressing a mutant ALS gene from tobacco or Arabidopsis were tolerant to sulfonylurea herbicides.

Detoxification of the herbicide

Herbicide-detoxification pathways exist in plant species that are naturally tolerant to specific herbicides. For example the detoxification of atrazine in tolerant maize lines involves its glutathione-S-transferase mediated conjugation with the tripeptide glutathione. These systems have been exploited in agriculture for selective use on crops. Detoxifying enzymes have also been identified in microorganisms and several soil microorganisms involved in herbicide degradation have been characterized as potential sources for herbicide resistance genes. In three cases the corresponding genes have been introduced into crops to inactivate the herbicide and prevent it from exerting its inhibitory effect in the plant cell. Resistance to glufosinate, bromoxynil and 2,4-D has been achieved by introducing bacterial genes encoding enzymes that inactivate the herbicides.

A gene encoding a nitrilase highly specific for bromoxynil has been isolated from Klebsiella pneumoniae ozaenae. The nitrilase acts on the cyano group of the molecule converting it to a nonphytotoxic compound. Chimeric gene constructs with the nitrilase gene have been introduced and transgenic tomato plants that expressed the nitrilase enzyme were resistant to bromoxynil (Stalker et al., 1988).

A detoxification enzyme has been found in the biosynthesis pathway of the herbicidal compound bialaphos. Bialaphos is a tripeptide antibiotic produced by Streptomyces hygroscopicus. It consists of phosphinothricin (PPT), which is an inhibitor of glutamine synthetase, and two L-alanine residues. Glufosinate, the chemical synthesized PPT, and bialaphos are used as non-selective herbicides. The bar gene, isolated as a resistance gene in the biosynthesis pathway was shown to encode a phosphinothricin acetyl transferase that acetylated the free amino group of PPT (Murakami et al., 1986; Thompson et al., 1987). The bar gene has been transferred in tobacco, tomato, potato, oilseed rape and sugarbeet plants and conferred complete resistance towards high doses of glufosinate and bialaphos on greenhouse plants (De Block et al., 1987; De Greef et al., 1989).

A gene encoding a 2,4-dichlorophenoxyacetate monooxygenase has been isolated from the soil bacterium Alcaligenes eutrophus. The gene product which catalyzes the side chain cleavage of 2,4-D was used to introduce a herbicide degrading mechanism into plants. Regenerated plants showed resistance when sprayed with 2,4-D (Streber and Willmitzer, 1989).

Conclusion

These examples clearly illustrate the progress with genetic engineering techniques to engineer herbicide tolerance. Resistance can in principle be introduced as a defined genetic characteristic, but in practice a number of problems may be encountered. Changes in the target of the herbicide requires that it is the unique target, and that mutant forms can still carry out their biological function. When multimeric enzyme complexes are involved, mixed populations of mutant and wild-type subunits might yield an inconsistent phenotype. Since both EPSP and ALS activities are present in wild-type plants, one argues that the possibility of deleterious effects on crop performance or product quality due to their reintroduction is unlikely.

When using heterologous detoxifying genes, the substrate specificity of the encoded enzyme is important and the second substrate for herbicide modification might not always be abundantly available. The fate and toxicology of the metabolized herbicide requires also careful examination. The biological activity of the specific herbicide conjugates and metabolites that may be present in the transgenic plants will have to be analysed according to existing chemical residue regulations.

HERBICIDE RESISTANCE IN TRANSGENIC CROPS UNDER FIELD CONDITIONS

Although the results with transgenic plants in the greenhouse looked very promising, different important questions were still to be addressed. Observations in the laboratory and the greenhouse, which are needed to monitor the expression of the introduced gene and the behaviour of the genetically modified crop in a first phase have to be followed by open field trials. They have to answer two recurring questions : 1) how stable is the expression of the introduced gene(s) under the highly variable conditions in the field, and 2) do plants suffer any undesired effects as a result of the genetic modification? Moreover, they provide statistical data on field performance and yield data and allow to compare qualitative and quantitative characteristics of engineered crops relative to competitive existing products. Important considerations are also the potential risks coupled with the cultivation of genetically engineered plants. The following risks are to be considered : 1) the offspring of the genetically engineered plant may become a pest; 2) the newly introduced information is transmitted to a related wild species; 3) the ecological impact on the crop. The most significant perceived risk with genetically modified plants is the transfer of newly-acquired genes to wild and weedy relatives. Several precautions can be taken during the small-scale field trials to eliminate the risk of spread of the recombinant genes : absence of relatives in test areas, prevention of pollen transfer (by early harvesting, buffer zones or deflowering), daily plant monitoring, access restricted to authorized personnel, destruction of the removed plant and seed material, control of volunteer weeds and follow-up of the field in subsequent seasons.

FIELD TRIALS

Since 1986, several small-scale field trials have been conducted in different countries. In 1988, about half of the field tests performed were for herbicide tolerance in tobacco and tomato. The remaining half were nearly all for insect and disease resistance. During 1989, the field experiments involve a greater range of crops, including potato, corn, soybean, oilseed rape and cotton. At present, it is difficult to estimate the number of field trials with transgenic plants and one can expect that requests for new trials will increase in the coming years. Presently, field trials 1) with glyphosate resistant tomato, oilseed rape, tobacco, cotton, soybean (Fraley, personal communication); 2) with sulfonylurea resistant tobacco and tomato (Falco, personal communication); 3) with bromoxynil tobacco, tomato and cotton (Stalker, personal communication) and 4) with glufosinate resistant tobacco, potato, tomato, poplar, sugar beet and alfalfa have been or are being conducted. In most cases a herbicide resistant or tolerant phenotype was observed and no yield penalties were observed. However, data of in-depth

analysis on qualitative and quantitative characteristics are not published yet.

In a first trial in 1987, P.G.S. evaluated glufosinate resistant tobacco and potato lines under field conditions (De Greef et al., 1989). Transgenic plants of the tobacco dwarf variety SR1 as well as untransformed control plants were planted in the field. Untransformed plants were used as control in order to evaluate the agronomic performance of transgenic crops. In the weeks following after herbicide applications, no visible effects or damage were observed on the resistant plants. The analysis of variance demonstrated that there was no significant difference between the treatments. From the subset of plants kept, it was seen that the flowering and seed set were not influenced by the herbicide treatments and confirmed previous greenhouse tests. Transgenic lines from the commercial potato cultivars Bintje, Berolina and Desiree were analysed in the field. In the weeks following the herbicide treatment there was no difference between unsprayed controls and transformed plants sprayed with the herbicide. At harvest, the number of surviving plants per plot and the tuber weight were recorded. In general, there was no difference between the unsprayed controls and the sprayed transformed lines in percentage of surviving plants. The analysis of variance for the fresh tuber weight per surviving plant for each plot indicated that there was no significant difference between the control and any of the sprayed herbicide resistant lines. This first field test proved the complete resistance to field dose applications of glufosinate, although the expression of the resistance gene in these lines varied by two orders of magnitude. The growth of the transgenic tobacco and potato lines was indistinguishable in all treatments from the non-transformed non-treated control lines over the whole crop cycle and they showed the same agronomic performance.

In 1988, field trials have been performed with transgenic commercial tobacco lines of cultivar PBD6. The results showed that the presence of the bar gene conferred total resistance to the herbicide; that no phenotypic differences were observed between the transgenic lines and the untransformed control lines. Moreover, weed control with Basta®, the commercial glufosinate-ammonium, allowed to perform optimal weed control during the whole growth season. In 1989 field trials are being conducted with tobacco, tomato, oilseed rape, alfalfa and sugar beet.

FUTURE PERSPECTIVES

This summary shows that the engineering of a herbicide tolerant trait in transgenic plants looks promising in several cases and is approaching commercialization. The commercial strategy in engineering herbicide tolerance is to gain market share through a shift in the application of herbicides and not to increase the overall use of herbicides. Herbicide-resistant plants will have the positive impact of reducing overall herbicide use through substitution of more effective and environmentally acceptable products. It is also important to notice that these traits will also be applicable as selectable markers in both basic research and plant breeding by physical linkage to other agronomic traits.

Before transgenic plants can be commercialized, important aspects will have to be considered. Factors such as herbicide performance, crop and chemical registration costs, potential for out-crossing to weed species, proprietary right issues and competing herbicide technologies must all be considered before final decisions on commercialization of specific herbicide-tolerant crops can be made (Fraley et al., 1987). Other issues that will affect the introduction of genetically engineered plants include regulatory approval, proprietary protection and public perception.

Although plant breeding products have always been freely distributed, transgenic plants require regulatory approval before even small scale field testing can be performed. In 1989, the regulatory situation in Europe varies from one country to another. Since the potential problems associated with genetically modified plants will not be confined to national territories, a common guideline for deliberate release of genetically engineered organisms in the environment instead of the current patchwork of national regulations is needed. It is of extreme importance that the process for evaluating field tests of genetically modified crops responds quickly to the need for testing plants at multiple locations and under normal agronomic practices including completion of the crop reproduction cycle in normal areas of production. Such a regulatory process should satisfy the concerns regarding environmental impact and health and the need to let research and development proceed in a rational and efficient way. Those regulatory structures should focus not on how a particular crop is made but on what new traits it has and how it will be used. Also, it is necessary that the regulation of the commercialization of these crops be formulated and harmonized in a way which does not discriminate the particular process used to improve the varieties.

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CURRENT ADVANCES IN BIOHERBICIDE RESEARCH

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ABSTRACT

Significant advances are being made in the discovery and development phases of novel bioherbicide products, but various factors have limited the deployment of bioherbicides in crop production systems. Many of the limitations to bioherbicide advancement have been suggested with low pathogen virulence and fastidious environmental conditions identified as the key restraints to overcome. Improvements in strain selection, bioherbicide formulation, and field use are being made and bioherbicides are being integrated with other weed control strategies to provide effective weed control. Overcoming these biological and technological limitations will significantly reduce the economic questions and concerns which should translate into increased industrial commitment and involvement, culminating in the deployment of effective, economically viable bioherbicide solutions to some of our major weed problems.

INTRODUCTION

Biological control of weeds is the deliberate use of natural enemies to suppress the growth or reduce the population of a weed species. Two primary strategies, the classical or inoculative strategy and the inundative or bioherbicide strategy, have evolved for biological weed control. Numerous review articles describe the fundamentals, the methodology, and the progress of biological weed control (e.g., Schroeder, 1983; Wapshere, 1982). Although insects, mites, plant pathogens, and aquatic and terrestrial herbivores have been used as biotic agents in biological weed control programs, the use of plant pathogens has become increasingly more prevalent. The progress and prospects of using plant pathogens in biological weed control programs is well documented in the literature (Adams, 1988; Charudattan, 1988; Hasan, 1988; Templeton, 1982; Templeton *et al*. (1986); TeBeest & Templeton, 1985; Scheepens & van Zon, 1982; Wilson, 1969).

This paper will attempt to address the current advances in bioherbicide research through a brief review and discussion of the basis, the progress, the restraints, and the prospects of this approach to weed control. Although fungi, bacteria, mycoplasmas, viruses and nematodes incite plant disease, the use of pathogens other than fungi as bioherbicides is limited. Therefore, the term "mycoherbicide" has often been used interchangeably with "bioherbicide." In addition the term "bioherbicide" is generally restricted to the use of plant pathogens and does not include attempts to augment populations of beneficial insects, nor does it generally include the use of naturally occurring compounds (phytotoxins) produced by microorganisms. Phytotoxins, however, may play an important role in bioherbicide development.

BASIS OF THE BIOHERBICIDE APPROACH

A bioherbicide is a preparation of living inoculum of a plant pathogen, formulated, and applied in a manner analogous to that of a chemical herbicide in an effort to control or suppress the growth of weed species. The use of bioherbicides is based on the fundamental epidemiological principles of plant pathology. Plant disease is the result of the interaction among the host plant, the pathogen and the environment, commonly referred to as the disease triangle. Although serious, devastating disease epidemics of crop plants occur, they are the exception rather than the rule and many factors can limit disease development. Pathogen factors such as low inoculum levels, weakly virulent pathogens, and poor spore dispersal mechanisms; environmental factors such as unfavorable moisture and/or temperature conditions; and plant factors such as low susceptibility of the host, and widely dispersed host populations often limit disease. The bioherbicide approach is an attempt to bypass many of these restraints on disease development by periodically dispersing an abundant supply of virulent inoculum uniformly onto a susceptible weed population. The application is timed to take advantage of favorable environmental conditions and/or the most susceptible stage of plant growth. Similarly the bioherbicide is formulated to avoid unfavorable environmental conditions and to facilitate application. As a consequence, the development of an effective bioherbicide requires a comprehensive understanding of the pathogen(s) involved, the biology and population dynamics of the target weed(s), the optimum requirements for disease initiation and development, and the complex interactions within the host-pathogen system.

In the development of any new pest control strategy, safety and efficacy are the two primary concerns (Watson & Wymore, 1989b). As a consequence, safety (in relation to crop plants, the environment, and human health) and efficacy (in relation to environmental tolerance, level of damage to the weed, and ability to be integrated within the crop production system) are the major criteria in the selection of suitable plant pathogens. The preferred characteristics of a potential bioherbicide pathogen include: 1) growth and sporulation on artificial media, 2) highly virulent, 3) genetic stability, 4) restricted host range, 5) broad tolerance range, 6) prolific propagule production, 7) capacity to damage its host plant, and 8) innocuous in ecological effects (Templeton et al., 1979).

In determining the suitability of a particular weed species as a target for bioherbicide development, native or naturalized weed species should have a larger complement of indigenous pathogens to select from as compared to fewer pathogens associated with recently introduced weeds. Templeton et al. (1986) suggest that bioherbicides have greatest potential for control of: a) weeds infesting small specialized areas where chemical herbicide development would be too costly, b) weeds that have been intransigent to chemical control, c) crop mimics, and d) parasitic weeds. Since potential return on investment is critical to industrial involvement in bioherbicide development, major weeds, presently not controlled by available technology, in major crops are perhaps the ideal targets for the bioherbicide approach.

Intuitively, annual weed species may be considered preferred targets when compared to perennial weed species. However, the growth habit, growth rate and other biological parameters which determine susceptibility and

subsequent disease development are more critical than whether the weed is an annual or a perennial. For example, annual weeds such as velvetleaf (Abutilon theophrasti) with their erect habit of growth and rapid rate of stem elongation may be less susceptible to disease development of foliar pathogens when compared to vigorous perennials such as field bindweed (Convolvulus arvensis) and dandelion (Taraxacum officinale) with their prostrate habits of growth.

STEPS IN BIOHERBICIDE DEVELOPMENT

The development of a biological herbicide involves three major phases or stages: 1) discovery, 2) development, and 3) deployment (Templeton, 1982). The discovery phase involves the collection of diseased plant material, isolation of the causal organism, demonstration of Koch's postulates, identification of the pathogen, culture of the pathogen on artificial media, and maintenance of the pathogen cultures in short-term and long-term storage. The development phase involves the determination of optimum conditions for spore production, determination of optimum conditions for infection and disease development, determination of host range and elucidation of mechanism of action of the pathogen. The final phase, deployment, often involves close collaboration between non-industrial and industrial sectors through the formulation, scale-up, field evaluation, and marketing stages of commercialization process of a new bioherbicide product.

The proposed close collaboration between industrial and non-industrial sectors is not always easy, especially when the objectives of the two groups are often not completely compatible. Both Baker (1986) and Scher and Castagno (1986) point out that despite intensive research and numerous apparently successful biological control agents, very few have reached the marketplace. Baker (1986) suggests the need for more research related to understanding the basic mechanisms of biological control, whereas Scher and Castagno (1986) suggest the reason for the paucity of marketable biocontrols is because most biocontrol agents have been developed from a scientific point of view only, without an industrial perspective.

PROGRESS

To date, two bioherbicides have been registered for weed control, both in the United States. DEVINE^{R}, a liquid formulation of Phytophthora palmivora was registered in 1981 for control of stranglervine (Morrenia odorata) in Florida citrus groves. COLLEGO^{R}, a dry powder formulation of Colletotrichum gloeosporioides f. sp. aeschynomene, was registered in 1982 for the control of Northern Jointvetch (Aeschynomene virginica) in rice and soybeans in Arkansas, Louisiana and Mississippi. To my knowledge no other bioherbicides are as yet registered for use, but active research programs in various laboratories within North America, Europe and elsewhere are making rapid progress towards the development and registration of additional bioherbicide products for specific weed problems.

