SESSION 6

TOXICOLOGICAL AND ENVIRONMENTAL ASSESSMENT OF PESTICIDES: CURRENT PERSPECTIVES

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THE NEED FOR MORE BALANCE IN THE SAFETY EVALUATION OF PESTICIDES

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The average actual daily intake of residues of a given pesticide is usually much lower than 0.1 mg. These tiny amounts should not pose great problems to human health, even in the case of pesticides, which are a particularly toxic class of compounds. The exposure of the farmer might be somewhat higher, but usually should not exceed 2 to 5 mg per day when up-to-date technology and personal protection is used. Taking this situation as starting-point, the necessary studies to safeguard human health should be rediscussed. We are of the opinion that the current requirements for these studies exceed the real needs. Financial and personnel resources ought rather to be invested in supplementary mechanistic investigations than in numerous expensive standard studies using large groups of animals. In addition, other areas such as nutritional, occupational, inhalatory or environmental toxicology deserve much more attention than mammalian pesticide toxicology, since in these fields the existing knowledge is limited and/or the impact on health is probably more important.

ENVIRONMENTAL ASPECTS OF PESTICIDES - THE DEVELOPMENT OF UNDERSTANDING.

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ABSTRACT

The assessment of environmental effects of pesticides and criteria for acceptability have evolved as knowledge and expectations have increased. Approaches to evaluating direct toxicity in the laboratory, small plot tests and field trials are generally well understood and widely accepted. Attention has turned increasingly to indirect and ecological effects which are much more difficult to assess. Experimental design and the definition of what constitutes a significant effect must be carefully considered in relation to defined objectives in order to make best use of resources and obtain interpretable results. Much can be achieved in identifying potential adverse effects by tiered pragmatic approaches, coupled with knowledge of the fate of pesticides in the environment.

INTRODUCTION

Agrochemicals have always had to satisfy simultaneous requirements for efficacy and freedom from unacceptable adverse side effects. Indeed the quest for selective toxicity has been a major force in pesticide development. The formidable potency against the target organism which was the basis for the remarkable success of the synthetic organic pesticides when they were first introduced was coupled with apparently relatively low hazard to major categories of non-target organism, compared with previous materials. Contrary to popular belief, broader environmental concerns were recognised early in the history of modern pesticides. For example, direct and indirect effects of DDT on aquatic invertebrates were being investigated in the 1940's and 1950's (see, for example, Cottam and Higgins, 1946).

Nevertheless the nature of environmental effects and methods for assessing them were at first not well understood. The initial wholehearted adoption of synthetic pesticides, resulting in their undiscerning introduction very widely in the environment, brought to the forefront deficiencies, highlighted potentially problematic properties and helped to define the conditions for acceptable use. An obvious example is seed treatment which offers the advantage of more economical pesticide use (and hence less general burden on the environment) but concentrates the active ingredient in a zone attractive to certain wildlife such as seed-eating birds. This places severe restrictions on the type of materials which can be used and inappropriate compounds (for example cyclodiene insecticides and certain organophosphate materials) were rapidly withdrawn for this use.

Stanley and Bunyan (1979) describe how policy in such cases can be determined by the interplay between effects on wildlife, availability of control agents with differential toxicity and species distribution and abundance.

From such experience and from extensive related research and investigation, much has been achieved in identifying environmental limitations. In consequence, just as requirements for efficacy have become more demanding and sophisticated (for example potency against target organisms has increased by two orders of magnitude since the 1940's; Graham-Bryce, 1981) so have criteria for environmental acceptability evolved. This is demonstrated by the proliferation of tests for environmental acceptability required by authorities for registration, illustrated in Table 1.

Table 1

Typical laboratory and small plot tests for environmental acceptability required by authorities.

1950s	1980s
Acute toxicity: birds.	Acute toxicity to fish (3 spp).
Acute toxicity: fish.	Fish early life stage tests.
Acute toxicity: bees.	Fish, bioaccumulation.
	Acute toxicity to aquatic invertebrates (4 spp).
	Acute toxicity to alga.
	Daphnia, reproduction study.
	Acute toxicity to bees.
	Acute toxicity to birds (2 spp).
	Dietary toxicity to birds.
	Effects on soil fauna.
	Residues in earthworms.
	Leaf litter decomposition.
	Effects on nitrification and CO_2 evolution from soil.
	Biodegradation rate, inhibition.
	Physicochemical properties, notably partition coefficient.

These studies are supplemented by additional tests and outdoor investigations appropriate to the compound and its intended use. These can be approached in a sequential tiered manner, with more detailed and complicated studies only being undertaken if initial tests indicate that they are needed.

There is an understandable desire for further progress. However, to ensure that this evolution continues to be genuinely fruitful and that resources are employed most effectively it is important to define clearly and to keep in mind the underlying reasons for concern about environmental behaviour of pesticides and to establish criteria for acceptability in relation to these reasons. In this context, the environment can be considered in terms of:

(1) Its value as a source of materials, including biological products and resources. These resources may be difficult to evaluate, for example the significance of the genetic resource represented by a particular species is often not clear.

(2) Its functions, such as nutrient cycling, providing a medium for crop production, maintenance of habitat for man and other species, decomposition of natural and synthetic substances.
(3) Its amenity and aesthetic value, including wildlife aspects.

Such a categorisation brings out a further point, namely that it may be difficult to assess the significance of effects on a local scale in relation to the overall impact on the environment. The development of environmental measures and future trends are considered below against this background.

DIRECT TOXICITY

The conventional starting point for evaluating potential environmental effects is determination of intrinsic toxicity as measured in a direct laboratory test. Such measurements clearly draw attention to areas of severe risk. They reveal that intrinsic activity can vary very widely between wildlife and pests. Even between closely related species and with compounds having a mode of action to which many species are vulnerable, a remarkable degree of selectivity can be obtained. This is illustrated by Table 2 (Graham-Bryce, 1987).

Table 2

Toxicity of representative insecticides against different insect species (values are LD50 ng/insect)

	Anopheles stephensi	Phaedon cochleariae	Glossina austeni	Stomoxys calcitrans	Musca domestica	Apis. mellifera
Endosulfan	12	-	5	17	-	7100
DDT	54	520	90	118	38	3900
Dimethoate	19	190	40	13	11	100
Malathion	8	-	>150	-	530	270
Biores-methrin	1	4	3	-	5	6

For the most part, the protocols for such direct tests of lethality (topical application, feeding tests, exposure to treated solution etc.) are well established and accepted. Their results are generally unambiguous. They may be supplemented by more subtle indicators such as the fish growth test developed by Crossland (1988). However, certain areas of uncertainty remain. For example the methodology for evaluating effects on soil microorganisms remains unresolved.

Greaves (1987) pointed out that the literature contains thousands of papers on the effects of pesticides on the composition and activity of the soil microbial population but in many cases it is difficult to draw confident conclusions because of soil variability and associated lack of reproducibility. Such observations underline the need for establishing what is a significant effect, which is particularly problematical in the case of effects on micro-organisms.