Published reviews of bioherbicides such as Charudattan (1988), Hasan (1988), Templeton et al. (1986) provide partial lists of current bioherbicide research projects. Information in Table 1 is not an attempt to provide a comprehensive, all-inclusive list of bioherbicide research, but rather a sampling of the recent literature to demonstrate the present

scope. Cited examples are from Europe, North America and Australia. This list illustrates to some extent the diversity of the target weeds, and conversely, the similarity of the plant pathogens being investigated.

TABLE 1. Examples of some current bioherbicide research projects.

Target weed	Plant pathogen	Reference
Abutilon theophrasti	Colletotrichum coccodes	Wymore et al. (1988)
Cassia obtusifolia	Alternaria cassiae	Walker & Boyette (1985)
Convolvulus arvensis	Phomopsis convolvulus	Ormeno-Nunez et al. (1988)
Cucurbita texana	Fusarium solani, f. sp. cucurbitae	Weidemann & Templeton (1988)
Desmodium tortuosum	Colletotrichum truncatum	Cardina et al. (1988)
Echinochloa crusgalli	Cochliobolus lunatus	Scheepens (1987)
Eleusine indica	Bipolaris setariae; Pirícularia grisea	Figliola et al. (1988)
Malva pusilla	Colletotrichum gloeosporioides f. sp. malvae	Mortenson (1988)
Pteridium aquilinum	Ascochyta pteridis; Phoma aquilina	Irvine et al. (1987)
Sorghum halepense	Colletotrichum graminicola Exserohilum turcicum Gloeocercospora sorghi	Chiang et al. (1989)
Xanthium spinosum	Colletotrichum orbiculare	Auld et al. (1988)

RESTRAINTS TO BIOHERBICIDE DEVELOPMENT

As with any developing technology, various problems and difficulties have been encountered by bioherbicide researchers. Some of these problems are biological or technological in nature, while others range from economic concerns to perception (Charudattan, 1988, Watson & Wymore, 1989b).

These restraints can be broadly characterized as biological, technological, economical, environmental and governmental (Watson & Wymore, 1989a). As mentioned earlier, few biological control agents have been developed to the marketable product stage and Baker (1986) concludes that one of the primary reasons is that biocontrol agents are less efficient than other control methods.

Most, if not all, plant species including weeds are attacked by plant pathogens. However, as indicated earlier in this report disease development is restrained by various plant, pathogen, and environmental factors. The inundative inoculation of the target weed with the bioherbicide is fashioned to overcome many of these restraints, but low virulence of the pathogen and fastidious environmental conditions are the two major biological hurdles to overcome (Templeton, 1982).

Most bioherbicide pathogens require rather exact moisture and temperature conditions for spore germination, and host penetration. Often these conditions are not provided naturally under field conditions and the resulting application of the bioherbicide is ineffective. Bioherbicides,

as with other biocontrol agents, are difficult to formulate which illustrates the primary technological constraint to the development of bioherbicides.

Large-scale production ("fermentation") of bioherbicide pathogens is another potential technological restraint to bioherbicide development. Although the precise requirements for sporulation are not known for most pathogens, and some pathogens do not sporulate readily in culture, technical expertise in the fermentation industry is available and is being utilized to some extent in the production of bioherbicide pathogens. However, there is a need to understand the basic mechanisms involved in the growth and sporulation of the various fungi being evaluated as possible bioherbicides.

To be acceptable to the producer, any pest control product, including a bioherbicide, must provide economic returns. Most of the available information to date on bioherbicide development indicates that development costs of a bioherbicide will be less than for a chemical herbicide. Similarly, registration costs should also be less. For example, the cost of developing COLLEGO^R has been estimated from $1 to 1.5 million U.S. (Templeton et al., 1986) which is substantially less than that for a chemical pesticide which has been estimated from $30 to 90 million. Unfortunately exact registration requirements for bioherbicides have not been finalized in some countries such as in Canada, and numerous other factors such as potential market, ease of formulation, competitive products, etc., are involved, most indications suggest that bioherbicides should be economically viable. However, many of the potential bioherbicide market niches are small, and these views may differ somewhat from those held by the large chemical pesticide industries (Jutsum, 1988) which are primarily interested in large market opportunities.

As with any other pest control strategy, or for that matter any perturbation, concerns and questions related to environmental issues are appropriate and necessary for the bioherbicide strategy. Although biocontrol agents are typically regarded as non-toxic to humans (Scher & Castagno, 1986), certain precautions and adequate testing are required prior to bioherbicide usage. In addition to human safety, environmental safety and crop plant safety are important parameters being addressed by regulatory authorities in various parts of the world.

The final suggested restraints to the development of bioherbicides are governmental aspects. Guidelines for the registration of biological pesticides and proposals for regulating biotechnology have been prepared and are being discussed. Unfortunately, in Canada we have a reputation of overregulation, and at times have been guilty of imposing political decisions rather than responding to sound scientific recommendations. Since much of the bioherbicide development relies on industrial involvement, overregulation may severely limit growth of this sector (Watson & Wymore, 1989b).

CURRENT ISSUES AND ADVANCEMENTS

Efficacy

Most discussions of biological control stress the importance of enhancing efficacy (Burge, 1988; Charudattan, 1988; Heale, 1988; Watson &

Wymore, 1989b). It is possible to screen for more efficient strains or isolates of bioherbicide pathogens and other genetic manipulations are being developed including recombinant DNA technology to enhance bioherbicide performance (Greaves et al., 1989; Templeton et al., 1986). Many aspects of the bioherbicide pathogen such as increased virulence, improved toxin production, altered host range, resistance to crop production chemicals, altered survival or persistence in the soil, broader environmental tolerance, increased propagule production in fermentation systems and enhanced tolerance to formulation processes are targets for genetic improvement of bioherbicide pathogens. Development of efficient transformation systems (Panaccione et al., 1988) and progress in the isolation and cloning of pathogenicity genes (Kronstad & Leong, 1989) of plant pathogenic fungi will encourage further advances. The advances and potential impact of fungi genetics on biocontrol have been recently reviewed (Greaves et al., 1989; Heale, 1988). The recent report by Miller et al. (1989) of the selection of a non-sclerotial mutant of Sclerotina sclerotiorium, one of the most ubiquitous and non-specific plant pathogens known, has interesting implications for bioherbicide development.

The efficacy of bioherbicide pathogens may be enhanced chemically, especially since many fungi are presumed to produce toxins or plant growth regulators which enhance disease development (Templeton et al., 1986). Many microorganisms, including some of the bioherbicide pathogens, produce toxic metabolites with herbicidal activity (Mishra et al., 1988; Huang et al., 1989). Reviews by Duke (1986) and Duke and Lydon (1987) have highlighted the progress and prospects of the development of herbicides from natural compounds. The relative importance of phytotoxins as new herbicide chemistry versus the use of the living organism as a bioherbicide is difficult to predict, but both approaches are likely to be major growth and development areas for the herbicide industry.

Enhancement of bioherbicide efficacy has been obtained with the addition of growth regulators and chemical herbicides to the bioherbicide (Templeton et al., 1986; Watson & Wymore, 1989b). The synergistic interaction of Colletotrichum coccodes and the growth regulator, thidiazuron for the control of Abutilon theophrasti (Wymore et al., 1987) and the tank mix of atrazine and Cochliobolus lunatus for the control of Echinochloa crusgalli (Scheepens, 1987) illustrate the approach.

Integration

Charudattan (1988) lists "incompatibility with chemical pesticides" as one of the problems associated with the use of bioherbicides. Certainly the potential toxicity of fungicides and other chemical pesticides or crop production chemicals may interfere with the use or integration of bioherbicides into intensive crop production systems (Templeton et al. 1986; Watson & Wymore, 1989a,b).

Due to the inherent narrow-spectrum nature of bioherbicides, they will likely be used in combination with other weed control strategies, particularly chemical herbicides. Normally a bioherbicide will be targeted against a dominant, troublesome weed, but will have limited or no effect on the complex of other weeds commonly found in most crops. Many bioherbicides can be effectively tank mixed with chemical herbicides, other biocontrol agents, or applied sequentially to provide the desired broad spectrum of weed control (Watson & Wymore, 1989a).

Formulation

Formulation has been identified as the major limiting factor in bioherbicide development (Watson & Wymore, 1989b). A bioherbicide formulation should maintain propagule viability for an extended period of time (shelf-life) and in some manner provide a suitable microenvironment at the propagule/target interface (e.g., leaf surface). Difficulties were encountered in the formulation of the bioherbicide DEVINE^{R} and it is formulated and sold as a "fresh milk" product on a contractual basis. Drying techniques, however, have successfully been used to formulate COLLEGO^{R} as a dry wettable powder and these techniques should be useful for many other bioherbicide pathogens. Success has been achieved with alginate, a water-soluble polysaccharide gum, to formulate bioherbicide pathogens (Connick et al., 1989; Fravel et al., 1985) and alginate gel technology will likely be increasingly important in attempts to develop suitable bioherbicide formulations. Attempts to use "invert emulsions" to bypass reliance on natural dew formation to facilitate infection have also been partly successful (Connick et al., 1989).

Host specificity

Host plant specificity is of prime importance in biological weed control and crop safety issues have been discussed by Charudattan (1988), TeBeest & Templeton (1985) and Watson (1985). Concerns of latent colonization by bioherbicide pathogens have been raised (Cerkauskas, 1988), but passage of some organisms through weed species has not resulted in predicted increased virulence on crop plants (McLean & Roy, 1988).

The degree or level of specificity required for a potential bioherbicide pathogen has not been clearly established. Absolute specificity to the target weed may be desirable, but is probably unrealistic and is often used as a criticism of the approach due to limited market potential. The active ingredient of COLLEGO, Colletotrichum gloeosporioides f. sp. aschenomene is not specific to a single plant species and is pathogenic, although not virulent, on other plant species (TeBeest, 1988; Weidemann et al., 1988). The potential bioherbicide pathogen certainly should not damage the crop plant(s) in which it is intended for use, but may have utilization even if it causes some disease on other desirable plant species. Product labels for COLLEGO^{R} and DEVINE^{R} provide restrictions on use of these products near areas where susceptible plants occur.

BIOHERBICIDE PROSPECTS

It is unfortunate that it is generally believed that rapid and complete weed control is required within intensively managed agroecosystems (Charudattan, 1988). Paul and Ayres (1987) have clearly demonstrated that a weed pathogen, although it did not increase mortality, effectively suppressed the competitive ability of a weed. Field evaluations with many of the prospective bioherbicides demonstrate that even if they do not cause high levels of mortality, effective weed control has been expressed in significant increases in crop yields (Wymore & Watson, 1989; Weidemann & Templeton, 1988).

In addition to numerous broad-leaf weed targets, more effort is being directed towards grass weeds (Chiang et al., 1989; Figliola et al. 1988)

and additional positive results are being obtained from field trials (Mortenson, 1988; Weidemann & Templeton, 1988; Wymore & Watson, 1989). Formulation difficulties are being addressed and application technologies need to be improved. In 1982 Scheepens and van Zon suggested that high pressure, small droplet sprays typical of fungicide application equipment provided superior results to those obtained with standard, low pressure, large droplet herbicide applications, which suggests that application technology could improve bioherbicide efficacy.

The prospects for improving the efficacy and performance of bioherbicides through genetic manipulation are encouraging (Greaves et al., 1988), and some studies are underway to understand and perhaps overcome host defense response (Irvine et al., 1987). Certainly, it has been demonstrated that bioherbicides can be effectively integrated into crop production systems (Watson & Wymore, 1989a).

Bioherbicides should not be viewed as alternatives to chemical herbicides, but rather as complementary tactics in integrated weed management systems (Templeton et al., 1986; Watson & Wymore, 1989a,b). To become acceptable weed control strategies, concerns of bioherbicide safety in relation to crops, the environment and human health and efforts to improve bioherbicide efficacy in relation to environmental tolerance (formulation), enhancement of level of control and integration into crop production systems are being addressed. Basic understanding of the disease cycle and of the mechanisms of pathogenicity, sporulation, host response, etc., are of paramount importance as are the concerns and views of industry and regulatory authorities as we strive to interact positively to achieve economic, environmentally sound, and effective weed control strategies.

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BIOCONTROL OF BRACKEN, PTERIDIUM AQUILINUM, IN THE U.K. : PROSPECTS AND PROGRESS

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ABSTRACT

We assess the current status of bracken as a weed in the U.K. and review the progress of the AFRC-funded biocontrol project. The diversity of the insect fauna attacking bracken in temperate regions of the world, suggests that potential exists for classical biological control of this weed in the U.K. Several bracken-feeding insects have been imported under strict quarantine and tested against a wide range of native U.K. plants and crops. The most promising agent for introduction is a noctuid moth, Conservula cinisigna, and results of the host range testing with this species are reported. Remaining problems involve rephasing the seasonality of this southern hemisphere moth and the development of a protocol for biocontrol introductions given the novelty of this pest control strategy for the U.K.

INTRODUCTION

Bracken (Pteridium aquilinum) is an invasive weed common over much of Britain (Page, 1976). It's range is thought to be spreading by as much as 3% per year in Scotland and Wales (Smith & Taylor, 1986). Recent work has highlighted the problems caused by bracken including stock poisoning, possible risks to human health and encroachment onto land used for grazing, conservation or recreation (Lawton, 1988; Marrs, 1987; Smith & Taylor, 1986; Taylor, 1989). In the first part of this paper we assess the status of this weed in the U.K., giving a summary of the results of a socio-economic survey of farms in England and Wales. In the second section we examine the potential for biocontrol of bracken in the U.K and summarize the progress to date and the problems that remain to be solved.

THE STATUS OF BRACKEN IN THE U.K.

The assessment of the economic losses caused by a target weed species is an essential part of a biocontrol programme. Few detailed figures on the extent of the bracken problem have been available until recently. Estimates of the current land area occupied by bracken stands have been variable (3500-7000 square km) as have the suggested rates of spread (1-3%)(Smith & Taylor, 1986). Existing evidence suggests that the major economic impact of bracken falls on hill farmers in the north and west of Britain. This impact has been quantified by carrying out a postal questionnaire survey of a stratified random sample of nearly 1000 farms in England and Wales (Lawton &

Varvarigos, 1989). Farms were selected by the Ministry of Agriculture in 'Less Favoured Areas' (LFA) in 6 English and 7 Welsh counties, classified according to the European Community as Type 3 (Hill and Upland sheep) and Type 4 (Hill and Upland Cattle and Sheep). Farmers were asked about the extent of bracken infestations on their own and associated common land, their estimates of its rate of spread, and a range of economic questions about cost of control, stock poisoning etc.

Despite claims that bracken is spreading in Britain, the survey reveals a much more complex picture. If farmers' perceptions of changes in bracken distribution on their own farms and associated commons are correct, bracken is declining in Wales (-1.78% in total over 10 years, Standard Error 0.91). The surveyed English counties show a net increase of 2.48% (S.E. 0.98) over the same period. Overall change is a mere 0.08% (S.E. 0.57). Although bracken may not be spreading, its present distribution is sufficient to cause economic problems. In the surveyed counties, total net losses to hill agriculture may approach £9m per annum. A more detailed breakdown is given in Table 1.

TABLE 1. Economic losses to agriculture from bracken in 13 counties in England and Wales.

Type of loss	Estimated total losses per annum in 13 counties (million U.K. pounds)
Production losses (from bracken)	1.6
Veterinary costs	0.16
Control costs (including equipment depreciation)	1.3
Lost land use opportunity	5.9
TOTAL	8.9

Estimated benefits from bracken are minor, amounting to £46,000 from harvesting bracken for bedding with resulting savings on straw. The largest part of the cost estimates represents lost opportunities for grazing on land affected by bracken. However, this figure is subject to a number of uncertainties in its estimation, and assumes that markets exist for extra production at current prices. It must therefore be treated with caution. At best it is an upper bound. Despite these uncertainties it is important to realise that these are merely the direct costs of bracken to agriculture. Bracken may be important in many other areas where economic or other losses are much harder to gauge. For example, no assessment of the potential risk to human health from bracken carcinogens leached into water supplies or inhaled in spores has been made, or of the problems bracken invasion may cause to land of conservation or recreational value. Similarly the direct economic costs only of alternative controls were included, e.g. cutting or herbicide treatment, with no assessment of any possibly harmful side-effects. Finally it may be socially desirable to control bracken to help to maintain the precarious economic viability of traditional hill-farming.