This in turn presents the authorities with difficulties which have been approached in different ways. In FRG a series of defined criteria have been adopted. These specify further tests which may be required on the basis of duration and magnitude of effects on soil respiration or dehydrogenase activity in initial investigations (Anderson et al, 1987). In the US and The Netherlands, for example, requirements for soil microflora information have been withdrawn, except for data on soil nitrification for soil incorporated chemicals (Greaves, 1987; van Doorn, 1987).

TOXICITY TESTING IN THE FIELD: THE IMPORTANCE OF EXPOSURE.

For many species and for the assessment of community structures, direct testing on laboratory maintained cultures is clearly impracticable. From an early stage, direct application as outlined above has been supplemented by outdoor toxicity testing, for example measurement of effects on honeybees (Felton et al, 1986) and on earthworms other soil fauna in small outdoor plots. Comprehensive assessment of species abundance may give insight into ecological affects (discussed more fully below). Such tests also bring into play some of the important factors which determine bioavailability.

It is a cardinal principle of ecotoxicology that hazard in practice is a function of both intrinsic toxicity and the exposure to the chemical experienced by receiving species. Exposure is a function of concentration and the period over which that concentration is experienced. The relevant concentration is, of course, the bioavailable concentration which may differ considerably from that measured on a bulk basis. Assessment of exposure must therefore take into account the processes of dilution/dispersion, partition/sorption and degradation by chemical photochemical and microbial processes. The important influence of exposure is well illustrated by the pond studies described by Crossland and Wolff (1988).

The pyrethroid insecticide cypermethrin provides a good example of how exposure can have a profound effect on hazard. This compound has a high acute toxicity to both fish and aquatic invertebrates, but is readily metabolised and has a low value for Henry's law constant and a high octanol/water partition coefficient. On the basis of laboratory data alone it is difficult to assess the potential acute hazard in practice. Studies in which experimental ponds were oversprayed at rates corresponding to those used in agriculture resolved the uncertainties. Only 5% of the applied cypermethrin was found dissolved in the pond water: the remainder was either lost to the atmosphere during spraying or sorbed onto vegetation and sediment, consistent with the physico-chemical properties (Crossland, 1982). Such studies underline two familiar points: firstly, that firm conclusions about potential impact in the environment cannot necessarily be drawn from acute toxicity measurements, which can only be regarded as tier 1 tests that draw attention to aspects requiring fuller consideration and secondly, that presence in the environment (as indicated by measurements of bulk concentration) is not in itself a cause for concern.

ASSESSMENT OF EFFECTS ON FUNCTION

Certain laboratory and small plot outdoor tests investigate what are considered to be key environmental functions, especially in relation to soil. Indeed, as discussed briefly above, tests on nitrification, respiration etc. have been a central element in approaches to evaluating effects on microorganisms, not only because they are considered to represent crucial indicators of the well-being of soil but also because measurements of direct toxicological effects are so problematic. Similarly in the case of soil arthropods there has been a move away from species structural analysis towards the functional approach of leaf litter degradation studies (Anon, 1986) which assess the functioning of a vital feature of soil: the degradation and recycling of organic debris. The effects which damage to these processes can have were revealed in the past in soils where heavy metal fungicides were used, particularly in vineyards and orchards which were sprayed repeatedly. In extreme cases the activity of the soil fauna was so severely disrupted that organic matter accumulated as a mat at the soil surface (Graham-Bryce, 1973). Certain synthetic organic pesticides may have similar effects: for example accumulation of plant debris was observed in New Zealand after applications of isobenzan (Kelsey and Arlidge, 1968). In terms of overall function, however, it should be noted that even in the badly contaminated orchard soils, established deep rooted trees continued to grow well.

From the agricultural standpoint, the most obvious functional aspect is indeed soil fertility. Various investigations have been undertaken in attempts to evaluate if soil fertility is being adversely affected, particularly by long-term repeated applications of pesticides. For example, plots treated annually with herbicides were maintained for some years at the Weed Research Organisation, Oxford while at Rothamsted Experimental Station, "Chemical Reference Plots" were established in the 1970's to evaluate the effects of annual treatments with representative pesticides singly and in all combinations.

The chemicals chosen were the fungicide benomyl, the insecticide chlorfenvinphos and the nematicide aldicarb incorporated into the seed bed and the herbicide chlortoluron applied as a pre-emergence spray to continuous barley given optimum fertiliser and a hormone weedkiller. Disease, residues and yield were measured annually and other aspects such as effects on soil micro-organisms determined at longer intervals (Briggs, 1975). The design and execution of such experiments can often give rise to much debate, for example over the choice of treatments. However they should help to reveal any major threats to soil fertility and have largely been reassuring on this score.

EVALUATION OF ECOLOGICAL AND INDIRECT EFFECTS

As the methodology for assessing direct effects has matured, allowing the early rejection of candidate pesticides likely to give problems from direct toxicity, attention has turned increasingly to indirect effects and ecological aspects. That this involves substantially greater complexity and uncertainty, both in methodology and interpretation, is obvious.

Factors to be considered include the influence of lethal and sub-lethal effects at the population, community and ecosystem level, the significance of pesticide impact in relation to other factors determining population dynamics, the difficulties of experimental design, especially with respect to replication and plot size, questions of how far individual results may be generalised and the definition of what constitutes significant damage to the overall ecosystem. In most cases the detailed ecological data are insufficient to allow rigorous criteria to be defined and it is difficult to avoid subjective judgement.

Certain important ecological aspects can be clearly studied and identified. For example pest resurgence and alteration of predator/prey balances give a definite indication of an adverse impact on ecological relationships. Investigation of such effects in the field is increasingly a component of the evaluation of pesticides for use in orchards. Work on control of the spider mite (Panonychus ulmi) on apples summarised by Cranham et al (1984) provides a good illustration of the principles.

The most important predator of P. ulmi is the phytoseiid mite Typhlodromus pyri which is capable of regulating the pest to non-injurious levels. Pesticides used as part of the intensive crop management programmes on apples are the most important factor likely to disrupt favourable relationships between predator and prey. Cranham and colleagues investigated insect pest management schemes aimed at minimising such damage. Pesticides used in orchards were classified according to their relative effects on predator and prey as (i) disruptive, leading to increase in spider mites (ii) suppressive, to both species (iii) neutral, non-disruptive (iv) corrective, reducing spider mites with little or no effect on the predators. Table 3 illustrates the application of this classification.

Table 3

Pesticides used in U.K. orchards classified according to their relative toxicity to organophosphate resistant T. pyri and P. ulmi (abbreviated from Cranham et al, 1984)

Category	Toxicity to		Pesticide
	T. pyri	P. ulmi	
Disruptive	high	high	Permethrin, cypermethrin, deltamethrin, pirimiphos-methyl
Suppressive	moderate	moderate	Binapacryl, dinocap, wettable sulphur
Neutral	no toxicity	no toxicity	Bupirimate, fenarimol, triadimefon, captan, dodine, dithianon, diflubenzuron, pirimicarb
Corrective	low	high	Cyhexatin

Assessment of such properties can be difficult in routine evaluation, where the advantage of long-term access to appropriate experimental plots may not be available. Experimental design can be of crucial importance. This question was addressed by Brown (1988) in assessing effects of the insect growth regulant, flufenoxuron on the beneficial fauna of orchards. Two field experiments were conducted in adjacent blocks of apples in Southern France. A small plot replicated experiment was established in one half of a large abandoned orchard and a large plot unreplicated study carried out simultaneously in the other half. The first experiment was intended to provide data on the more sedentary taxa of beneficial arthropods.

The results of this first experiment demonstrate surprisingly few effects of any treatment on either pest or beneficial species, suggesting that the small plots were particularly vulnerable to recolonisation following treatment.

In contrast, in the large plots, effects of insect growth regulator treatments and a toxic organophosphate reference compound were in line with expectations from known toxicology. However, Brown underlines the need for careful interpretation of such results from unreplicated plots and for caution in extrapolating to other situations. Such studies indicate the need for more information about the mobility of different life-stages of beneficial species in order to estimate an appropriate plot size. Where it is not possible to achieve the desired plot size, unreplicated experiments may provide information which would draw attention to possible major effects on beneficial species, but cannot indicate quantitatively the likelihood of such effects.

Such examples illustrate some of the difficulties in studying even well defined "ecological" questions. The problems are multiplied when the objective is to assess the more general ecological impact of pesticides. In a widely quoted review, Bunyan and Stanley (1983) concluded that there was little evidence to indicate that the survival of individual species was threatened by the direct effects of pesticides used on British farmland. Two notable approaches demonstrate the considerations involved in seeking more detailed information.

The Cereals and Gamebirds Research Project of the Game Conservancy, designed to provide information on effects of pesticides on farm wildlife has involved annual sampling of populations of many species in 100 cereal fields for over 15 years (for a recent review, see Oliver-Bellasis and Sotherton, 1986). The study has provided much information and has for example, addressed the use of key indicator species and the value for game and wildlife of leaving headlands in cereal fields unsprayed. However it has proved difficult to distinguish the effects of pesticide usage from the overall effects of farming systems.

Somewhat later, benefiting from the experience gained at that stage from the work of the Game Conservancy, the Agricultural Development and Advisory Service (ADAS) initiated the ambitious and well-known Boxworth project with the original aim of developing suitable methodology for examining the ecological and economic implications of various pest, weed and disease management systems for cereals (Hardy, 1986).

Again, a wide range of ecological, economic and agronomic information has been collected. In the present context, however, aspects of the experimental design are of particular interest.

At an early stage, it was decided on the basis of faunal mobility, not to use small plots. A working farmland system, including whole fields and boundaries, was adopted to provide large treatment areas in which edge effects were reduced to a minimum. Because of inevitable resource limitations, this restricted the degree of replication that was possible. Steps were therefore taken to minimise variability other than that due to the treatments, for example by matching fields between different treatments for all non-treatment operations such as sowing date.

Even with the substantial resources deployed and the considerable thought which has gone into this project, it is accepted by those involved that it cannot provide definitive answers about the economic and ecological significance of pesticide use strategies. The approach demonstrates the unavoidable compromise in such investigations between the need for replication and the requirement for plots large enough to obtain unambigious effects. Critics point to the lack of statistical rigour in the results. However, the project is a test bed for methodology and serves to identify aspects requiring more detailed study in fully replicated trials, for example indicator species.

CONCLUSIONS

It is desirable that appropriate research on the nature of effects of pesticides continues, in order to identify optimum and cost-effective assessment techniques and to clarify the important factors and key species. As discussed, particularly for some indirect and ecological effects, satisfactory approaches have not been established and the central question of what constitutes a significant effect remains.

It is accepted that investigations such as the Game Conservancy and Boxworth Projects could not be applied routinely, despite their merits, because the resources required would be prohibitive, the methodology is uncertain and the results are not definitive. Indeed it is important to examine whether elaborate ecological studies for emerging pesticides will ever represent a worthwhile use of resources. It can be argued that at present the best approach to environmental assessment is the familiar tiered or stepwise method, referred to earlier and described by Brown (1986) which should reveal significant risks. This involves initial determination of physico-chemical properties and environmental fate, which will indicate potential exposure, together with acute and chronic toxicity tests on standard species in the laboratory and small plots. These may be extended as appropriate in the light of intended use patterns and pointers from the initial studies and would include more detailed population studies on key indicator species in the field. A final component is monitoring both of wildlife incidents, which may involve increasing use of biochemical and sub-lethal indicators (Walker, 1985), and of indirect effects in appropriate long-term experiments.

With the reassurance which comes from the increasing knowledge of fate and behaviour of pesticides in the environment, such a pragmatic approach already goes a long way to identifying the environmental and ecological impacts (such as interference with leaf litter degradation, pest resurgence and disturbance of predator/prey relationships) which are likely to be significant in relation to criteria such as those discussed in the introduction.

Looking to the future, some further refinement in the methods for evaluating conventional organic pesticides to overcome limitations discussed above can be expected. It must also be hoped that full advantage will be taken of experience acquired in developing evaluation methods and regulations for such materials when developing approaches to new contol agents, for example those derived from biotechnology.

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SESSION 7A

RECENT ADVANCES IN THE DEVELOPMENT AND USE OF BIOLOGICAL CONTROL AGENTS

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REGULATING THE RELEASE OF GENETICALLY MANIPULATED PEST CONTROL ORGANISMS IN THE UK

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ABSTRACT

Organisms used to control pests must be able to kill or interfere with the growth of pest species to be effective. Genetic manipulation will be used to improve these properties, either through an enhancement of aggressive characteristics or through modifications to alter persistence or formulation properties. With the exception of manipulations that reduce host range or viability, most genetic modifications could have the potential to produce environmentally undesirable organisms. Problems associated with regulating the release of such organisms are discussed.

INTRODUCTION

Recent developments in molecular genetics have altered completely our ability to exploit organisms because it is possible to modify an existing species genetically to produce progeny with new and desirable characteristics derived from unrelated species. The use of such gene cloning techniques offers us the opportunity to develop biological control systems much more quickly than at present - or would do so if we had a better understanding of how such organisms function, so that specific useful genes could be isolated and used to generate "improved" strains for commercial use.

Examples of the types of manipulation that would be useful are those that would enable inoculants to survive longer or kill susceptible species more rapidly. However, if water stress is a problem for survival how can an improved strain be produced unless the biochemistry and physiology of water stress are understood sufficiently well to suggest how particular genes may be altered or introduced? Similar problems exist for most theoretical objectives.