POTENTIAL BIOCONTROL OF BRACKEN

Bracken has a worldwide distribution and is attacked by a different set of insect herbivores in different parts of it's range (Kirk, 1982; Lawton, 1982). Hence classical biocontrol, the introduction of beneficial agents from one region of the world to control a pest problem in another, is a possibility. Classical biocontrol has rarely been attempted against native plants such as bracken although there are cases when it has been successful (Goeden & Ricker, 1980). The most obvious problem is that the presence of an indigenous fauna may interfere with an introduced species by interspecific competition or more likely by providing a reservoir of parasites, predators or diseases that may attack the introduced herbivores. Choosing agents that are dissimilar to the indigenous fauna in taxonomy or biology, i.e. filling 'vacant niches' in the indigenous fauna, should minimise this risk.

Despite the near ubiquitous distribution of bracken in the world, the need to choose an accessible region with the same bracken subspecies aquilinum as the U.K. and with a similar climate, shortlisted South Africa as the most promising source of biocontrol agents. Three species of insects have been considered : Conservula cinisigna (Lepidoptera; Noctuidae), Panotima nr. angularis (Lepidoptera; Pyralidae) and Eupteryx maigudoi (Homoptera; Cicadellidae). Conservula larvae feed externally on the pinnae early in the season when no indigenous noctuids are present on bracken in the U.K. Panotima larvae first graze the back of the pinnae, and then in the third instar migrate to the rachis (stem) and complete their development as a stemborer. No U.K. bracken-feeding insects have a similar ecology. Cicadellid leafhoppers were also unrecorded in the U.K. bracken fauna. All three species can be found commonly in the Katberg Mountains in South Africa and can inflict damage to stands of bracken, both attributes of many successful biocontrol agents in other weed control programmes (Crawley, 1987; Harris 1973). None of these species has been recorded from hostplants other than bracken in South Africa, suggesting that they may be foodplant specific (Compton et al., in press). The tests described below provide further evidence of the specificity of these insects.

HOST RANGE TESTING

Establishing whether potential control agents are foodplant specific is the most important part of any weed biocontrol programme. Plants for testing are selected using a series of internationally accepted criteria such as taxonomic relatedness to the target weed, similar morphology and biochemistry or attack by closely related insect species (Wapshere 1975). Plant species of economic, conservation or ornamental value are given priority in testing. The 71 test plants used in this programme included representatives of nearly all the fern or closely related plant families found in the U.K., plants found in similar habitats to bracken and a range of crop plants used in the U.K. The full list is given in the Appendix.

The first stage of testing uses the most appropriate and practical life stage of the herbivore in no-choice feeding trials, where the alternative to feeding on a novel foodplant is starvation. Eupteryx maigudoi adults were found to feed and oviposit on several U.K. fern species and this herbivore was therefore rejected as a potential biocontrol agent. Of the two lepidopteran species, to date we have concentrated on Conservula cinisigna because Panotima has proved difficult to rear and sporadic in its availability in the field in South Africa.

Host range testing of C.cinisigna used freshly emerged first instar larvae offered cut young foliage of each plant species. Where possible, 5-10 replicates of 5-10 larvae were set up using different individual plants. Controls used young bracken foliage, older foliage and water only (starvation controls). Testing has been conducted in South Africa using field collected eggs and in the U.K. in quarantine with airfreighted eggs.

Conservula cinisigna larvae failed to survive beyond the first instar and showed minimal feeding on nearly all plants tested other than the U.K. or South African bracken controls (Table 2.). The exceptions were on several ferns, Pellaea viridis, Phyllitis scolopendrium, Athyrium filix-femina, Matteucia struthiopteris, Gymnocarpium dryopteris and Blechnum spicant, where occasional larvae survived into the second instar. Mean survival beyond first instar in the controls fed young foliage of Pteridium aquilinum aquilinum was 86%. One slow growing larva survived to 5th instar on Blechnum but failed to pupate and showed severe cuticular and developmental abnormalities for most of its life. Four larvae fed Pellaea produced abnormally small pupae that failed to produce adults. Only larvae fed young bracken eventually produced adults.

TABLE 2. Results of the 1st instar no-choice feeding trials using C.cinisigna larvae. With bracken, young and old (o) foliage was used. Survival to adult occurred only with young foliage of bracken, Pteridium aquilinum aquilinum. Figures show percent survival to the maximum stage achieved by the larvae.

TEST PLANTS ON WHICH MAXIMUM SURVIVAL OCCURRED ONLY TO :		
2nd instar	P.scolopendrium	1.7%
	A.filix-femina	19%
	M.struthiopteris	12%
	G.dryopteris	1.3%
5th instar	Pteridium aquilinum aquilinum (old foliage)	22%
	Blechnum spicant	1%
Pupation	Pellaea viridis	11%

The larval host range testing is therefore complete for one potential control agent and the project is at a stage when a release could be considered given the political decision to proceed.

BARRIERS TO A BIOCONTROL RELEASE IN BRITAIN

Large numbers of eggs of Conservula cinisigna can be imported from South Africa from October to December each year, but a direct release is not feasible for two reasons : (1) The season is incorrect and (2) because cultures should pass one generation in quarantine to prevent transmission of diseases and parasites. C.cinisigna larvae can suffer a high mortality from an unidentified disease (Lawton 1988) and large numbers of pupae have been lost in quarantine in the U.K. from attack by a Paecilomyces fungus. Surface sterilisation of the eggs is a possible solution that has

succeeded with other biocontrol programmes (R.Hill pers. comm.). However, the rephasing of the seasonality of the cultures from southern to northern hemisphere would remain a problem. In South Africa there is a partial second generation of C.cinisigna so it may prove possible to ship eggs at the start of the northern hemisphere spring. Alternatively, we can attempt to break, or substantially alter, diapause to achieve rephasing quickly. At present this is a problem because the cues that cause the moth pupae to enter or terminate diapause are not understood. The necessity to provide young bracken foliage in large quantities in winter to feed the voracious larvae is a further problem. However we are confident that these technical hurdles can be overcome assuming that the political decision to release is made. We believe that the political case for an attempt to biocontrol bracken in the U.K. is strong. The economic gains to agriculture are clear and the present economic value of bracken is negligible. Successful biocontrol would only reduce the land coverage of bracken, not eradicate it, so sufficient bracken should remain to provide habitats for the animal and plant species that presently utilise it. Finally, the risk of an introduced agent attacking a non-target plant we believe is minimal given the careful host specificity testing reported in this paper.

To summarize, despite some remaining biological problems, we have one agent fully and successfully screened. If bracken biocontrol proceeds it may require further agents : multiple releases are a feature of many successful weed biocontrol programmes (Hokkanen 1986). At present, no protocol exists regarding biocontrol releases against weeds in the U.K. because it is not an established technique. In contrast, countries such as Australia and New Zealand, with similar legislature to the U.K., have used weed biocontrol with considerable success for over 50 years. The Australian approach has resulted in specific enabling legislation following from the controversy surrounding the attempts to biocontrol Echium plantagineum (Cullen & Delfosse, 1985). Such costly and time consuming legislation should be unnecessary if we follow the approach taken in New Zealand, where information and intentions are made public at all stages. The pros and cons of a release can then be widely debated and assessed prior to a decision by the appropriate government body. This paper represents part of this process of dissemination of knowledge.

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APPENDIX

Plants used in starvation tests with Conservula cinsigna larvae. English names given for U.K natives and crops.

		Total larvae	No. of replicates
LYCOPODIACEAE			
Huperzia selago	FIR CLUBMOSS	57	10
Lycopodium clavatum		25	5
SELAGINACEAE			
Selaginella kraussiana		65	10
SCHIZAEACEAE			
Mohria caffrorum		30	6

HYPOLEPIDACEAE		
Pteridium aquilinum aquilinum UK BRACKEN(young)	221	35
P. aquilinum aquilinum UK BRACKEN(old)	27	5
Hypolepis sparsisora	30	6
PTERIDACEAE		
Pteris cretica CRETAN FERN	60	8
P.dentata	30	6
OPHIOGLOSSACEAE		
Ophioglossum vulgatum ADDERSTONGUE FERN	35	6
ADIANTACEAE		
Adiantum pedatum	80	11
A.poirettii	25	5
Pellaea rotundifolia	70	11
P.viridis	35	7
Cheilanthes hirta	20	4
DAVALLIACEAE		
Nephrolepis cordifolia	30	6
CRYPTOGRAMMACEAE		
Cryptogramma crispa PARSLEY FERN	5	1
THELYPTERIDACEAE		
Thelypteris palustris MARSH FERN	50	7
Amouropelta bergiana	30	6
ASPLENIACEAE		
Phyllitis scolopendrium HARTSTONGUE	80	13
Asplenium aethiopicum	30	6
ATHYRIACEAE		
Athyrium filix-femina LADY FERN	165	24
Onoclea sensibilis SENSITIVE FERN	51	7
Cystopteris fragilis BRITTLE BLADDER FERN	25	5
Matteucia struthiopteris	50	7
DRYOPTERIDACEAE		
Dryopteris filix-mas MALE FERN	100	14
D.inaequalis	30	6
Polystichum aculeatum HARD SHIELD FERN	40	7
P.setiferum SOFT SHIELD FERN	65	10
P.lucidum	30	6
Gymnocarpium dryopteris OAK FERN	60	9
Rumohra adiantiformis	30	6
BLECHNACEAE		
Blechnum spicant HARD FERN	61	13
POLYPODIACEAE		
Polypodium vulgare COMMON POLYPODY	183	23
OSMUNDACEAE		
Osmunda regalis ROYAL FERN	87	12
CRUCIFERAE		
Brassica oleracea CABBAGE	25	5
CARYOPHYLLACEAE		
Melandrium dioicum RED CAMPION	40	4
LEGUMINOSAE		
Trifolium hybridum CLOVER	25	5
Pisum sativum PEA	25	5
Lathyrus pratensis MEADOW VETCHLING	40	4
Phaseolus vulgaris FRENCH BEAN	25	5

ROSACEAE			
Potentilla erecta	TORMENTIL	40	4
Malus pumila	APPLE	22	5
UMBELLIFERAE			
Anthriscus sylvestris	COW PARSLEY	25	5
Heracleum sphondylium	HOGWEED	25	5
Petroselenium crispum	PARSLEY	34	5
CURCURBITACEAE			
Cucumis melo	CANTALOUPE MELON	25	5
FAGACEAE			
Quercus robur	OAK	25	5
BETULACEAE			
Betula pubescens	BIRCH	25	5
B.pendula	BIRCH	18	4
ERICACEAE			
Calluna vulgaris	LING	40	4
Erica tetralix	CROSS-LEAVED HEATH	40	4
E.chamissonis		25	5
E.demissa		25	5
Vaccinium myrtillus	BILBERRY	25	5
PRIMULACEAE			
Primula veris	COWSLIP	27	5
SOLANACEAE			
Lycopersicon esculentum	TOMATO	25	5
SCROPHULARIACEAE			
Linaria vulgaris	COMMON TOADFLAX	40	4
CAMPANULACEAE			
Campanula glomerata	CLUSTERED BELLFLOWER	40	4
C. rotundifolia	HAREBELL	40	4
RUBIACEAE			
Galium saxatile	HEATH BEDSTRAW	57	10
G.verum		25	5
DIPSACEAE			
Dipsacus fullonum	TEASEL	40	4
COMPOSITAE			
Cirsium arvense	CREEPING THISTLE	25	5
Hypochoeris radicata	COMMON CATSEAR	50	10
Lactuca sativa	LETTUCE	31	5
LILLIACEAE			
Asparagus plumosus		40	6
Allium fistulosum	WELSH ONION	24	5
GRAMINAE			
Zea mays	MAIZE	25	5
Cynosurus cristatus	CRESTED DOGS TAIL	50	10
Holcus mollis	CREEPING SOFT GRASS	50	10
Triticum aestivum	WHEAT	25	5
STARVATION CONTROL		81	18

COMMERCIAL PROSPECTS FOR BIOLOGICAL AND BIOTECHNOLOGICAL WEED, PLANT DISEASE AND PEST CONTROL

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ABSTRACT

Over a decade ago there was a considerable optimism in scientific and commercial circles about the scope for applied biotechnology in agriculture - especially for pest, weed and disease control. Many of the forecasts then made have proved grossly over-optismistic. Over-optimism was followed by over-pessimism, especially during a period when the value of venture capital companies in this field seemed to be a multiple of their losses. There is evidence, however, that the increasing collaboration between large companies, specialist ag-biotech companies and research institutes is now beginning to bear fruit.

INTRODUCTION

A recently published report, commissioned by the EC, predicts a 'biotechnological revolution' during the next 15 years which will significantly affect producer profitability and product quality. It is estimated that since World War II average crop yields have increased by approximately 160 per cent. Further large yield increases attributable to agrochemical use are considered unlikely. Instead biotechnology is expected to play a major rôle in yield enhancement in the twenty-first century through the introduction of genetically-engineered plants and effective biopesticides. To date, however, no engineered varieties have appeared on the market although genetically engineered seed is expected to make an impact in Europe in the 1990s.

The first genetically engineered plants to enter the market are likely to be herbicide-resistant varieties planned for release by 1994. Calgene, for example, expects to market herbicide-tolerant cotton varieties in the USA in the early 1990s. Conservative estimates suggest that the market for engineered crop varieties could be worth in the region of US $75 mn per year by the late 1990s. By contrast, a number of biopesticides have been available for several years with a range of new products under development. The following sections will discuss biopesticides currently available for the control of weeds, insects and pathogens and identify likely future trends and markets.

WEEDS

The range of currently available mycoherbicides is somewhat limited.

TABLE 1. Currently available bioherbicides

Product/Manufacturer	Active ingredient	Target
Devine/Abbott	*Phytophthora palmivora*	Milkweed vine
Collego/Ecogen	*Colletotrichum gloeosporioides*	Northern jointvetch

Research into the development of bioherbicides is concentrated in North America and Europe although there is some interest in Israel and Australia. Fungi have attracted the most interest as potential control agents for weeds although there have been a few, to date, unsuccessful attempts to exploit bacteria and viruses. Many projects investigating weed biocontrol are still at the early stage of pathogen screening and are primarily of academic rather than commercial interest. In a number of cases the rationale behind the choice of target has been unclear and indeed the approach taken has been opportunistic rather than market driven and has resulted in studies on the biocontrol of so-called 'niche weeds'. There is, however, increasing interest in the biocontrol of major crop weeds including *Cyperus* spp and *Cirsium* spp, which have a wide distribution both in terms of affected crops and geographical area and are becoming increasingly difficult to control by conventional methods. Selected opportunities are shown in Table 2.

TABLE 2. Selected commercial opportunities for weed biocontrol

Weed	Crop	Putative control agent(s)	Commercial interest
Chenopodium album	Maize Potatoes Soya beans	*Ascochyta* spp	
Convolvulus arvensis	Citrus Grapes Wheat	*Phomopsis* spp	CIL AGC CIBA-Geigy
Cyperus spp	Cotton Rice Sugar cane	*Puccinia* spp	
Galium aparine	Wheat Oilseed rape	?	AGC
Sorghum halepense	Soya beans Sugar cane	?	CIBA-Geigy

A number of significant opportunities for mycoherbicide control in field crops, rather than for niche-weeds, can be suggested; *Convolvulus arvensis*, for example, is often poorly controlled within the broad-leaved complex and as such represents a good opportunity for biocontrol. In a number of cases major emphasis is being placed on the development of fungicide-resistant and herbicide-tolerant mycoherbicides enabling these biological agents to be used in tank mixes with existing control treatments. Several companies are known to have interests in the development of mycoherbicides and are funding external contracts.

INSECTS

The market for bioinsecticides is dominated by Bt-based products (Table 3).