Despite limitations in knowledge of biological processes, progress is being made in developing modified pest control organisms and we can expect to see examples in the near future. This is because such systems of pest control are academically very interesting (see other chapters in this volume) and because there is a desire to reduce the use of pesticides. The exploitation of existing methods of biological control appears attractive because their use would be "natural" and thus should not cause harm to the environment. Unfortunately, in reality, most natural pest control processes are unreliable, slow and do not eliminate the pest, and thus need to be modified to produce practical and commercially attractive alternatives to chemicals. It is the duty of genetic manipulation advisory committees to determine if appropriate modifications can produce environmentally damaging species before such organisms are released into the environment.

U.K. REGULATIONS

These operate at two levels. One is a well established, mandatory, scheme run by the Ministry of Agriculture, Fisheries and Food (MAFF) to regulate pesticides. The other is a new, presently voluntary, scheme run by the Health and Safety Executive to screen all types of genetically manipulated organisms before they are released into the environment.

For the purposes of regulating pest control organisms the MAFF regulations (Anon 1986a) are clear if the organisms are recognized as pesticides, but are probably inadequate if organisms are produced and marketed without a clear understanding of their function. An example of the second type of organism would be bacteria that are fed to animals to reduce their susceptibility to disease (probiotics). If such organisms are not genetically manipulated and can not be described as pesticides their use and release into the environment is unregulated. There is some controversy at present whether the Wildlife and Countryside Act (Anon 1984a) includes microorganisms. If not, there appears to be a gap in the U.K. regulatory framework that needs to be filled. It is clear that the Bern Convention (Anon 1984b) of 1984 can be used to regulate the import of non-indigenous species, but this is probably unworkable with microoganisms because they are very widespread and non-indigenous biotypes are likely to be far more important than species. In an attempt to plug any gaps that might exist in regulating the release of organisms in the U.K. the Department of the Environment has just established an advisory committee to advise the Department on proposals for releasing organisms.

Regulating the release of genetically manipulated organisms is the responsibility of the Health and Safety Executive because it has been responsible for the Advisory Committee on Genetic Manipulation (ACGM) which controls laboratory and industrial work involving genetic manipulation. A Planned Release Subcommittee (which reports to ACGM) is responsible for assessing potential risks associated with the release of genetically manipulated organisms (Anon 1986b). This committee contains members of relevant government departments (Department of the Environment, Ministry of Agriculture Fisheries and Food, Department of Trade and Industry, Forestry Commission, Health and Safety Executive and Environmental Health Department) together with ecologists and other scientists with relevant skills. At present, seeking approval for the release of genetically manipulated organisms from this subcommittee is voluntary, but will become mandatory early in 1989.

The role of the Planned Release Subcommittee is to determine whether genetic manipulation has altered an organism so that its potential to cause harm to the environment is enhanced. This is done by eliciting a series of responses to questions about the organism, the genetic manipulation and possible environmental effects of the wild-type and manipulated derivative. These, plus any further information deemed necessary, are considered by the members of the Subcommittee and also the government bodies that they represent. Approval for release can only be given if all members and government bodies represented agree.

PARTICULAR PROBLEMS ASSOCIATED WITH PEST CONTROL ORGANISMS

An important criterion in assessing whether an organism should be released or not is that it will not harm other organisms in the environment. However, by definition, pest control agents are designed to control pests and will therefore harm them. A most important aspect for regulating such organisms must be that

they have clearly defined host ranges and that the host range is not altered accidentally by the genetic manipulation to produce organisms with novel and unwanted host ranges.

Unfortunately, for both regulators and gene manipulators, host range is not a clearly definable property and can almost never be defined precisely in biochemical terms which would enable a genetic modification to be assessable simply by analysis of known charges in the biochemistry of an organism. It appears likely, therefore, that for many years to come an assessment of environmental harm will depend upon laboratory tests with potential target species; a procedure that is time consuming and expensive. For "well known" organisms, such as Bacillus thuringiensis and the baculoviruses there is a history of large scale use of wild-type strains that can be used to facilitate regulatory processes. For totally new types of biological control agent it is hard to predict how difficult it will be to bring together adequate scientific information to demonstrate to regulatory bodies that the wild-type organism is safe, let alone a genetically manipulated derivative. In general the larger an organism is the lower the apprehension that it may become an environmental problem.

Gene manipulation offers scientists the opportunity to produce completely novel organisms with previously untested properties; for example baculoviruses that produce the B. thuringiensis toxin. Derivatives of natural biological control agents carrying toxin, or other rapidly acting genes, are attractive because they offer the opportunity to speed-up biological control. Important questions that must be asked by regulatory authorities are whether such organisms will have enhanced host ranges, how toxin production will affect the normal predator-host balance and whether the new genes will modify the persistence of the control agent.

Our ability to introduce genes into plants and animals introduces a completely new problem in relation to regulating pesticides. Is a potato plant which carries and expresses the B. thuringiensis toxin gene a pesticide? Should such organisms be subject to normal pesticide testing? If so how? Production of such toxins by plants or animals would inevitably lead to a persistence problem related to the period of growth of the organism. Low level production in some tissues or whole organisms, as well as expected rates in target tissues, will facilitate selection for toxin-resistant pest species. While this can be seen as largely a commercial problem, it could also have ecological implications for those species (if any) whose populations are controlled naturally by the same toxins. Particularly where manipulated organisms are able to mate with indigenous species there is concern about the spread of novel genes, and in the case outlined above how the inheritance of such genes may alter natural predator-host balances.

It should also be recognized that the introduction of genes to confer pest resistance into organisms eaten by man or animals will pose problems in relation to the nutritional qualities of such foods. Problems, such as this are to be considered by the recently formed Advisory Committee on Novel Foods and Processes.

CONCLUSIONS

It is clear that genetically manipulated pest control organisms will be constructed and used in the future. For many, based on well-known and studied organisms, regulation will be a relatively straightforward process. With others our incomplete understanding of biological control, ability to construct entirely novel combinations of genes, or other problems, will lead to regulatory problems that

may delay their use for considerable periods of time. There will be no substitute for developing a better understanding of biological control if we are to benefit from the opportunities offered by genetic manipulation.

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BIOLOGICAL CONTROL OF PLANT DISEASES: ACHIEVEMENTS AND PROSPECTS

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ABSTRACT

Several bacterial and fungal preparations are now available to farmers and growers to control plant diseases. There is much scope to expand the list, especially in the control of seedling and root diseases of horticultural and tree crops and in speciality crops such as mushrooms. Many agrochemical and biotechnology companies are now investing in disease biocontrol R & D, especially with a view to incorporating it into integrated crop management programmes. After the selection or genetic engineering of potential antagonists, development depends on understanding the mode of action of the agents and in carrying out a sequence of laboratory and field studies including toxicology.

INTRODUCTION

There is a long history of research on the biological control of plant diseases. Many of the early studies related only to the characterization of possible agents involved in the reduction of disease levels from so-called "suppressive soils". However, since the initial attempt by Hartley (1921) to control damping-off of pine seedlings by inoculation with antagonistic fungi there have been many attempts to control disease by inoculation. Much of this early work has been reviewed in the books by Baker & Cook (1974), Cook & Baker (1983) and Chet (1987), as well as several symposium volumes.

Implicit in many of the early studies was the recognition that antibiosis might be a mode of action, following the initiatives of Sir Alexander Fleming and the soil microbiologist Selman Waksman in the field of pharmacology. Some early studies were initiated by Dr W F Bewley and carried out by Dr E Grossbard at the Experimental Station of Cheshunt which was the forerunner of the Glasshouse Crops Research Institute and now the AFRC Institute of Horticultural Research (Littlehampton). The first reports appeared in annual reports of the Station between 1945-49 and subsequently published in the open literature (Grossbard 1952). Penicillium patulum was shown to have the potential to control damping-off diseases of tomato by producing the antibiotic patulin. It is perhaps fortunate that it was not developed further at that time because patulin had not been recognized as a mycotoxin and carcinogen. With the impetus today to produce inoculants rather than to exploit suppressive soils directly it is crucial that any agents which are developed are completely safe as well as being efficaceous. Indeed, there are more factors to consider in the development and registration of disease biocontrol agents than are necessary for the development of chemical agents.

Several chemical agents (e.g. methyl bromide) have become unacceptable environmentally because residues can be retained in the soil or transported through food chains. With some others (e.g. benomyl) disease resistance has

built-up. However, another major justification for the development of disease biocontrol agents is to extend the crop protection weaponry where no chemical agents exist. Many opportunities exist for minor uses where industry has found it difficult to justify the cost of R & D and registration.

In reviewing the general field of biocontrol research recently (Payne & Lynch 1988), it became clear that there has been considerably more development and commercial exploitation of microbial insecticides than of disease biocontrol agents. The R & D spend in the former area has been considerably greater than in the latter but perhaps also the 'right attitude' has not been adopted for disease biocontrol. The main purpose of this article will be to analyse the reasons for this and how such attitudes need to change.

ACHIEVEMENTS

On a small scale, a range of inoculants are already available to farmers and growers in some parts of the world (Table 1). The most successful in terms of a long market life and consistency of effect has been Peniophora gigantea which has been used against Heterobasidion (Fomes) annosum since 1963 (Rishbeth 1988). This is distributed as tablets or fluid suspensions to treat pine tree stumps. The antagonist seems to act simply by pre-emption (establishment before the pathogen) and by hyphal interference. The method has been used successfully over an area of about 62 000 ha, but it is restricted to use with Pinus species.

Agrobacterium radiobacter var tumefaciens strain K84 has been used since 1973 when it was first applied to bare root nursery stock or seed of stone fruit and roses to control crown gall. The disease is caused by Agrobacterium tumefaciens, A. rhizogenes and A. rubi (all biotypes of A. radiobacter) (Kerr 1980). The pathogenic strains have the tumour-inducing (Ti) plasmid. This plasmid is absent from strain K84 which has another large plasmid coding for the production of the bacteriocin, agrocin (an antibiotic active between closely related bacterial strains). Some problems have arisen because the plasmid coding for agrocin can be transferred to pathogenic recipients, in the presence of nopaline which is contained in gall tissue. This has caused a breakdown in the biocontrol system in some situations where disease incidence has been high, notably Greece. However, as a consequence of extensive scientific inputs in understanding both the physiology and genetics of the disease and its control over many years an antagonist strain with a deficient plasmid-transfer system has been engineered (Jones et al. 1988) and tested (A. Kerr, personal communication). It is hoped that this will replace strain K84.

Trichoderma spp. have been investigated as biocontrol agents on a wide scale (Papavizas 1985) but the only commercial development has been Binab T product aimed at various tree diseases. The claims of disease control by the producer company are fairly wide, and, whereas efficacy has been proven in many situations, it would be surprising if consistency is obtained for diseases such as Dutch elm. Extensive Eastern European interest in Trichoderma is aimed at protection of horticultural crops to reduce import costs of chemicals, and, in Bulgaria alone, eleven laboratories started producing Trichoderma two years ago for distribution to growers for the control of Botrytis cinerea on strawberries as well as a range of other diseases (E. Mirkova, personal communication). It appears that in the Soviet Union there are very many more outlets for this inoculant. Extensive

TABLE 1

Availability of disease biocontrol agents to farmers and growers

Antagonist	Product name/supplier	Target	Host crop
BACTERIA			
Agrobacterium rhizogenes strain K84	Diegall (Fruit Growers Chemical Co., NZ) Galltrol (Agbiochem Inc., California, USA) No gall (Root Nodule Pty Ltd, Australia) Norbac 84C (New Bio-Products, USA)	*Agrobacterium radiobacter* biotypes (Crown gall)	Fruit trees, roses
Pseudomonas fluorescens	Dagger G (Ecogen Inc., PA., USA)	*Pythium*, *Rhizoctonia*	Cotton (and others)
Pseudomonas fluorescens	Conquer (Mauri Foods, Australia)	*Pseudomonas tolaasii* (Bacterial blotch)	Mushrooms
FUNGI			
Fusarium oxysporum (non-pathogenic)	Japanese Government	*Fusarium oxysporum*	Sweet potato
Peniophora (*Phlebia*) *gigantea*	P.g. SUSPENSION (Ecological Laboratories Ltd, UK)	*Heterobasidion* (*Fomes*) *annosum*	Pine
Pythium oligandrum	Polygandron (Vyzkummy ustav rostlinne vyroby, Czechoslovakia)	*Pythium*	Sugar beet
Trichoderma spp.	Trichodermin (Bulgarian & Soviet Governments)	*Botrytis*, *Pythium*, *Verticillium*, *Sclerotinia*	Fruit, vegetables
Trichoderma viride	Binab T (Bio-Innovation AB, Binab, Sweden)	*Armillaria mellea* (honey fungus)	Trees
		Ceratocystis ulmi (Dutch elm)	Elm
		Chondrostereum purpurpeum (Silver leaf)	Fruit trees and eucalyptus
		Heterobasidon annosum (stem and root rot)	Pine

interest in Europe, Israel and North America, scientifically and commercially, makes Trichoderma a likely candidate for successful exploitation in the near future. Certainly we have found it easy to establish the organism on cereal straw in the field (Magan et al. 1988). Added to this is the potential of this inoculant as a stimulator of plant growth (Baker 1988), a phenomenon confirmed in our own laboratory.