TABLE 3. Currently available bioinsecticides

Product/Manufacturer	Active ingredient	Target
Dipel/Abbott	Bt var *kurstaki*	Lepidopteran larvae
Vectobac/Abbott	Bt var *israelensis*	Mosquitoes/blackfly
Javelin/Sandoz	Bt var *kurstaki*	Armyworms/loopers
Thuricide/Sandoz	Bt var *kurstaki*	Forestry pests
Teknar/Sandoz	Bt var *israelensis*	Mosquitoes/blackfly
Certan/Sandoz	Bt var *aizawai*	Wax moth larvae
Bactospeine/Solvay	Bt	Lepidoptera
Bactimos/Solvay	Bt	Mosquitoes
M-One/Mycogen	Bt var *san diego*	*Leptinotarsa decemlineata*
BioSafe/BioSys	*Steinernema feltiae*	Soil pests
Bionem/Koppert	*Heterorhabditis* spp	Vine weevil
Mamestrin/Calliope	*Mamestra brassica* NPV	*Heliothis* spp
Spodoptera/Calliope	*Spodoptera littoralis* NPV	
?/Kemira Oy	*Neodiprion sertifer* NPV	Forestry pests
MicroGermin/CHR Hansen's Biosystems	*Verticillium lecanii*	Aphids
NoloBait/Evans Biocontrol	*Nosema locustae*	Grasshoppers, locusts

Although the majority of work on the biocontrol of insects concentrates on the use of Bt, both viruses and entomopathogenic fungi are steadily attracting increasing attention. The main areas of current research can be summarised as renewed searches for exploitable pathogens, improvement of available pathogens through strain selection and/or genetic manipulation, improved formulations, improved application technology and monitoring the

fate of released pathogens. Integrated pest management strategies are of increasing importance in both tropical and temperate regions. Over 400 sexual attractant or aggregation pheromones are currently available and are marketed by over 50 companies worldwide. As pheromones are based on naturally occurring products the EPA, for example, waives much of the data requirement and gives these products priority classification over conventional pesticides. In addition, attractants, mating disruptants and attracticides have limited crop contact and so are treated accordingly in terms of toxicology data requirements. Most currently available pheromones are relatively unsophisticated products and the development of effective, patentable controlled release technology will be important in the successful commercialisation of these products, which have a major rôle to play in IPM strategies.

In view of current R and D activity and expenditure it is likely that microbial insecticides will continue to dominate the biopesticide market for the foreseeable future with Bt-products maintaining or increasing their market share. Selected opportunities are shown in Table 4.

TABLE 4. Selected commercial opportunities for insect biocontrol

Pest	Crop	Putative control agent(s)	Commercial interest
Cydia pomonella	Apples Pears	CpGV	Hoechst MicroGeneSys AGC/Calliope
Heliothis spp	Cotton Maize Tobacco Tomatoes	HzNPV	MicroGeneSys Calliope Repligen-Sandoz?
Leptinotarsa decemlineata	Potatoes Tomatoes	Bt	Mycogen Ecogen Sandoz Abbott
Nilaparvata lugens	Rice	Fungi	ICI
Ostrinia nubilalis	Maize	*Beauveria bassiana* Bt	Calliope ?
Otiorhynchus spp	Ornamentals	Nematodes	AGC BioSys Koppert
Spodoptera spp	Cotton Maize	NPV Bt	Calliope Kyodo Shiryo

Bt is considered to be a prime target for genetic manipulation and thus improvement given the ease of large-scale production and the fact that the

major toxin coding genes are plasmid-borne. Recent studies into the molecular biology and genetics of Bt toxins are providing information on observed host ranges but further research is necessary to identify relationships between toxin structure, biological specificity and variation in potency.

PLANT PATHOGENS

Despite intense research activity into the biocontrol of plant pathogens there has been relatively little success in the commercialisation of such agents (Table 5).

TABLE 5. Currently available biofungicides

Product/Manufacturer	Active ingredient	Target
NoGall/Bio-Care Technology	*A tumefaciens*	Crown gall/stone fruit, nuts and roses
Binab-T/Bio Innovation	*Trichoderma viride*	Forestry pathogens
Dagger/Ecogen	*Pseudomonas* spp	Damping off/cotton

The lack of commercialised biofungicides can be attributed to a number of factors including problems of target specificity and inconsistent performance in the field, but perhaps most important has been the *ad hoc* approach to much of the research. Generally speaking R & D effort regarding biological disease control has not been as comprehensive as that for bioinsecticides. A concerted, integrated, international effort is necessary at ecological, physiological and molecular levels if products with significant market credibility are to be developed. Details of the mode of action of antagonism eg niche competition, induced host resistance and/or antibiosis, should ideally be established thus enabling strain selection or improvement through genetic manipulation.

It is evident from research literature that the current emphasis is on the biocontrol of rhizosphere pathogens. The lack of effective chemical treatments of these pathogens has stimulated research in this area, coupled with the fact that the rhizosphere represents a slightly more constant environment than the phylloplane. The most widely studied soil pathogens are *Pythium* spp, *Rhizoctonia* spp, *Fusarium* spp and *Sclerotinia* spp, with *Trichoderma* spp and *Pseudomonas* spp being the most investigated antagonists. A wide range of temperate crops, both agricultural and horticultural, have been studied but to date, in-depth studies on the biocontrol of diseases affecting tropical crops have been limited.

Despite limited progress in this field, a number of commercial opportunities for the biocontrol of plant pathogens can be identified (Table 6).

TABLE 6. Commercial opportunities for disease biocontrol

Disease/ pathogen	Crop	Putative control agent(s)	Commercial interest
Damping off eg *Pythium* spp *Rhizoctonia* spp *Phytophthora* spp	Brassicas Cotton Lettuce Oilseed rape Ornamentals Sugar beet Wheat	*Trichoderma* spp *Pseudomonas* spp	Ecogen AGS
Take-all	Wheat	*Bacillus subtilis* *Psialophora* spp *Microdochium bolleyi* *Pseudomonas* spp	ICI Monsanto Abbott
Plasmidophora brassicae	Brassicas	*Pythium oligandrum*	
Pseudomonas tolaasii	Mushrooms	*Pseudomonas* spp	AGC Mauri Foods
Sclerotia-producing pathogens eg *Botrytis* spp *Sclerotinia* spp	Lettuce Oilseed rape Onions Sunflowers	*Trichoderma* spp *Gliocladium* spp *Teratosperma oligocladum* *Coniothyrium minitans*	WR Grace AGC Philom Bios

DISCUSSION

Biopesticides have been under consideration for many years and, although no longer seen as a complete alternative to conventional chemicals, potentially exciting market opportunities exist. Outlets for their use exist in IPM strategies, in situations where conventional methods are, or become, unacceptable either in terms of poor performance (due to the development of resistance) or increasingly, because of perceived problems associated with their impact on the environment.

Biopesticides are no longer considered to be a soft option in terms of R & D effort and inputs. These costs are likely to increase, particularly if genetic manipulation is necessary, but should still be considerably lower that those associated with the development of conventional pesticides. Fundamental research into the mechanisms of observed biocontrol is seen as being important in strain selection and subsequent genetic modification and could result in the identification of new, potentially exploitable, active ingredients.

There appear to be a number of exploitable opportunities for both small, specialist agbiotech companies and the larger, multinational agrochemical companies. The reputation, expertise, marketing skills and distribution facilities of the agrochemical companies are considered necessary for the introduction of new biopesticides into large scale field and plantation crops, whilst the smaller operations might be expected to succeed, at least initially, in the introduction of these products into defined niche markets. In both cases the transition from R & D to commercialisation is critical and it is at this stage that smaller companies could benefit from collaboration with multinationals; indeed, such arrangements are already in evidence eg, AGS/Rohm & Haas, AGC/CIBA-Geigy, Ecogen/American Cyanamid, Mycogen/Monsanto.

Given the requirements of fungal pathogens for high humidity it is considered that mycoherbicides could be commercially available for use in irrigated crops such as rice and sugar cane by the mid 1990s. Genetic modification of fungal pathogens is seen as a pre-requisite for the development of mycoherbicides for use in temperate crops and consequently such agents are unlikely to become widely available until the late 1990s by which time improved formulations should be available and guidelines for the use of engineered organisms firmly established.

The scope for mycoherbicide use on field crops may be limited by the fact that weeds are usually controlled as part of a complex. However, *Chenopodium album* in maize, for example, remains a major problem despite considerable expenditure on broad-leaved herbicides and seriously infested crops could therefore warrant specific treatment with a mycoherbicide either alone or tank-mixed with conventional products. Recently Landell Mills Market Research have analysed the market potential of a number of mycoherbicides. Significant markets for the control of *Chenopodium album* on maize in France were identified; the current cost of controlling the broad-leaved complex is estimated at over US $35m pa. In the USA, however, the maize herbicide market is in decline although the broad-leaved sector is worth over US $900m pa with atrazine commanding a major share. Major opportunities for mycoherbicide control of *Chenopodium album* could exist in maize and other field crops if atrazine resistance becomes more widespread or if restrictions are placed on its use on environmental grounds. Similar opportunities exist for several broad-leaved weeds in a range of field crops.

Herbicide-tolerant crop varieties are being developed by a number of agrochemical manufacturers, many with major investments in the seeds business. It is predicted that over the next 15 years broad-spectrum herbicide-tolerant varieties of cereals, oilseeds and sugar beet will become widely available giving more flexibility in the timing of herbicide application. As in other areas of crop management there is a trend towards integrated weed control in which both herbicide-tolerant varieties and mycoherbicides have a major rôle to play.

It is considered that a number of Bt-based products containing new, improved strains will be marketed by the mid 1990s. Transgenic plants incorporating Bt toxin-coding genes are expected to be available by the year 2000 and strategies designed to optimise and prolong their efficacy are currently under consideration.

Viral-based products are under development to control a range of major pests including *Cydia pomonella, Heliothis* spp and *Spodoptera* spp. The genetic manipulation of baculoviruses to incorporate genes that enhance speed of kill coupled with protective formulations may lead to a new generation of viral products by the late 1990s. It is estimated that worldwide the market for control of *Cydia pomonella*, a major pest of apples and pears, is over US $40m. In Europe due to the trend towards integrated strategies in orchard pest management, high specificity but low cost bioinsecticides could achieve a significant market share. In Italy for example, if a bioinsecticide were used on 10 per cent of the currently treated area at an arbitrary cost of US $25/ha this would represent an annual market of US $0.3m (assuming only one application). The value of these products in "environmentally sensitive" areas is likely to receive greater recognition and their increased use is predicted initially in grassland and forestry sectors in northern temperate regions.

A major outlet for Bt-products at the present time is the public health sector and a significant market opportunity exists for cheap, but effective, products for use in large scale mosquito eradication programmes. It is particularly important that microbial insecticides are compatible with current agronomic practices and research into growers' attitudes is seen as being a pre-requisite for the development of integrated strategies and also in identifying perceived problems that could perhaps be overcome by effective user education.

It is generally agreed that by the year 2000 a number of genetically-unmodified biological agents will be available for the control of damping off, wilts and sclerotia-producing pathogens. Such products will initially be introduced for use in high value protected crops such as lettuce, tomatoes and ornamentals. A potentially lucrative opportunity exists for these products in the domestic/amateur grower sector which given the current 'green' philosophy is immediately exploitable. Such niche markets are likely to be developed by smaller, specialised companies. The development and acceptance of effective biocontrol strategies in the horticultural sector is seen as essential prior to their exploitation in field crops.

Diagnostics could play a major rôle in the grower's acceptance of biofungicides. Detection of a pathogen prior to symptom expression could be invaluable in determining the optimum application time. A number of companies have expressed interest in this area; Stirling Diagnostics, for example, intend to launch a product for the detection of eyespot (*Pseudocercosporella herpotrichoides*) on cereals in 1989. Given the widespread availability of effective chemical treatments and the current lack of R & D activity into the biocontrol of aerial pathogens, biological products are unlikely to be developed for use in this sector in the foreseeable future, despite an increasing public aversion to conventional pesticides.

The future success of biopesticides will be strongly influenced by the fate of conventional pesticides as determined by environmental pressures and public opinion. Although the public are voicing concern over pesticide use, such awareness is strongly influenced by the media. If biopesticides are to attain their potential it will be essential to ensure that the public are educated regarding their use, particularly in the light of the recent bad press over food 'contamination'.

SESSION 8C

LOOKING FORWARD TO WEED CONTROL IN HORTICULTURAL CROPS DURING THE NINETEEN-NINETIES

SESSION ORGANISER DR R. W. P. HIRON

POSTERS 8C-1 to 8C-7

PROBLEMS OF HERBICIDE REGISTRATION AND USAGE ON HORTICULTURAL CROPS

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ABSTRACT

Herbicide use in horticultural crops has mostly in the past developed from the use of the same products in major agricultural crops. This situation is not likely to change in the next decade. This paper reviews the problems and restrictions placed upon, and largely overcome by the industry. The effects of crop areas and species, economic considerations by the herbicide manufacturers, legislation and controls on agrochemicals, and the withdrawal of public funds from development work are discussed, and some of the successes and possible solutions noted.

INTRODUCTION

In the few decades since the use of herbicides became commonplace in the agricultural industry, horticulturists have generally found it necessary to make use, where appropriate, of the herbicides that have been developed and introduced for the more major agricultural crops. In worldwide terms, the UK does not grow large areas of any of the major world crops; the closest we get is with cereal crops. Horticultural crops in the UK, and similarly in the rest of the world are very minor in terms of area, and seldom if ever give sufficient potential usage of a herbicide to make the cost of development and introduction worthwhile to the manufacturer. The UK industry will consequently continue to rely primarily on molecules developed for major agricultural crops, and the mechanisms for obtaining registration and approval for minor crop usage will almost certainly limit the herbicides available to horticulturists by the next century.

HORTICULTURAL CROPPING AND HERBICIDE DEVELOPMENT

Crop areas

Table 1 shows how over the past 2 decades the total area of farm land, including grassland, has remained more or less stable. The area under agricultural crops has increased by 12%, whilst the area under horticultural crops has decreased by 35%, a fact which itself has added to the problems of the industry justifying the development of specific herbicides for this decreased area.

TABLE 1. Crop areas in the UK 1970-1987 ('000 ha)

Crop	1970	1980	1986	1987
Total farm areas including grazing	18097	17739	17459	17436
Cereals	3719	3938	4024	3941
Oilseed Rape	4	92	299	391
Sugarbeet	188	213	205	203
Potatoes	271	205	178	178
Vegetables	206	190	146	130
Fruit	84	65	53	53
Ornamentals	15	12	12	12
Glass or plastic houses	2	2	2	2
Total agricultural crops	4182	4448	4706	4713
Total horticultural crops	307	269	213	197
Horticulture as a % of Agriculture + Horticulture areas	6.8	5.7	4.3	4.0

Crop growing techniques

In any system of crop production where the evolution of growing techniques is moving fast, the science of crop protection can easily lag behind other agronomic practices, at least in respect of manufacturer's recommendations. Horticulture represents one such area of rapidly changing techniques. This delay in the consequential change to recommendations may be brought about by the manufacturer's unwillingness to apply their recommendations to the new and probably fundamental changes in crop husbandry which could affect the safety of their product. Examples of this situation in horticulture during the past few years have been the upsurge in the use of cellular or modular transplants, and the vast increase in the use of plastic crop covers. Until recent years most transplanted horticultural crops were bare root plants. Over the past decade, many crops, particularly in some of the more intensive vegetable areas such as Holland Lincs, have moved almost completely to transplants raised in small plug modules or cells. Most product recommendations were drawn up from information relating to bare root plants and in 1984 the United Kingdom Pesticide Registration Department drew to manufacturers' attention the possibility of greater phytotoxicity, due to the different morphology of the new type of transplant. (Tucker 1984). As a result some manufacturers restricted the use of their products on these trans-

plants. Similarly, the use of unsupported plastic crop covers in the United Kingdom has increased from practically zero 10 years ago to an estimated 8000 ha in 1989. The use of herbicides on crops covered in this manner has outstripped recommendations generally, though information is being gathered, and a few manufacturers have felt able to make recommendations for their products on these crops (Greenfield 1989).

New crop species

New crop species introduced or yet to be introduced present another challenge to the industry. It is easy to extrapolate from some crops eg cabbage or Brussels sprouts to the new crop, Chinese cabbage, with some hope of success in the use of herbicides. In other cases this may present more of a problem; for example from which crop do you extrapolate when searching for likely candidates to use on evening primrose? There are no closely related crops that presently have recommendations, therefore relatively expensive trial work is the only way to obtain the necessary data to enable safe usage and eventually recommendations.