The most common rhizosphere organisms are pseudomonads, especially Pseudomonas fluorescens. With their diverse metabolic properties and ecological 'fitness', there has been considerable interest in their biocontrol properties. In 1987 'Dagger G' became the first product to reach the market. Another product is available in Australia for use against bacterial blotch on mushrooms and we have also found useful isolates against this target (Fermor & Lynch 1988) which have been successful in a long series of cropping trials.

The Fusarium oxysporum product in Japan, based on the work of Ogawa & Komada (1985), which acts as a cross-protectant, is used widely by growers. This is made available without charge by the Japanese government and appears to have reduced the incentive for the Japanese agrochemical industry to develop saleable products. Polygandron, based on the work of Veseley & Hejdanek (1984) has been available to growers in Czechoslovakia, but appears to have limited success at present.

TARGETS

Some targets for disease biocontrol are listed in Table 2. Maximum control of the cropping environment, short cropping period (to allow for maximum number of field trials) and minimum duration of necessary protection in the cropping cycle are important factors which are likely to improve the potential success rate of biocontrol agents. Mushroom diseases, following the success with blotch control, are therefore ideal targets, especially as the crop is so valuable (farm gate value of about £140 million in the UK alone, which is about the fourth largest world producer). In contrast, control of take-all in wheat which is of even greater economic significance, is at the other end of the spectrum in satisfying the criteria for likely success.

TABLE 2

Some targets for disease biocontrol

1	Dry bubble disease of mushrooms (Verticillium fungicola)
2	Damping-off of horticultural crops (Pythium spp., Rhizoctonia spp.)
3	Fusarium wilts and rots
4	Grey mould of horticultural crops (Botrytis cinerea)
5	Sclerotium-forming pathogens (Sclerotinia spp., Sclerotium spp.)
6	Take-all of wheat (Gaeumannomyces graminis var tritici)
7	Bacterial soft-rots of vegetables, especially potatoes (Erwinia caratovora var atroseptica)
8	Fireblight of apples and pears (Erwinia amylovora)
9	Virus diseases carried by fungal vectors

Bacterial agents are favoured organisms as inoculants for the biological control of take-all (Gauemannomyces graminis var tritici). At Washington State University, in work supported by Monsanto, Pseudomonas fluorescens is preferred (Cook 1988), whereas at Bristol University in association with ICI, Bacillus spp. are preferred (Capper & Campbell 1986). None has yet reached the market place and entry time could well depend on the success or failure of products such as 'Dagger G'.

It should be recognised that there are few soil-acting fungicides against root diseases. Even though there is considerable opportunity for biocontrol agents in this area, damping-off diseases (where action is necessary over a much shorter time-frame) are perhaps better goals for companies aiming at getting products to the market place rapidly.

Sclerotial pathogens are active against a wide range of horticultural crops and these appear good targets for biocontrol, especially if the antagonist can destroy the sclerotia themselves and prevent carry-over to the next crop. Pathogens epiphytic on leaves are likely to present major challenges for antagonist establishment because the water potential on the leaf surface frequently drops below that which is suitable for many, otherwise potentially useful, antagonists.

Fusarium wilts and rots are extremely widespread and common, especially in tropical climates. Included amongst the crops here should be, for example, Panama disease of bananas (Fusarium oxysporum f. sp. cubense) because there is increased disease incidence in varieties which have been resistant.

The list excludes nematodes as targets. It includes however agents which could be used indirectly in the control of viruses by restricting the fungal vectors on which they are carried such as Polymyxa betae which carries beet necrotic yellow vein virus (BNYVV) causing "rhizomania" ("root madness") of sugar beet (Brunt & Shikata 1986).

ANTAGONISTS

A range of antagonists have been isolated and evaluated. Table 3 is a catalogue compiled from Cook & Baker (1983) and Wood & Way (1988) with my evaluation of those showing most promise. There is however a vast number of potential antagonists waiting to be developed for specific needs or, if necessary, genetically modified for enhanced activity.

COMMERCIAL INTERESTS

A wide range of companies, large and small, from different backgrounds, or having been set up specifically for the purpose, are now making investments into disease biocontrol research (Table 4). Agrochemical companies are, of course, ideally placed to include biocontrol options in integrated crop management programmes. Much work in biocontrol is however at a pre-competitive stage, especially mode of action studies and it would make sense for consortia of companies to supplement relatively meagre public spending in these areas.

TABLE 3

Potential antagonistic genera

BACTERIA	*Agrobacterium, Alcaligenes, Arthrobacter, *Bacillus, Bdellovibrio, *Enterobacter, Erwinia, Hafnia, *Pseudomonas, Rhizobium, Serratia, Streptomyces
FUNGI	Ampelomyces, Ascocoryne, Chaetomium, *Coniothyrium, *Fusarium, Gaeumannomyces (hypovirulent), *Gliocladium, Glomus, Hansfordia, Hyphochytrium, Laetisaria, Leucopaxillus, *Microdochium, Myrothecium, *Penicillium, *Peniophora (Phlebia), Phialophora, Pisolithus, Pythium, Scytalidium, Sphaerellopsis, *Sporodesmium, *Talaromyces, Teratosperma, *Trichoderma, Trichothecium, Tuberculina, *Verticillium

*; genera considered to show greatest promise

TABLE 4

Companies with active interests in disease biocontrol

Company	Country
Advanced Genetic Sciences	USA
Agricultural Genetics Company Limited	UK
Agrobiochem	USA
Allelix	Canada
Bio-Innovation AB Binab	Sweden
Biotechnology General	Israel
Ciba-Geigy	Switzerland & USA
Eastman-Kodak	USA
Ecogen	USA
Ecological Laboratories Limited	UK
Fermenta Plant Protection	USA
FMC	USA
Fruit Growers Chemical Company	New Zealand
WR Grace	USA
ICI	UK
Mauri Foods	Australia
Makhteshim Agan	Israel
Monsanto	USA
Mycogen	USA
New BioProducts	USA
Orsan	France
Philom Bios	Canada
Plant Genetic Systems	Belgium
Ricerca	USA
Root Nodule Pty	Australia
Shell International	UK & Netherlands

MODE OF ACTION

With chemicals used against plant diseases, antibiosis is usually the mode of action, whether by striking the target organism directly or by prior translocation. In contrast, biocontrol agents can act against the pathogen by a variety of mechanisms, including antibiosis (Table 5). It is impossible to review evidence here for modes of action but considerations are available elsewhere (Whipps 1986, Lynch 1987). These modes of action are not necessarily mutually exclusive and successful antagonists might exhibit more than one mode of action. In order to optimise disease control, it is important to understand the mode(s) of action of the antagonist so that the effective attribute(s) in the organism can be stabilised to give consistency of effect (or even elevated, by genetic manipulation, if suitable gene products can be identified).

TABLE 5

Some potential modes of action in disease biocontrol

1	Competition for active sites/substrates
2	Antibiosis
3	Lytic enzymes
4	Physical restriction
5	Cross-protection
6	Ionophore production
7	Growth stimulation

If an organism produces antibiotics or lytic enzymes, it is reasonable that it should be treated, for registration and trials clearance, in exactly the same way as a chemical agent. However, the picture is less clear when the mode(s) of action are other than these, especially if the organism that is being introduced into the environment is indigenous to that environment and has not been genetically manipulated. For example, if simple competition is the sole mode of action, the practical challenge is to elevate the population of the antagonist against the pathogen in a niche such as the root surface. Registration should then perhaps be treated more leniently, provided the antagonist of choice does not present any health risk.

EXPLOITATION

Generally speaking, the R & D on disease biocontrol agents has been concentrated on selection procedures and, to a lesser extent, mode of action. Herein lies the likely explanation as to why the considerable body of research on biocontrol has not as yet resulted in many successful products. If such agents are to be commercially exploited, many other factors need to be considered (Table 6). Realistically, each of these factors could take as long to investigate as the selection procedure itself, but it is important to generate R & D programmes where factors such as toxicology and shelf-life are investigated alongside the more fundamental aspects of the R & D programme. Industry should provide the driving force

for the extended spectrum of research and especially development activity. These diverse interdisciplinary approaches are at best likely to be only marginally quicker and less expensive to study than the approaches needed to evaluate potential chemical agents and therefore it is crucial that patentability of organisms or processes is investigated at an early stage to protect the technology. If the antagonist itself is to be protected, DNA fingerprinting of strains would certainly be helpful in preventing piracy.

It has often been found that consistent results have been obtained in successive glasshouse trials but field trials have then given highly variable effects of inoculation. This is where multi-disciplinary approaches, especially with co-operating soil scientists and crop physiologists, are needed to evaluate the boundary conditions of efficacy. This would then lead to effective recommendations being made on the labels of products, in a similar manner to that already made for many chemicals.

TABLE 6

Factors in the development of disease biocontrol agents

1	Selection
2	Mode of action
3	Fermentation/production
4	Delivery/formulation
5	Side effects/toxicology
6	Registration
7	Genetic stability (phenotypic and genotypic)
8	Strain improvement
9	Patent protection/fingerprinting
10	Shelf-life
11	Compatability with existing agrochemicals
12	Environmental conditions/soil types
13	Crop physiology/agronomy
14	Marketing/profitability

CONCLUSIONS

There appears to be a very bright future for disease biocontrol agents, provided industry's needs are recognized (Scher & Castagno 1986, Jutsum 1988, Lethbridge 1988). Whereas industry is now putting increasing amounts of funds into investigating disease biocontrol, it is essential that public funding is sustained and industry support is increased to investigate the fundamental scientific factors governing efficacy, mode of action and safety of released organisms, be they genetically engineered or wild-type organisms. It seems most unwise to attempt to run to the market place too quickly with disease biocontrol agents; research is needed first to demonstrate that biocontrol is a credible means of reducing chemical inputs into the environment, and of enhancing the reliability of integrated crop protection schemes.

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DEVELOPMENT OF 'NOLO BAIT' (NOSEMA LOCUSTAE) FOR THE CONTROL OF GRASSHOPPERS AND LOCUSTS

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ABSTRACT

Research and development on 'NOLO Bait', a formulation of the microsporidian Nosema locustae Canning for the control of grasshoppers and locusts, is described. Recent advances in large scale in vivo production of Nosema have made possible its commercial application in the United States for control of economic infestations of grasshoppers. Two field trials in 1987 showed a 58-79% reduction in density three weeks after application of 1.1 kg product/ha to moderate populations of rangeland grasshoppers. Where the product was applied at a third location to a high-density population of adult grasshoppers, no significant reduction was seen. The surviving populations showed a 10% infection rate at the end of the season.

INTRODUCTION

'NOLO Bait' (hereafter referred to as 'the product') is a formulation of the grasshopper and locust parasite Nosema locustae. N. locustae was first described by E. Canning (Canning 1953) from laboratory-infected African migratory locusts (Locusta migratoria migratorioides R. & F.) and has since been reported to occur naturally in grasshoppers or locusts from Canada (Smith 1965), the Soviet Union (Issi and Lipa 1968), the United States (Henry 1969a), and India (Srivastava and Bhanotar 1985). Currently over 95 species of grasshoppers and locusts from North and South America, Africa, Australia, and India have been shown to be susceptible.

N. locustae is a microsporidian which is classified as an obligate intracellular entomophilic protozoan that produces resistant unicellular spores for persistence in the environment. The spores, after ingestion by a host, extrude a coiled polar filament that penetrates the mid-gut of the host insect and injects it sporoplasm into a host cell. It grows in host tissues and new spores, with a chitinous wall, are produced approximately two weeks after initial penetration. N. locustae primarily infects fat body tissue in grasshoppers and locusts and competes with the host for this energy reserve. Typically, grasshopper populations in North America are reduced 20-30% within 3 to 6 weeks and 30-70% 6-9 weeks after application of N. locustae bait (Henry 1971, Henry et al. 1973, Henry and Oma 1974, Ewen and Mukerji 1979, Johnson 1987). Of the surviving grasshoppers, typically 20-50% are infected. If a grasshopper is infected, then development (Henry 1969b), mobility (Canning 1962), fecundity (Henry and Oma 1981, Ewen and Mukerji 1980) and food consumption (Oma and Hewitt 1984, Johnson and Pavlikova 1986) are substantially reduced. Grasshoppers or locusts that are debilitated

by N. locustae are more susceptible to cannibalism (Henry, 1969b) especially during moulting which is lengthened by infection. Because Nosema is spread by spore ingestion, cannibalism is a main route of transmission through a population.

It has been shown that an introduced Nosema infection in a population persists at a level higher than the normally low enzootic natural infection at least I year after the initial inoculation (Ewen and Mukerji 1979) Nosema spores are carried in the egg pod or on the surface of eggs. Johnson (1987) found 75% of the foam plugs from Melanoplus bivittatus had mature spores present, but only 9% of the eggs carried spores. Germida et al. (1987) found that low levels of Nosema spores persist in soils for up to 3 years, but not on vegetation from treated fields. It is concluded that transmission to the next generation depends primarily on overwintering spores in the matrix of a foam plug, in soil, and in cadavers. However it is doubtful that significant transmission within the egg (transovarian) occurs with Nosema locustae. Infection of second generation grasshoppers may help control grasshopper populations for more than the season of use.

'The product' is produced by growing Nosema locustae in a selected strain of grasshoppers. The strain is tolerant of a heavy infection by N. locustae and produces abundant spores. Spores are harvested, concentrated, purified and formulated onto wheat bran flakes, and then packaged according to market requirements. Currently, shelf life of formulated product is only about 13 weeks at room temperature, but our research indicates that this can be substantially improved. Shelf life of frozen Nosema spores is at least three years, so inventory of the active ingredient is not a problem.