THE AGROCHEMICAL INDUSTRY

The manufacturers of herbicides and other pesticides are in business to make profits for their owners or shareholders, not for altruistic reasons, nor in the first instance for the benefit of the agricultural industry. It is accepted that their products must benefit the industry to be economically successful, but regrettably for horticulture, the latter does not always follow the former.

The value of herbicides sold in the agricultural and horticultural sector in 1988 is shown in Table 2 and compared with other agrochemicals.

TABLE 2. Value of Agrochemicals sold in UK. 1988 (Source B.A.A)

Agrochemical	M£	% of total
Herbicides	201.6	53.0
Insecticides	28.9	7.6
Fungicides	108.8	29.0
Molluscicides	8.9	2.4
Seed Treatments	11.3	3.0
Plant growth regulators	11.4	3.0
Others	7.5	2.0
Total	378.4	

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WEED CONTROL IN CARROTS AND SALAD ONIONS UNDER LOW-LEVEL POLYETHYLENE COVERS

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ABSTRACT

Covering spring-sown carrots and salad onions with polyethylene sheeting, increased the number and fresh weight of naturally-occurring weeds. Broad spectrum pre-emergence herbicides generally controlled weeds as well or better under the covers than in the open. There was little evidence of increased herbicide damage to the covered crops.

INTRODUCTION

Low-level polyethylene covers promote earliness in spring-sown vegetables. However, conditions which favour crop establishment and growth also promote germination and development of naturally-occurring weeds. Weed control and the lack of suitable herbicides are considered to be important factors limiting the use of covers (Antill, 1987). The present report summarises the results of weed control experiments made with carrots and salad onions grown under perforated polyethylene covers.

METHOD AND RESULTS

The field experiments, made on a sandy loam soil with 2% o.m., were of a split-plot randomised block design with three replicates. Herbicide treatments, including unsprayed controls, were assigned to main plots and covering treatments to sub-plots. Main plot size was 1.5 x 8 m. The herbicides were commercial wettable powder formulations of linuron (50% a.i.), chlorbromuron (50% a.i.) and chlorthal-dimethyl (75%), emulsifiable concentrate formulations of trifluralin (48% a.i.), pendimethalin (33% a.i.) and flurochloridone (25% a.i.), and suspension concentrate formulations of aclonifen (60% a.i.), propachlor (48% a.i.) and propachlor + chloridazon (40% + 8.6% a.i.). Beds were prepared and treatments for incorporation were applied by knapsack sprayer in 450 litres/ha. The experimental area was cultivated with a rotary power harrow working to a depth of 10 cm which prepared the seedbed and mixed the previously-applied herbicide treatments into the soil. The seedbed was rolled and four crop rows were drilled in shallow furrows 25 cm apart in each bed. Pre-emergence treatments were applied by knapsack sprayer in 450 litres/ha shortly after drilling. Perforated polyethylene covers (Polycrop Coverall; 200 holes/m^2 were laid over appropriate sub-plots within 24 h of spraying, and the edges secured with soil. At a time appropriate for the crop, the sheeting was slit along the length of the bed prior to complete removal the following day. After the covers had been removed the weeds in a 1 m^2 area of each sub-plot were counted and their total fresh weight recorded (weed data were transformed (log (n+1)) before analysis). The whole experiment was then hand weeded. Irrigation was applied to minimise the effect of cover removal on crop growth. At harvest, 2 X 2 m lengths of the inner crop rows of each subplot were counted and weighed.

Carrots

Carrot cv. Touchon Ideal Red was drilled on 7 March 1984 (Trial 1). Linuron and flurochloridone were applied pre-emergence. The seedbed was moist at the time of covering, 37 mm rain fell in the following 3 wk but conditions later became dry. Covers were removed and the experiment weeded on 10 May when the protected crop had 5-7 leaves. The carrots were harvested on 10 July (covered crop) and on 19 July (open crop). Soil capping reduced carrot numbers in the open but crop weights were similar to those from the covered subplots harvested 9 days earlier (Table 1). There was no effect of herbicides on the crop. On the untreated plots there were three times as many weeds and the total fresh weight was much greater under polyethylene than in the open. The main species were Tripleurospermum inodorum and Polygonum aviculare with smaller numbers of Viola arvensis, Stellaria media, Lamium amplexicaule, Veronica persica and Thlaspi arvense. Both herbicides gave good weed control. With flurochloridone, the main survivor was T. inodorum. On plots treated with linuron, T. inodorum and V. persica survived under covers while in the open P. aviculare also remained.

In a second experiment cv. Touchon Ideal Red was drilled on 3 April 1984 (Trial 2). Linuron and pendimethalin + aclonifen were applied pre-emergence. Conditions were dry and irrigation was applied on 24 April. Covers were lifted on 23 May and the weeds recorded and removed. The carrots were harvested on 19 July (covered crop) and on 24 July (open crop). Crop stand was similar under covers and in the open but crop weights were heavier on the sheeted subplots (Table 1). Herbicide treatments had no effect on crop growth. The main weeds were V. arvensis, Poa annua, Lamium amplexicaule and, especially under the polyethylene, Solanum nigrum. In the absence of herbicide, twice as many weeds emerged under the polyethylene as in the open and weed fresh weight was six times greater (Table 1). Neither herbicide treatment was outstanding in killing weeds but with pendimethalin + aclonifen most survivors were stunted and non-competitive.

In 1985, carrot cv. Toudo was drilled on 2 April. Linuron, flurochloridone and pendimethalin + linuron were applied pre-emergence. Rain fell daily for the next two weeks. Covers were removed and plots weeded on 4 June. The carrots were harvested on 9 July (covered crop) and on 18 July (open crop). Low crop numbers on the unsheeted plots resulted mainly from attack by cereal thrips at crop emergence but phytotoxicity of linuron and flurochloridone may have contributed. Crop weights at harvest were also low (Table 2, 1985 trial). None of the herbicides affected crop growth under the covers but weed competition reduced yields on the unsprayed subplots. The weeds included P. aviculare, S. media, P. annua and L. amplexicaule, together with Senecio vulgaris, Papaver rhoeas and Fumaria officinalis. Twice as many weeds emerged on the covered unsprayed subplots as in the open and weed fresh weight was 15 times greater (Table 2). Flurochloridone gave excellent weed control with no survivors in the open and a few seedlings of S. vulgaris remaining under the covers. Pendimethalin + linuron performed almost as well. Linuron alone gave better weed control under covers, where F. officinalis was the only survivor, than in the open where P. aviculare and L. amplexicaule also survived.

In 1986, cv. Toudo was drilled on 28 April. Pre-emergence herbicide treatments were linuron, chlorbromuron, flurochloridone, pendimethalin + linuron and trifluralin + linuron. In addition, trifluralin incorporated before drilling followed by linuron pre-emergence was also included. The

TABLE 1. Response of carrots and weeds to herbicides under polyethylene covers (C) and in the open (U).

Trial 1 1984

Herbicide treatments kg ai/ha	Carrots/4 m row			
	Number		Weight (kg)	
	C	U	C	U
Flurochoridone 0.75	174	71	3.5	3.5
Linuron 0.56	155	73	4.0	3.6
Untreated	163	83	3.8	3.5
L.S.D. (5%) between columns	24		0.7	
within columns	25		0.9	

	Weeds/m^2			
	Number*		Weight (g)*	
	C	U	C	U
Flurochloridone	0.56(3)	0.42(2)	0.62(3)	0.68(4)
Linuron	0.96(8)	1.14(13)	1.54(34)	0.78(5)
Untreated	2.70(504)	2.17(146)	3.41(2570)	2.04(109)
L.S.D. (5%) between columns	0.70		0.65	
within columns	0.56		0.75	

Trial 2 1984

Herbicide treatments kg ai/ha	Carrots/4 m row			
	Number		Weight (kg)	
	C	U	C	U
Pendimethalin 0.67 + aclonifen 1.2	137	149	5.3	4.4
Linuron 0.56	116	118	4.3	3.5
Untreated	123	132	4.3	3.6
L.S.D. (5%) between columns	41		0.5	
within columns	53		1.7	

	Weeds/m^2			
	Number		Weight (g)	
	C	U	C	U
Pendimethalin + aclonifen	1.45(28)	1.46(28)	1.67(45)	1.20(15)
Linuron	1.29(18)	1.24(17)	1.96(90)	1.51(31)
Untreated	2.20(157)	1.89(76)	2.46(290)	1.70(49)
L.S.D. (5%) between columns	0.39		0.50	
within columns	0.45		1.24	

* Transformed data (log(n+1)), back transformed data in parenthesis

soil surface was dry at the time of sheeting and crop emergence was erratic. Covers were removed on 13 June but plots were not weeded until 1 July. The carrots were all harvested on 29 July. Herbicide treatments did not affect crop growth. In this relatively late-sown experiment, under dry conditions, there was little difference in weed numbers between sheeted and unsheeted subplots but fresh weight was greater under the covers. Weed competition on the untreated controls reduced yield on both sheeted and unsheeted subplots (Table 2, 1986 trial). The main weeds were F. officinalis, V. persica, T. arvense, S. media and the mayweeds. Linuron and chlorbromuron applied alone gave the poorest weed control, the main survivors being F. officinalis, V. persica and the mayweeds. The other herbicides worked well.

TABLE 2. Response of carrots and weeds to herbicides under polyethylene covers (C) and in the open (U).

1985 Trial

Herbicide treatments kg ai/ha	Carrot/4 m row			
	Number		Weight (kg)	
	C	U	C	U
Pendimethalin 0.67 + linuron 0.25	383	190	8.8	3.2
Linuron 0.56	311	121	7.5	2.7
Flurochloridone 0.50	332	158	7.1	2.7
Untreated	352	224	6.4	3.7
L.S.D. (5%) between columns	51		0.7	
within columns	45		0.8	

Herbicide treatments kg ai/ha	Weeds/m^2			
	Number*		Weight (g)*	
	C	U	C	U
Pendimethalin + linuron	0.82(6)	0.70(4)	1.39(24)	0.71(4)
Linuron	0.83(6)	1.41(24)	1.46(28)	1.66(45)
Flurochloridone	0.20(1)	0.00(0)	0.36(1)	0.00(0)
Untreated	2.52(330)	2.23(167)	3.59(3925)	2.43(268)
L.S.D. (5%) between columns	0.35		0.47	
within columns	0.39		0.57	

1986 Trial

Herbicide treatments kg a.i./ha	Carrots/4 m row			
	Number		Weight (kg)	
	C	U	C	U
Trifluralin 1.1 inc. + linuron 0.75	187	213	5.6	4.4
Trifluralin 1.1 pre. + linuron 0.75	173	208	5.3	4.3
Linuron 0.75	228	211	5.5	4.2
Chlorbromuron 0.75	173	194	5.4	3.9
Flurochloridone 0.50	187	169	5.6	4.5
Pendimethalin 1.1 + linuron 0.75	202	198	5.0	4.5
Untreated	203	211	3.4	2.8
L.S.D. (5%) between columns	44		0.7	
within columns	56		1.5	

Herbicide treatments kg a.i./ha	Weeds/m^2			
	Number*		Weight (g)*	
	C	U	C	U
Trifluralin inc. + linuron	0.16(1)	0.32(1)	0.35(1)	0.69(4)
Trifluralin pre. + linuron	0.48(2)	0.44(2)	1.02(9)	0.69(4)
Linuron	1.05(19)	1.09(11)	2.17(146)	1.86(72)
Chlorbromuron	1.32(20)	1.39(24)	2.81(648)	2.62(418)
Flurochloridone	0.10(1)	0.00(0)	0.35(1)	0.00(0)
Pendimethalin + linuron	0.30(1)	0.59(3)	0.79(5)	1.29(18)
Untreated	1.83(68)	1.82(65)	3.37(2354)	3.13(1342)
L.S.D. (5%) between columns	0.41		0.89	
within columns	0.49		0.93	

* Transformed data (log(n+1)), back transformed data in parenthesis

TABLE 3. Response of salad onions and weeds to herbicides under polyethylene covers (C) and in the open (U).

1984 Trial

Herbicide treatments kg ai/ha	Onions 4/m row			
	Number		Weight (kg)	
	C	U	C	U
Propachlor 4.3 + chlorthal-dimethyl 4.5	565	511	10.3	10.2
Propachlor 4.3 + pendimethalin 0.67	512	473	9.3	8.6
Propachlor 4.0 + chloridazon 0.86	542	531	10.6	10.0
Untreated	553	476	10.3	9.3
L.S.D. (5%) between columns	87		1.4	
within columns	85		1.8	

	Weeds/m^2			
	Number*		Weight (g)*	
	C	U	C	U
Propachlor + chlorthal dimethyl	0.39(2)	1.74(54)	0.36(1)	1.10(11)
Propachlor + pendimethalin	0.10(1)	1.66(45)	0.10(1)	1.00(9)
Propachlor + chloridazon	1.99(96)	1.69(48)	1.55(34)	1.04(10)
Untreated	2.79(614)	1.96(90)	2.85(707)	1.60(39)
L.S.D. (5%) between columns	0.26		0.40	
within columns	0.42		0.37	

1985 Trial

Herbicide treatments kg a.i./ha	Onions/4 m row			
	Number		Weight (kg)	
	C	U	C	U
Propachlor 4.3 + chlorthal-dimethyl 4.5	165	165	2.4	1.9
Propachlor 4.3 + pendimethalin 0.67	152	159	2.3	1.6
Propachlor 4.0 + chloridazon 0.86	177	179	2.5	1.4
Untreated	173	177	1.8	1.6
L.S.D. (5%) between columns	18		0.5	
within columns	30		0.5	

	Weeds/m^2			
	Number*		Weight (g)*	
	C	U	C	U
Propachlor + chlorthal dimethyl	1.01(9)	1.28(18)	1.50(31)	1.22(15)
Propachlor + pendimethalin	0.75(5)	0.83(6)	1.19(14)	0.70(4)
Propachlor + chloridazon	1.50(31)	1.05(10)	1.99(98)	0.85(6)
Untreated	2.67(471)	2.44(271)	3.50(3132)	2.43(267)
L.S.D. (5%) between columns	0.52		0.70	
within columns	0.48		0.60	

* Transformed data (log(n+1)), back transformed data in parenthesis.

Salad onions

Onion cv. White Lisbon was drilled on 3 April 1984. Propachlor + chlorthal dimethyl, propachlor + pendimethalin and propachlor + chloridazon were applied pre-emergence before covering. The covers were removed on 18 May and weeds counted and weighed. The onions were harvested on 27 June (covered crop) and 17 July (open crop); none of the herbicides had a significant effect on yield (Table 3, 1984 trial). The main weeds were V. arvensis, P. annua, T. inodorum and P. aviculare. Solanum nigrum was also present especially on the covered plots. There were 7 times as many weeds and weed fresh weight was 18 times greater on the covered untreated subplots than in the open (Table 3). Under the covers, weed control with propachlor + pendimethalin and propachlor + chlorthal dimethyl was good but propachlor + chloridazon did not control V. arvensis. On the unsheeted subplots the level of weed control appeared poor because none of the treatments completely controlled V. arvensis, although many survivors were stunted.

In 1985, cv. White Lisbon was drilled on 2 April and the same pre-emergence treatments applied as in 1984. The sheeting was removed and the crop weeded on 4 June. The whole experiment was harvested on 2 July. There were no differences in crop stand between treatments but weed competition on covered untreated subplots reduced crop weight at harvest (1985 trial, Table 3). Cereal thrip damage at onion emergence may have contributed to the lower yields on the unsheeted plots; covered plots were not affected. Weed species present included P. aviculare, P. annua, L. amplexicaule, Matricaria matricariodes, S. media, F. officinalis and T. arvense. There were almost twice as many weeds under the covers and the fresh weight was 12 times that on the uncovered control plots (Table 3). Propachlor + pendimethalin and propachlor + chlorthal dimethyl gave the best weed control. Propachlor + chloridazon controlled weeds better in the open than under the covers where P. aviculare was the main survivor.

DISCUSSION

Although post-emergence herbicide applications are feasible with fibrous covers, pre-emergence treatments offer the best option for weed control under plastic, and since surviving weeds are likely to grow rapidly broad-spectrum treatments are essential. However, differences in weed emergence and in the movement and breakdown of herbicides under the covers may affect weed control. In the early-sown trials reported here, weed numbers under covers far exceeded those in the open. In the late-sown crop of carrots this was not so but weed growth was greater under covers. In additional trials (Bond and Walker, 1989), there were differences in the distribution of the herbicides, but percentage weed kill was similar under covers and in the open when compared with the appropriate controls. In the present trials, the percentage weed kill averaged for herbicide treatments under covers was 94 ±8%, and in the open 84 ±17%.

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WEED CONTROL WITH FLUROXYPYR IN SPRING SOWN BULB ONIONS.

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ABSTRACT

The potential addition of fluroxypyr to the small and declining number of herbicides approved for use in spring sown onions in the United Kingdom, offers the opportunity of controlling a wider spectrum of weeds in this crop. In four replicated trials in 1988, a single application of fluroxypyr was made at two timings and at up to three rates, between 100-400 g a.i./ha. The percentage weed control by species, was recorded at regular intervals up to a month after treatment. Assessments of crop vigour were made at similar timings. Yield and bulb size assessments were undertaken on sub samples of the crops at harvest time. Results showed that, at all rates tested, no reduction in bulb size, yield or long term vigour of plants was recorded, whilst good control of *Solanum nigrum, Convolvulus arvensis, Stellaria media, Galium aparine, Aethusa cynapium* and *Solanum tuberosum* was achieved .

INTRODUCTION

Excellent weed control throughout the life of an onion crop is a pre-requisite for successful commercial production. The addition of fluroxypyr (*Starane** 2 herbicide) in an onion weed control programme offers potential as a valuable asset in controlling a wide range of broad leafed weeds especially *Solanum nigrum, Convolvulus arvensis, Stellaria media, Galium aparine, Aethusa cynapium* and *Solanum tuberosum.*

This paper describes the results from four replicated small plot field experiments undertaken in Kent and Cambridgeshire to assess the activity of fluroxypyr in commercial onion crops.

MATERIALS AND METHODS

The trials were laid out in a randomised block design, four replicates per treatment, each consisting of a five metre length of raised onion bed.

Four trial sites were located, two in Kent and two in Cambridgeshire. On all sites the crop was drilled to a stand of four or five rows per bed.

* Trade mark of The Dow Chemical Company

When the crop was at the one to one and half leaf stage, on two of the sites, an early application of fluroxypyr was made to four replicate plots at a rate 200 g a.i./ha. The remaining treatments were at 100, 200 and 400 g a.i per hectare applied when the crop reached the two and a half leaf stage, two weeks later.

Applications were made in late May and early June, with a hand held propane displacement Azo sprayer. Delivery was through three, 8003 flat fan nozzles at volume rates of water equivalent to 200 l/ha. On each trial site replicate plots were left untreated. In order to compare yields all plots were hand weeded four to six weeks after treatment and an application of commercial rates of aziprotryne and/or bentazone were subsequently made to all plots to help keep them weed free up to September and harvesting.

Regular weekly or fortnightly assessments were made of overall weed vigour on linear scales of 0-10. Mean percentage control ratings were derived from these values.

Counts of onion plants per meter row were made. Crop vigour, was calculated as a mean value from a sub sample of 20 onion plants per replicate plot.

At harvest time the diameter of a sample of 20 bulbs per plot was recorded, as was the fresh weight of a sample of topped onions derived from a total run of four meter row per replicate plot.

Table 1 Summary of Treatments

Treatment	Rate g a.i./ha	Timing
Untreated		
Fluroxypyr	200	T 1: 1 - 1.5 leaves
Fluroxypyr	100	T 2: 2.5 leaves
Fluroxypyr	200	T 2: 2.5 leaves
Fluroxypyr	400	T 2: 2.5 leaves

RESULTS.

Table 2 Mean % Control of Weed Species from Four Replicated Trial Sites

Species (No of sites)	Rate of Fluroxypyr g a.i./ha	Assessment Timing Days after Treatment		
		T1+7-18	T2+14-18	T2+29-30
Solanum nigrum (2) Weed size	100 200 400		50.0 100.0 100.0 **4 lvs	100.0 100.0 100.0
Convolvulus arvensis (2) Weed size	100 200 400	52.5 coty~	15.0 95.0 80.0 3-4lvs	100.0 100.0 100.0
Stellaria media (2) Weed size	100 200 400	90.0 coty~-12lv	6.4 91.5 80.0 15cm-flwr	32.5 100.0 100.0
Galium aparine (3) Weed size	100 200 400	68.0 4-7 #wrls	43.0 55.3 62.0 6-10 wrls	30.0 55.3 65.0
Sonchus oleraceus (2) Weed size	100 200 400	70.0 3-4lvs	28.0 55.0 60.0 2-5lvs	5.0 15.0 15.0
Aethusa cynapium (2) Weed size	100 200 400	68.0 3lvs	17.5 40.0 50.0 4-8lvs	31.0 38.0 41.5
Solanum tuberosum (4) Weed size	100 200 400	60.0 2-6 lvs	23.7 36.5 48.2 6-8 lvs	59.2 52.5 61.7
Polygonum persicaria (2) Weed size	100 200 400		3.0 35.0 30.0 2-6lvs	6.5 0.0 3.5
Chenopodium album (2) Weed size	100 200 400		11.5 11.5 2.5 4- 6 lvs	5.0 0.0 0.0

Weed size at application T1 and T2. **lvs = leaves; #wrls = whorls of leaves: ~coty = cotyledons

Solanum nigrum was controlled by fluroxypyr at all rates tested with complete control having been achieved four weeks after treatment.

Solanum tuberosum was controlled at T1 + 7-18. Similar activity was obtained at T2 +29 in plots treated with fluroxypyr at all rates under investigation.

Convolvulus arvensis treated from the cotyledon stage was very susceptible to fluroxypyr at all rates tested.

Stellaria media was completely controlled by rates of fluroxypyr of 200 g a.i./ha and above.

Galium aparine was effectively controlled when between 4-10 whorls. Similar levels of control were achieved from late applications of fluroxypyr at 200 g a.i./ha and above, to that achieved by 200 g a.i./ha applied earlier.

Sonchus oleraceus was suppressed initially, recovering within a month of treatment.

Aethusa cynapium at up to the three expanded true leaf stage, was controlled well by fluroxypyr at 200 g a.i./ha, however larger plants were not as susceptible to treatment.

Polygonum persicaria sprayed at the two to six expanded true leaf stage was only checked for up to a month, but subsequently recovered.

Chenopodium album at the 4-6 expanded true leaf stage was resistant to fluroxypyr at all rates tested.

Several other weeds including *Senecio vulgaris* (5 leaf stage), *Malva sylvestris* (12 leaf stage), *Capsella bursa - pastoris* (flowering) *Matricaria perforata* (10 leaf stage) and *Fumaria officinalis* (8 leaf stage) were recorded in only one of four sites. All the above were suppressed by fluroxypyr for varying periods especially at the higher rates tested.

Selectivity

Table 3 Mean Crop Vigour Rating as % of Control: Four Trials

Treatment	Rate g a.i./ha	Assessment Date and Crop Stage		
		T1+15-18 2 lvs	T2+14-18 2-5 lvs	T2+29-30 3-5 lvs
Fluroxypyr	100		94.3	96.3
Fluroxypyr	200	86.9	93.9	97.9
Fluroxypyr	400		90.1	96.0

A slight reduction in vigour was noted two to three weeks after the early treatment T1, with 200 g a.i./ha fluroxypyr, with the general appearance of treated plants being poorer than that of the untreated crop. Some transient distortion of leaf tips was recorded. Within a month the effect was outgrown. Where later applications (T2) were made to more healthy and mature plants, fluroxypyr had a detrimental effect on their vigour. Generally the higher the rate applied the more marked this became. Within 28 days leaf tip distortion and necrosis were outgrown.

Table 4 Mean number of onion plants per meter row: Four trials.

Treatment	Rate g a.i./ha	Assessment Date and Crop Stage		
		T1+15-18 2 lvs	T2+14-18 2-5 lvs	T2+29-30 3-5 lvs
Untreated		21.1	17.1	19.2
Fluroxypyr	100	-	16.3	18.8
Fluroxypyr	200	20.6	16.7	18.4
Fluroxypyr	400	-	16.6	17.6

Counts were taken of the number of plants per meter row. On no site was there a significant reduction in plant stand, when compared to the untreated control plots. Slightly lower plant numbers were recorded from treated plots.

Table 5 Mean onion diameter and yield

Treatment	Rate g a.i/ha	Diameter (mm) from 20 replicate bulbs	Yield (kg) from meter row
Untreated		48.1	1.1
Fluroxypyr	100	51.8	1.2
Fluroxypyr	200	52.1	1.3
Fluroxypyr	400	51.6	1.5
Fluroxypyr (early application)	200	57.9	1.4

The increase in bulb diameter was probably a combination of two factors, successful weed control and a marginally lower plant stand in the fluroxypyr treated plots.

Yield was enhanced by the fluroxypyr treatments, compared to untreated control plots. The general increase in bulb diameter was reflected in an increase in yield.

DISCUSSION

Though some distortion of onion leaf tips was noted shortly after treatment these effects were outgrown within a month. " Pig tail" spiralling of leaves, induced by the early treatment, was rapidly outgrown. The effects of later treatments were not visible after one month. No detrimental effect was recorded from any of the treatments, on crop stand, yield or bulb size one month or more after any treatment.

At rates of fluroxypyr between 100 and 400 g a.i. /ha, good activity against a range of weeds including *Solanum nigrum*, *Polygonum convolvulus*, *Stellaria media*, *Galium aparine*, *Aethusa cynapium*, and *Solanum tuberosum*, was noted. It was shown that fluroxypyr could be used safely and to advantage, as a post emergent herbicide on spring sown bulb onion crops, with the potential of uniquely extending the weed control spectra of other herbicides commonly used in commercial onion crops.

METHODS OF IMRPOVING THE EFFICACY OF GLUFOSINATE-AMMONIUM

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ABSTRACT

The non-selective herbicide glufosinate-ammonium, which can be used for weed control in various crops and situations, was tested in the form of the standard formulation containing 200 g a.i./l. To investigate ways of improving the product efficacy, varying methods were used. One involved adding an anionic wetting agent containing the sodium salt of an alkyl-polyglycol ether sulphate, and another one was to develop a new formulation with a lower content of active ingredient (150 g/l). Field trials in Germany showed, that the new formulation at equal rates of formulated product was equally effective as the standard formulation. The addition of the surfactant increased the efficacy of the standard formulation on a greater number of test plants.

In other trials, the influence of the addition of ammonium sulphate at a rate of 10 kg/ha was tested, and it was found, that the efficacy of glufosinate-ammonium was improved considerably: In more than 20 trials, the efficacy of the standard formulation was increased by 20 - 30 %, if ammonium sulphate was added.

INTRODUCTION

The non selective foliar herbicide glufosinate-ammonium, trade names ®Basta, ®Buster, ®Finale or ®Ignite, code number Hoe 039866, is widely used for weed control in orchards, vineyards, tropical plantation crops and other fields. The good weed control was described in a number of publications, for instance by Langelüddeke *et al*, 1984, Goetz *et al*, 1984, Langelüddeke *et al*, 1985, Ceconi *et al*, 1986, Erny *et al*, 1986, or Purusotman *et al*, 1987. In order to improve the efficacy of the standard formulation, (200 g a.i./l), in model and field studies ammonium sulphate or an anionic wetting agent, trade name Genapol LRO, were added. First reports given by Langelüddeke, Baedelt and Bieringer 1988, and by Langelüddeke *et al*, 1989, demonstrated the positive effects of these additives, and the good efficacy of a recently developed formulation containing 150 g a.i./l. The objective of the experiments reported here was to confirm these results and to investigate further details under Central European field conditions.

MATERIALS AND METHODS

All trials were conducted using normal field trial techniques, i.e. the chemicals were applied with a hand held boom sprayer; spray volume was 300 l/ha (unless otherwise indicated) using a flat fan 11004 Teejet nozzle; plot size was mostly 10 m^2, number of replicates mostly 3. Evaluations were made by means of visual assessments using the normal 0 to 100 % scoring scale. Test plants were naturally occuring weeds, in some cases crop plants were sown to get a uniform stand of test plants.

®: Basta, Buster, Ignite, Finale and Genapol LRO: registered trade marks of Hoechst AG.

Two formulations of glufosinate-ammonium were used: The standard formulation with 200 g a.i./l, and a new formulation with 150 g a.i./l. In one basic study a solution of the unformulated Hoe 039866 was applied. The wetting agent used was an anionic surfactant with the trade name ®Genapol LRO; this wetter is available as a paste or as a fluid product with 68 or 29 %, resp., content of the sodium salt of an alkyl-polyglycol ether sulphate.

RESULTS

1. Model studies

In a field trial, conducted on lambsquarters (*Chenopodium album*) and pale smartweed (*Polygonum lapathifolium*), unformulated Hoe 039866 at rates of 500 and 800 g a.i./ha plus increasing amounts of Genapol LRO fluid was sprayed with 200 or 1000 l/ha water. One or two weeks after application it could be shown especially at the high water volume, that increasing rates of surfactant resulted in increasing efficacy. This effect was very clear on lambsquarters, where a marked difference between 200 and 1000 l/ha spray volume could be noted: At the lowest surfactant rate used (0.4 l/ha), the efficacy was very good (98 %) with 200 l/ha spray volume, whereas at the same rate, sprayed with 1000 l/ha, only a very marginal control rate was found (13 %). At higher surfactant rates, and on smartweed, the differences were less dramatic, but still visible (table 1). The best assessment dates to show the differences between different treatments, were 2 weeks after application in lambsquarters and 1 week in smartweed.

TABLE 1: Influence of increasing rates of surfactant (Genapol LRO fluid) on the efficacy of unformulated Hoe 039866 at 2 water volumes; % weed control.

treatments	*C. album* 2 weeks after applic. Hoe 039866 g a.i./ha 500	800	*P. lapathifolium* 1 week after applic. Hoe 039866 g a.i./ha 500	800
A. at 200 l/ha spray volume				
surfactant				
0.4 l/ha	98	100	88	92
0.8 l/ha	98	100	88	94
1.2 l/ha	98	100	88	95
B. at 1000 l/ha spray volume				
surfactant				
0.4 l/ha	13	60	65	80
0.8 l/ha	73	93	80	87
1.2 l/ha	80	96	78	89

TABLE 2 : Influence of spray volume on the herbicidal efficacy of Hoe 039866 at 400 g a.i./ha; weed control 14 days after application

formulation	*P.sativum* 200 l/ha	1000 l/ha	*C.album* 200 l/ha	1000 l/ha
standard form.	88	40	92	67
new form.	96	86	97	86

In a further field trial (table 2), the efficacy of the standard (200 g/l) and of the new (150 g/l) formulation were compared at 200 and 1000 l/ha spray volume on two test species, lambsquarters and peas (*Pisum sativum*). Both formulations were very effective on both test species when sprayed with 200 l/ha, the new formulation being clearly better than the standard. At 1000 l/ha, the efficacy of the standard formulation decreased drastically by 48 or 25 %, whereas the new formulation still showed good weed control rates with a decrease of only ca. 10 % on both species.

Another field trial was conducted to show the influence of the surfactant Genapol LRO paste on the efficacy of the standard formulation; test plants were three cereals, spring barley (*Hordeum vulgare*), spring wheat (*Triticum aestivum*) and oats (*Avena sativa*), all at the growth stage 31, and a mixture of volunteer dicot weeds (*C. album, P. persicaria, Thlaspi arvense, Matricharia chamomilla, Galium aparine*). The figures given in table 3 show, that the efficacy of the standard formulation was improved by adding the wetting agent Genapol LRO paste at a rate of 1.5 l/ha (= 0.5 % in the spray liquid). This effect was very clear on barley at both rates, and on wheat or oats at the lower rate, as the level of efficacy was lower. When higher control rates were achieved by the product alone (see the 5 l/ha rate on wheat or oats, or both rates on dicots), the degree of improvement was smaller.

TABLE 3: Efficacy of the standard formulation (200 g Hoe 039866/l), without and with addition of the surfactant; % efficacy 7 and 19 days after application.

product	l/ha	barley		wheat		oats		dicots	
		7	19	7	19	7	19	7	19
Hoe 039866	2.5	42	40	57	77	60	77	89	92
Hoe 039866 + surf.	2.5 1.5	50	58	62	82	67	83	90	96
Hoe 039866	5.0	57	68	73	92	83	92	94	98
Hoe 039866 + surf.	5.0 1.5	75	86	83	96	90	97	95	98

2. Broad field trials

In 1987 and 1988, broad field tests were conducted in Germany under a wide range of different conditions, in orchards and vineyards, in non-crop land, in vegetables prior to seeding or transplanting, in vegetables or potatoes immediately before emergence of the crop, prior to direct drilling, in trials with application at different growth stages, or in specially sown crops for conducting comparison tests, both in the warm and in the cold season; certainly in these trials dose rates were used which were adapted to the particular situation. A summary of all results obtained is given in the following table 4. Based on a great number of results, it can be shown that there were no differences on dicot and only slight differences on monocot species.

TABLE 4: Comparison of the new and the standard formulation of Hoe 039866 at equal rates of product; summary of a l trials in Germany 1987/88;
n = number of results (average control per trial and species).

formulated product l/ha	2 weeks after applic. n	standard formul.	new formul.	4 weeks after applic. n	standard formul.	new formul.
Dicotyledonous species						
1.5 - 2.0	136	82.1	82.3	85	69.5	69.9
2.5 - 3.5	136	82.8	81.5	85	82.6	81.3
4.0 - 5.0	86	80.3	79.6	63	88.0	86.9
Monocotyledonous species						
1.5 - 2.0	30	70.9	70.4	12	74.0	70.1
2.5 - 3.5	60	69.2	64.0	45	66.3	63.5
4.0 - 5.0	43	78.6	76.1	37	76.9	72.5

3. Trials with ammonium sulphate

Twenty two trials were conducted in orchards and vineyards at rates of 3 and 5 l/ha of the standard formulation with and without addition of 10 kg/ha ammonium sulphate. In table 5, average weed control figures are given for both trial programs, one conducted in spring with applications in April or first half of May (7 trials), the second one in early summer, i.e. late June or July (15 trials). The weed spectrum occuring in these trials included annual and perennial species, dicotyledonous as well as grassy weeds.

TABLE 5: Average efficacy (% weed control) and average coverage (% regrowth) figures of 2 trial series conducted in Germany:
(AS = 10 kg/ha ammonium sulphate, WAA = weeks after application)

assessments type	WAA	Hoe 039866 standard formulation 3 l/ha	3 l/ha + AS	5 l/ha	5 l/ha + AS
A. spring applications (7 trials)					
% weed control	2	60	78	89	91
% weed control	4	48	63	71	78
% regrowth	8	77	75	72	66
% regrowth	12	89	85	73	78
B. summer applications (15 trials)					
% weed control	2	85	90	93	95
% weed control	4	85	90	94	96
% regrowth	8	46	38	29	24
% regrowth	12	67	61	55	49

The addition of ammonium sulphate revealed a better efficacy. In average, 3 l/ha Hoe 039866 + ammonium sulphate were clearly better than 3 l/ha without, whereas the difference between 5 l/ha and 5 l/ha + ammonium sulphate was smaller, as 5 l/ha alone came up to a relatively high average weed control of approx. 90 % or more. The rate of 3 l/ha + ammonium sulphate was slightly weaker than 5 l/ha alone.

DISCUSSION and CONCLUSIONS

Since the first introduction of glufosinate–ammonium, the rate of active ingredient necessary for a good weed control ranged from 0.75 to 1.5 kg/ha (Schwerdtle *et al*, 1981) to rates as low as 0.2 or o.375 kg/ha (Kassebeer *et al*, 1983; Purusotman *et al*, 1986). These low rates of a.i./ha correspond to rates of 1.0 to 1.875 l/ha of the standard formulation (200 g a.i./l). Connected with low rates of formulated product certainly is a low surfactant concentration in the spray liquid. This was followed by considerations as to whether the efficacy of glufosinate–ammonium could be improved by adding surfactants or improving the formulation. Some special effects achieved in model studies by the addition of Genapol LRO, a specific anionic surfactant, were reported last year by Langelüddeke *et al* (1988) and supported by further pot and field trials (Langelüddeke *et al*, 1989), which included already first results with a new formulation containing 150 g a.i./l.

Now it could be shown in further field trials (tables 1 and 2), that the efficay of Hoe 039866 is influenced by the rate of surfactant and by the spray volume: If the efficacy of the formulated product is compared at different spray volumes, a drastic decrease of efficacy may be found at high water volumes and low product rates; this is shown by the results described in table 1, where equal amounts of surfactant were added to the unformulated Hoe 039866. On the other hand, the addition of 0.5 % surfactant to the spray liquid improved the efficacy of the standard formulation on a number of different test species, as shown in table 3. This again supports the suggestion, that rate or concentration of surfactant is one important parameter of the efficacy of Hoe 039866. This confirms effects described by Langelüddeke *et al* (1989) for South–East Asian conditions. This method deserves, however, further investigations under Central European conditions, as this is a way to reduce the rate of active ingredient per hectare.

Another way is the development of a new formulation with a lower content of a.i. per litre. In broad field trials in Germany it was found, that this new formulation was more or less equally active as the standard formulation if applied at equal rates of formulated product. This implies a reduction of 25 % of the rate of active ingredient. A slight loss of grass weed activity could be accepted (table 4).

The results presented in table 5 indicate that the addition of ammonium sulphate can increase the efficacy of Hoe 039866 to a considerable extent. This effect is especially clear if the general level of activity is below the optimum; even if 3 l/ha of the standard formulation plus ammonium sulphate did not reach the control level of 5 l/ha, it was clearly superior to that of 3 l/ha alone, and it can be concluded, that the recommended rate of 5 l/ha can be reduced to approximately 3.5 or 4 l/ha, if ammonium sulphate is added at a rate of 10 kg/ha.

In summary, it can be concluded, that the efficacy of glufosinate–ammonium can be improved by

a. a new formulation with a lower content of a.i. per liter;
b. by adding a suitable wetting agent, for instance Genapol LRO or similar, to the standard or to the new formulation ;
c. by adding ammonium sulphate to the standard formulation.

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POST-HARVEST WEED CONTROL AND RUNNER CONTROL WITH GLUFOSINATE-AMMONIUM IN STRAWBERRIES

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ABSTRACT

This paper reports results of two years experiments with glufosinate-ammonium either alone, or in mixture with ammonium sulphate on post-harvest weed control, runner control, growth of the strawberry plants and yield (quantity and quality). It was established that glufosinate-ammonium provided good post-harvest weed control and suppressed regrowth of runners.

INTRODUCTION

Post-harvest weed control and control of unwanted strawberry runners is a serious problem for commercial strawberry prodution in Bulgaria.

According to Lawson and Wisemen (1980, 1985), paraquat and dinoseb-in-oil are currently widely used in the United Kingdom for the chemical runner control in strawberries. The new contact herbicide glufosinate-ammonium (HOE 39866) has also shown promise for this purpose. Glufosinate-ammonium has been recommended for weed control in horticultural crops until harvest, although summer application was more efficient than a spring application (Langeluddeke et al, 1985; Majek, 1985).

This paper reports results of two years experiments with glufosinate-ammonium either alone, or in combination with ammonium sulphate. Assessments were made on the effect of herbicide on post-harvest weed control, runner control, growth of the strawberry plants and yield (quantity and quality).

MATERIALS AND METHODS

During 1987-1988 field trials were carried out at the Plant Protection Institute in Kostinbrod on an alluvial soil (om 1.9% and pH 6.5). The experiment was laid down on a two year old strawberry plantation of cv Red Gauntlet, and treatments were replicated three times, the area of test plot being 16 sq m. The interrow spacing was 0.80 m. Glufosinate-ammonium, 20% ec, was applied at 0.4, 0.6 and 0.8 kg/ha either alone or in a mixture with ammonium sulphate at a rate of 10 kg/ha. The herbicide was applied with a hand sprayer at a rate of 600 l/ha. All treatments were applied on 9 July 1987 and 25 July 1988. The density and number of different weed species per square metre were recorded at the beginning of spraying. Weed control and runner control were assessed 10, 20, 40 days after treatment by using the usual scoring system from 0 to 100, where 0 indicates no effect and 100 the complete extermination of green parts of plants. The effect of glufosinate-ammonium either alone or in combination with ammonium sulphate on the growth of strawberry plants, yield and quality of the fruit were determined the year following spraying by using standard methods.

RESULTS AND DISCUSSION

The effect of glufosinate-ammonium either alone or in combination with ammonium sulphate on post-harvest weed control in strawberries is shown in Table 1.

Table 1 Average percentage control of annual and perennial weeds (1987-1988) - 40 DAT

Weed species	Treatments - kg ai/ha Glufosinate-ammonium			Glufosinate-ammonium + ammonium sulphate*		
	0.4	0.6	0.8	0.4 + 10	0.6 + 10	0.8 + 10
Amaranthus retroflexus	59	61	63	63	72	78
Chenopodium album	91	94	98	99	100	100
Euphorbia peplus	89	91	94	98	100	100
Galinsoga parviflora	70	72	78	90	98	100
Polygonum lapathifolium	54	61	67	61	66	72
Sinapis arvensis	58	72	79	64	78	82
Stellaria media	71	79	82	84	95	100
Galium aparine	85	93	96	94	100	100
Matricaria maritima	87	95	98	96	100	100
Datura stramonium	81	89	94	95	100	100
Polygonum convolvulus	74	81	93	89	94	100
Senecio vulgaris	77	88	91	86	89	99
Cirsium arvense	61	64	72	69	77	85
Convolvulus arvensis	58	60	63	66	68	74
Elymus repens	52	59	65	58	61	69
Setaria sp	89	92	98	100	100	100
Echinochloa crus-galli	90	91	96	100	100	100

*Rate of ammonium sulphate is given as a product

The data from Table 1 shows clearly that glufosinate-ammonium at rates 0.8 kg ai/ha provided a good herbicidal effect within 40 days of treatment. Annual dicotyledonous weeds, with the exception of A. retroflexus were destroyed from 78 to 98%. The combination of glufosinate-ammonium with ammonium sulphate was more efficient than glufosinate-ammonium alone. C. arvensis and E. repens could not be efficiently controlled at 0.8 kg ai/ha glufosinate-ammonium, but the effect of glufosinate-ammonium may have been slightly improved by the addition of 10 kg/ha ammonium sulphate.

Table 2 Average percentage desiccation score of strawberry runners (1987-1988)

Treatments kg ai/ha	Dat 10	20	40
Glufosinate-ammonium			
0.4	5	48	64
0.6	6	67	82
0.8	11	69	86
Glufosinate-ammonium + Ammonium-sulphate			
0.4 + 10	7	70	77
0.6 + 10	9	73	89
0.8 + 10	16	86	100

After application, glufosinate-ammonium was slow acting on the suppresssion of runners, but by 40 days after treatment it had suppressed regrowth of treated runners. In comparison with the glufosinate-ammonium alone, the effect of combination with ammonium sulphate is a slight improvement.

During the year following the application of glufosinate-ammonium treatments, observations were made to determine the action of glufosinate-ammonium either alone or in mixture with ammonium sulphate on the strawberry plants. No significant differences were established for number, width, length and fresh weight of the leaves from treated and untreated plants. (Table 3).

Mean strawberry yields and an assessment of the quality of the fruit for treated and untreated plants is shown in Table 4.

Table 3 Effect of glufosinate-ammonium alone and with ammonium sulphate on the growth of strawberry plants

Treatments kg ai/ha	Leaves* Number	Width (cm)	Length (cm)	Fresh weight (g)
Glufosinate-ammonium				
0.4	15	5.8	7.3	27.8
0.6	14	5.9	7.8	26.9
0.8	15	5.4	7.6	27.1
Glufosinate-ammonium + ammonium sulphate				
0.4 + 10	14	5.9	7.5	27.6
0.6 + 10	16	5.6	7.7	27.2
0.8 + 10	15	5.8	7.8	27.9
Control	15	5.7	7.5	28.1

*Average of 20 plants

Table 4 Yield recorded and quality of the fruit (average 1987-1988)

Treatments kg ai/ha	Mean yield as % of control	Quality of fruit Ascorbic acid Mg %	Titratable acid % as citric acid	Glucose %	Sucrose %
Glufosinate-ammonium					
0.4	100	67.83	0.672	7.32	0.89
0.6	101	67.78	0.623	7.21	0.91
0.8	100	67.62	0.662	6.98	0.90
Glufosinate-ammonium + Ammonium sulphate					
0.4 + 10	101	67.78	0.658	7.38	0.92
0.6 + 10	104	67.65	0.661	7.35	0.90
0.8 + 10	103	67.43	0.653	7.42	0.87
Control	100	67.88	0.642	7.53	0.91

Glufosinate-ammonium either alone or in combination with ammonium sulphate did not have any effect on the quality of the strawberries.

The results of this work show that glufosinate-ammonium alone or in mixture with ammonium sulphate is a promising herbicide for post-harvest weed control and runner control in fruiting strawberry crops. Further investigations are required on the relationship of dose and persistence of runner suppression of treated plants.

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TERBACIL SELECTIVITY FOR WATERMELON

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ABSTRACT

Terbacil was efficacious at 0.06 to 0.22 kg/ha pre-emergence for seeded watermelon in three years of field studies on a Norfolk loamy sand, 0.6% o.m. 'Crimson sweet' watermelon size, number/ha and yield with terbacil, pre-emergence, were not significantly different from the handweeded controls. The small-seeded watermelon, cv. Crimson sweet was 25% more susceptible to terbacil than the large seeded cv. Charleston gray and Jubilee. Terbacil tolerance of cantaloupe and cucumber was 60 and 90% less than watermelon, respectively. Pre-emergence combinations of terbacil with bensulide or diclofop provided improved weed control compared to either herbicide singularly. Terbacil at 0.10 kg/ha controlled Ambrosia artemisiifolia, Chenopodium album, Portulaca oleracea and Ipomoea hederacea.

INTRODUCTION

Watermelons have few registered herbicides and weed control is essential for maximum yield and melon size. Terbacil at 1.12 kg/ha pre-emergence, a 2X rate, was efficacious with an activated charcoal barrier for Charleston gray watermelons in a sandy loam soil (Glaze et al., 1979). Broadleaf weeds are a major concern in watermelons as they interfere with harvest and weed tissues that contact the melon cause a blemished coloration of the rind. Activated carbon is tedious to apply and may decrease weed control, therefore, its elimination would be advantageous. The objective of this study was to determine efficacious terbacil rates for seeded watermelon and other vine crops.

MATERIALS AND METHODS

The studies were conducted on a Norfolk loamy sand, 0.6% o.m. and sprinkler irrigation supplemented rainfall, to maintain normal growth.

Logarithmic rates of terbacil, pre-emergence were applied from 1.12 to 0.03 kg/ha with half-rate changes at 4.5 m intervals at 935 l/ha spray volume. Growth ratings were made six weeks after treatment in 1984 and 1985. The treatments were replicated twice each year. The sprayer had three 8004 flat fan nozzles spaced 50 cm and the propellant was compressed air at 170 kPa. The planting and treatment dates were June 13, 1984 and June 7, 1985.

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Yield trials were established with three replications in 1986 and 4 replications in 1987 and 1988 in a randomized complete block design with one row plot sizes of 2.4 by 23 m in 1986 and 2.3 by 10 m in 1987 and 1988. Herbicide treatments were applied with a hand held spray boom of four 8003 flat fan nozzles spaced 50 cm. CO_2 was the propellant at 275 kPA to deliver 206 l/ha spray volume. 'Crimson sweet' watermelon was planted and treated on May 5, 1986; May 7, 1987 and April 28, 1988. Terbacil was applied pre-emergence as a tank mix combination with diclofop, 1.1 kg/ha, in 1986 and bensulide, 4.5 kg/ha, in 1987 and 1988. All treatments had four mechanical cultivations in 1986 and 1988 and two in 1987. Early and total yields were obtained from three harvests.

RESULTS AND DISCUSSION

Small seeded Crimson sweet watermelon had 50% growth reduction at 0.16 kg/ha in the logarithmic rate screening trial; whereas, the large seeded varieties Jubilee and Charleston gray required 0.30 and 0.22 kg/ha, respectively, for 50% growth reduction (Table 1). Cantaloupe and cucumber were 60% and over 90% less tolerant than watermelon. Broadleaf weeds were controlled at terbacil rates of less than 0.1 kg/ha.

TABLE 1. Logarithmic rate evaluation of several crop and weed species for terbacil tolerance.

Crop or Weed Species	Terbacil, kg/ha, rate to elicit observed % control			
% Control:	100	90	50	0
Watermelon:				
Crimson sweet	0.30	0.20	0.16	0.12
Jubilee	0.60	0.40	0.30	0.20
Charleston gray	0.40	0.30	0.22	0.20
Cantaloupe:				
Jumbo Hale's Best	0.09	0.07	0.06	0.05
Cucumber:				
Poinsett 76	0.05	<0.03	----	----
Ipomoea hederacea	0.08	0.05	<0.03	----
Chenopodium album	<0.03	----	----	----
Portulaca oleracea	<0.03	----	----	----
Ambrosia artemisiifolia	<0.03	----	----	----

Crimson sweet watermelon yields of terbacil treatments were not significantly different than the handweeded control or the bensulide alone treatments (Table 2). The cultivations and lack of intense broadleaf competition enabled bensulide alone to prevent a yield loss from weed competition. However, yields with herbicide treatments were significantly greater than the untreated control which showed that cultivation alone was inadequate to prevent yield loss from weed competition. Watermelon size was unaffected by terbacil (Table 3) and the yield of large melons was significantly increased by terbacil treatments compared to the untreated

control in 1987. Terbacil improved the control of broadleaf weeds compared to bensulide alone (Table 4). Although not reported, grass control was acceptable with bensulide and diclofop; however, terbacil improved the control.

Table 2. Crimson sweet watermelon yields with pre-emergence terbacil combinations on a Norfolk loamy sand, 0.6% o.m.

Pre-emergence Treatment		Yield (1000 kg/ha)					
		Early			Total		
Herbicide	kg/ha	1986	1987	1988	1986	1987	1988
Untreated	--	8 b	2 b	--	29 b	5 b	--
Handweeded	--	29 a	11 a	25 a	45 a	20 a	60 a
Bensulide	4.5	25 a	12 a	--	44 a	16 a	--
Terbacil*	0.06	--	7 ab	--	--	21 a	--
Terbacil*	0.11	--	9 a	--	--	19 a	--
Terbacil*	0.17	27 a	9 a	--	48 a	15 a	--
Terbacil*	0.22	--	12 a	44 a	--	19 a	68 a
Terbacil*	0.30	25 a	--	--	41 a	--	--

Means within columns followed by the same letter are not significantly different with Duncan's New Multiple Range Test at 0.05 probability.

*Tank mix combination with diclofop, 1.1 kg/ha in 1986 and bensulide, 4.5 kg/ha in 1987 and 1988.

Table 3. Crimson sweet fruit quality: melon size and yield of large melons.

Pre-emergence Treatment		Melon Size (kg/melon)			Large Melons[1/] (number/ha)		
Herbicide	kg/ha	1986	1987	1988	1986	1987	1988
Untreated	--	9.1 a	5.8 b	---	1430 a	100 c	---
Handweeded	--	9.5 a	6.5 ab	9.5 a	2540 a	1220 ab	2950 b
Bensulide	4.5	9.5 a	6.3 ab	---	2220 a	1120 ab	---
Terbacil[2/]	0.06	---	7.3 a	---	---	2040 a	---
Terbacil[2/]	0.11	---	6.9 a	---	---	1630 a	---
Terbacil[2/]	0.17	10.0 a	6.9 a	---	3040 a	1330 a	---
Terbacil[2/]	0.22	---	6.7 ab	9.7 a	---	1530 a	4100 a
Terbacil[2/]	0.30	8.6 a	---	---	1430 a	---	---

Means within columns followed by the same letter are not significantly different with Duncan's New Multiple Range Test at 0.05 probability.

1/Large melon weights >6.8 kg in 1987 and >9.0 kg/ha in 1986 and 1988.
2/Tank mix combination with diclofop, 1.1 kg/ha in 1986 and bensulide, 4.5 kg/ha in 1987 and 1988.

Table 4. Weed control with terbacil in Crimson sweet watermelons and four cultivars in 1986 and 1988 and two cultivations in 1987.

Pre-emergence Treatment Herbicide	kg/ha	% Weed Control[1/] *I. hederacea*	*A. artemisiifolia*	*C. album*	*P. oleracea*
Untreated	---	0	0	0	0
Handweeded	---	100	100	100	100
Bensulide	4.5	0	70	85	60
Terbacil[2/]	0.06	85	100	100	100
Terbacil[2/]	0.11	85	100	100	100
Terbacil[2/]	0.17	90	100	100	100
Terbacil[2/]	0.22	95	100	100	100
Terbacil[2/]	0.30	100	100	100	100

1/Average for all study years.

2/Tank mix combination with diclofop, 1.1 kg/ha in 1986 and bensulide, 4.5 kg/ha in 1987 and 1988.

Terbacil was efficacious for watermelon at rates of 75 to 85% less than normal use rates and activated carbon was unnecessary for protection. Terbacil could be an alternative for the recently discontinued herbicide, dinoseb.

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WEED CONTROL IN EARLY SUMMER CAULIFLOWERS GROWN UNDER POLYTHENE COVERS

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ABSTRACT

Seven pre- and post-planting residual herbicide combinations were compared with a hand-weeded control on field-grown early summer cauliflowers covered with perforated polythene. In 1988, two plant raising module sizes were compared; in 1989 covering immediately after planting or 48 hours later were compared. Good weed control up to cover removal (six to eight weeks later) was achieved with most treatments. In 1988 good weed control up to harvest was achieved using trifluralin plus propachlor or tebutam, and propachlor plus tebutam. Pendimethalin plus metazachlor, trifluralin plus tebutam and chlorthal-dimethyl plus high rate metazachlor showed some phytotoxic effects compared to the hand-weeded control, reducing yield or quality. Other treatments showed no adverse effects. Covering immediately reduced quality compared to delaying covering in 1989, however, in 1988 plants were planted, sprayed and covered on one day and five of the seven treatments showed no reduction in yield or quality over hand weeded control plots.

INTRODUCTION

Investigations into the use of pre- or post-planting residual herbicides on crops grown under polythene covers have been reported for lettuce and courgettes (Antill 1987), but not for cauliflowers.

Polythene crop covers are being used on early summer cauliflowers to produce earlier crops and growers need to use herbicides for weed control. Under normal growing conditions early summer cauliflowers are particularly sensitive to environmental conditions, producing more buttons (small, immature heads), lower levels of marketable yield and higher levels of defects than summer and autumn cauliflowers. Some growers will not use herbicides at all on block-raised crops, preferring to hand-hoe, for fear of checking the plants and increasing the risk of buttoning. Early summer cauliflowers treated with residual herbicides and grown under polythene were examined for phytotoxic effects. Currently, the use of residual herbicides on transplanted brassicas grown under polythene are not recommended (Greenfield and Williams in press).

Polythene crop covers increase soil temperatures and herbicide volatilisation which coupled with reduced dispersal of gases and reduced air movement may be expected to lead to an increased risk of phytotoxicity. Alternatively, weed control may be less effective if materials are broken down more quickly under the warmer, moister soil conditions (Walker 1987), or fail to reach their site of activity because of reduced water movement (Bond and Walker 1989). These possible effects on the efficacy of pre and post-planting residual herbicides were investigated in two trials. In 1988 a range of possible herbicide combinations was tested, but in 1989 only combinations of chemicals approved for use on brassicas were used and higher rates of propachlor and metazachlor in combination with chlorthal-dimethyl were included.

MATERIALS AND METHODS

Early summer cauliflowers were sown in early October and raised overwinter in unheated glasshouses. The trials were grown at Kirton Experimental Horticulture Station on Lincolnshire silt soils of the Wisbech series (OM 2.1% pH 7.8) using perforated polythene covers, 10 m wide with 500 holes/m^2.

Each plot contained 30 plants and the trial was laid out as a three replicate randomised block design (1988) or split plot design (1989), each block or main plot was covered by one sheet of polythene.

1988 trial

The variety Perfection was raised in 6 cm peat blocks and Hassy 308 cellular trays with an individual cell volume of 13.5 cm^3. The trial was planted on 4 April 1988 and pre- and post-planting herbicides were applied and covers laid on the same day. Covers were removed six weeks later (19 May) and weed cover assessments were made. The untreated control was hand-weeded. Harvesting was from 25 May to 27 June, weed cover was assessed again on plots of cellular tray-raised plants which were not yet harvested.

Herbicide treatments

(a) Propachlor (as Albrass) at 9 l/ha plus chlorthal-dimethyl (as Dacthal) at 3 kg/ha post-planting.

(b) Pendimethalin (as Stomp) at 3 l/ha surface application pre-planting plus propachlor at 9 l/ha post-planting.

(c) Trifluralin (as Tristar) at 2.3 l/ha incorporated pre-planting plus propachlor at 9 l/ha post-planting.

(d) Metazachlor (as Butisan S) at 1.5 l/ha plus chlorthal-dimethyl at 6 kg/ha post-planting.

(e) Pendimethalin at 3 l/ha surface application pre-planting plus metazachlor at 1.5 l/ha post-planting.

(f) Propachlor at 9 l/ha plus tebutam (as Comodor) at 4 l/ha post-planting.

(g) Trifluralin at 2.3 l/ha incorporated pre-planting plus tebutam at 4 l/ha post-planting.

(h) Hand-weeded control.

1989 trial

The variety Jubro was sown on 17 October 1988 in GPG 308 cellular trays individual cell volume of 12 cm^3. Pre-planting herbicide treatments were applied on 16 March and the trial was planted on 28 March following a spell of wet weather. Post-planting herbicide treatments were applied on 29 March and covered that day or two days later. Untreated controls were hand-weeded on 9 May and again at cover removal on 24 May, when mean curd diameter was 5.4 mm. Weed cover assessments were made on 25 May and the crop was harvested between 16 and 30 June.

Herbicide treatments

(a) Propachlor (as Ramrod Flowable) at 9 l/ha plus chlorthal-dimethyl (as Dacthal) at 6 kg/ha post-planting.

(b) Propachlor at 13.5 l/ha plus chlorthal-dimethyl at 6 kg/ha post-planting.

(c) Trifluralin (as Tristar) at 2.3 l/ha incorporated pre-planting plus propachlor at 9 l/ha post-planting.

(d) Chlorthal-dimethyl at 6 kg/ha plus metazachlor (as Butisan S) at 1.5 l/ha post-planting.

(e) Chlorthal-dimethyl at 6 kg/ha plus metazachlor at 2.5 l/ha post-planting.

(f) Trifluralin at 2.3 l/ha incorporated pre-planting.

(g) Trifluralin at 2.3 l/ha plus tebutam (as Comodor) at 4 l/ha tank-mixed and incorporated pre-planting.

(h) Hand-weeded control.

RESULTS

1988 trial

Good weed control was achieved with all combinations up to cover removal but by harvest only trifluralin plus propachlor or tebutam and propachlor plus tebutam gave good weed control. Pendimethalin plus metazachlor produced lower yields because of higher levels of missing plants and unmarketable heads. Trifluralin plus tebutam produced more heads with green bracts. Other treatments did not affect yield or quality compared with the hand-weeded control.

TABLE 1. Results from 1988 trial

Herbicide treatment	Weed Cover (%) 20 May	7 Jun	Mktbl yield crates/ ha	% Class I	% Un-mktbl	% Missing	% Green bracts	% Loose
Propachlor + chlorthal-dimethyl	7*	38	2304	20	22	1	22	23
Pendimethalin + propachlor	6*	25	2244	23	24	5	16	19
Trifluralin + propachlor	2*	6*	2376	21	19	3	20	18
Metazachlor + chlorthal-dimethyl	10*	45	2475	22	16	0	23	23
Pendimethalin + metazachlor	10*	55	2007*	19	32*	9*	20	21
Propachlor + tebutam	5*	11*	2172	22	26	3	19	21
Trifluralin + tebutam	4*	8*	2321	29	22	2	25*	15*
Control (hand-weeded)	95	40	2376	19	19	1	14	28

* Denotes significant difference from hand-weeded control at 5% level

1989 trial

Trifluralin alone gave very poor weed control. Trifluralin plus tebutam also gave less good weed control than all the other treatments which gave adequate control. By harvest, chlorthal-dimethyl plus high rate metazachlor had noticeably less weed than other treatments. Yield was not affected by herbicide treatment or time of covering but the split between Class 1 and 2 was affected by time of covering. Covering immediately produced more Class 2 heads, down-graded from Class I because of yellow discolouration. This trend was apparent for all herbicide combinations, even where soil incorporation was 3 days before planting and covering. There was a significant reduction in percent Class 2 and yellow from delayed covering when trifluralin plus tebutam was used. Chlorthal-dimethyl plus high rate metazachlor produced a higher percent green bracts than other treatments. Herbicide treatments had no effect on time of harvest.

TABLE 2. Results from 1989 trial

Treatment	Weed cover (%)	Yield	% Class 2 ** imm	% Class 2 *** +48 hrs	% Yellow imm	% Yellow +48 hrs	% Green bracts
Propachlor (9 l/ha) chlorthal-dimethyl	11.0	2590	33	31	31	30	2
Propachlor (13.5 l/ha) + chlorthal-dimethyl	6.0	2640	36	30	38	30	3
Trifluralin + propachlor	6.3	2541	41	32	40	34	3
Chlorthal-dimethyl + metazachlor (1.5 l/ha)	11.0	2673	42	37	39	26	4
Chlorthal-dimethyl + metazachlor (2.5 l/ha)	8.3	2673	44	42	42	43	8*
Trifluralin	78.0*	2541	43	41	43	38	2
Trifluralin + tebutam	23.3*	2590	57	31+	58	32+	2
Hand-weeded control	0	2541	33	42	34	41	3
Cover immediately	16.8	2582	41		41		3
Cover +48 hrs	24.4	2615	36+		36+		3

* Denotes significant difference from hand-weeded control at 5% level
\+ Denotes significant difference between pair at 5% level
** Covers put on immediately following herbicide application
***Covers put on 24 hours after herbicide application

DISCUSSION

Weed control was generally effective using recommended rates of approved herbicide combinations in both years. Using trifluralin alone, included in the second year as it was present in successful combinations in the first year, did not control weed growth up to cover removal because trifluralin does not control a broad enough spectrum of weed species. Where higher rates of propachlor or metazachlor were used in the second year, the latter appeared to have prolonged weed control but also resulted in higher levels of green bracts.

To achieve effective weed control it is suggested that covers should not be applied for one to seven days following herbicide application and irrigation should be applied if the soil surface is dry (Antill 1987). This is to provide moisture to activate chemicals and move them to the site of action. In Lincolnshire early summer cauliflowers are planted when the soil surface becomes dry enough to work, but the soil into which they are planted is still moist. This would explain why no signs of herbicide inactivity were observed in these trials.

There was generally no reduction in yield or quality when using approved herbicides for cauliflowers. Using trifluralin plus tebutam increased the level of defects in both years. In 1988, the application method did not follow manufacturer's recommendations but this was corrected in 1989 and defects still occurred. This combination appeared to be too active to use under covers. Pendimethalin plus metazachlor also increased defects in 1988 but as it is not approved for brassicas, it use under covers was not pursued.

In 1989, covering immediately after planting produced more Class 2 heads than delaying covering for 48 hours, even for pre-planting herbicides applied 13 days earlier. The intervening day, 30 March, was hot (max 17.1 ^{0}C) and sunny (8.8 hours) and some damage may have occurred as a result of young plants being covered, the next hottest day was 1 May and plants were well established by then. In other trials on the same field, covered versus uncovered treatments were compared which experienced the same hot, sunny day with no effect on quality, but these trials were planted earlier. These results suggest there are no reasons for manufacturers or growers to be cautious of the use of propachlor plus chlorthal-dimethyl, or trifluralin on cauliflowers under covers although there are no recommendations for their use under low level plastics at present.

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