'The product' and the concentrate 'NOLO BB' have been registered by the United States Environmental Protection Agency for use in suppressing grasshoppers. A registration package has been submitted to Agriculture Canada for approval in that country. No adverse response has been shown in any of the test species studied as required by the Environmental Protection Agency or Agriculture Canada for registration of a biological insecticide. Honey bees (Menapace et al. 1978) and the silkworm Bombyx mori (Raina et al. 1987) are not susceptible to N. locustae. Although little research has been done concerning the susceptibility of insect parasites and predators of grasshoppers to N. locustae, it is not expected that they are susceptible because there is an apparently high specificity of N. locustae towards Orthopterans, primarily Acrididae (grasshoppers and locusts) and some crickets.

Timing of product application is important in order to maximize density reduction of North American grasshoppers. Grasshoppers are most susceptible when young. Optimal timing is when the majority of grasshoppers are 2nd to 4th instar. If Nosema is applied at the adult stage, little mortality is obtained, although infection rates can actually be higher than for an earlier application (Johnson 1987).

The best way of ensuring good Nosema infection in a grasshopper population is to achieve complete coverage of the treatment area. It has been shown, in 4 ha plots that 50% coverage with Nosema bait resulted in infection similar to 100% coverage (Henry and Oma 1981). However, it has been the experience of company personnel that early density reduction on large treatment areas is reduced if full coverage is not used and this application method is not recommended for population suppression or control.

'The product' is recommended to be used where long-term suppression or control of grasshoppers is warranted. This need is most evident in large scale IPM (integrated pest management) programs. Since Nosema does not kill quickly, but produces a rather slow debilitating disease, the product is not recommended where quick control of adults is needed or high populations threaten a crop.

METHODS AND MATERIALS

In 1987, three large-scale government-sponsored IPM demonstration trials in the U.S., totalling about 18 500 ha, were conducted with 'the product'. In 1988, government IPM trials covering about 27 000 ha were planned, but only 7 000 ha were actually treated because of the drought in the Western U.S. which substantially reduced grasshopper populations. Currently, only results of the 1987 trials are available.

'The product' was applied in early June at the rate of 1.1 kg/ha to rangeland typical of Western North Dakota and Eastern Montana. Application was made by air using a Brockwell Thrush with a modified granule spreader. Bridging of flakes in the plane's hopper was prevented by using a ram-air injection system installed at the top of the spreader which forced air into the bottom of the tank to fluff the bait. Within each solid block treatment area, 4 sample sites were established. An equal number of sampling sites in untreated areas were established adjacent to the treated area. Density sampling consisted of placing 0.25 sq. m rings every 10 m along two, 100 m transects at every sample site for a total of 80 rings/treatment. Counts were taken 1-2 days before treatment, then again at 3, 6, and 11 weeks after application. Also from each site at each sampling, at least 100 grasshoppers, if available, were taken for species identification and infectivity analysis.

RESULTS AND DISCUSSION

In the three test locations, the predominant grasshopper species included Melanoplus sanguinipes, Ageneotettix deorum, Aulocara elliotti, and Camnula pellucida. All species are economically important grasshoppers in the Western U.S.

At Beach, North Dakota, site of c. 1 640 ha, the average number of grasshoppers/m^2 for both the control and treatment plots at pre-treatment were moderate at 7.4 and 6.3 per sq. m, respectively. Grasshoppers were 3rd-4th instars.

Table 1 shows the results at 3 and 6 weeks post-treatment. The 3 week count showed that the grasshopper population was still increasing at the time of treatment, but that 'the product' produced a significant 57% reduction in density. At 6 weeks post-treatment, it had reduced the population a significant 79% compared to the pre-treatment density.

The second location of c. 9 430 ha was at Watford City, North Dakota. A moderate population of 4th-5th instar grasshoppers was present at the time of treatment. Table 2 shows that at 3 weeks post-treatment, a significant 79% reduction in population density was recorded compared to the pre-treatment density.

TABLE 1

Mean number of grasshoppers per m^2 in controls and after treatment with 1.1 kg product/ha at Beach, North Dakota, 1987. (c. 1 640 ha treated)

Weeks after application	Treatment	
	Control	Product
0 - (Pre-Treatment)	7.4	6.3
3	10.5	2.7*
6	7.7	1.3*

* Differs significantly from the control ($P \leq 0.01$; t-test)

TABLE 2

Mean number of grasshoppers per m^2 in controls and after treatment with 1.1 kg product/ha at Watford City, North Dakota, 1987. (c. 9 430 ha treated)

Weeks after application	Treatment	
	Control	Product
0 - (Pre-Treatment)	5.5	7.5
3	4.9	1.6*
6	3.5	1.0*

* Differs significantly from the control ($P \leq 0.01$; t-test)

The third site at Winnett, Montana encompassed an area of about 7 380 ha. Unlike Beach and Watford City, the grasshopper population was 90% adult. Densities were also quite high at 30/m^2 in the treated areas. At 3 and 6 weeks post treatment, treated populations declined slightly more rapidly than in the untreated areas although the differences were not significant.

TABLE 3

Mean number of grasshoppers per m^2 in controls and after treatment with 1.1 kg product/ha at Winnett, Montana, 1987. (c. 7 380 ha treated)

Weeks after application	Treatment	
	Control	Product
0 - (Pre-Treatment)	30.8	33.1
3	18.6	14.7
6	8.5	7.0

Infection levels at the end of the season ranged from 8 to 19% (mean c. 10%) in the various treated sites at the three locations. Population densities and Nosema infection are being monitored during the 1988 grasshopper season and indications are that, at Beach, (the site of significant Nosema density reduction in 1987) populations in the treated area have been reduced compared to the untreated areas. The Watford City site shows low populations in both treated and untreated areas, presumably due to the effects of the 1988 drought and because populations were quite low at the end of the 1987 season. Indications are at the Winnett site where adult grasshoppers were treated in 1987 that there are no significant differences in low population densities between treated and control.

In summary, a growing awareness and concern over the routine and often environmentally damaging use of chemical insecticides for control of grasshoppers and locusts has increased the need for an alternative biological control. The standard approach to controlling grasshoppers and locusts has been one of crisis management instead of trying to prevent or manage outbreaks. The chemical eradication approach has not worked as evidenced by recurring locust and grasshopper plagues throughout the world. 'NOLO™ Bait' formulation of N. locustae offers the opportunity to either prevent serious outbreaks by establishing the parasite in the population early in its development phase or suppressing the population later as part of an integrated pest management program which also judiciously employs chemical sprays or baits. The fact that N. locustae is a specific parasite of grasshoppers, locusts, and a few related Orthopterans and not harmful to man, wildlife, and the environment should be a strong incentive for grasshopper and locust control agencies to learn how to use this biological control agent to everyone's advantage.

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TWO MICROORGANISMS FOR THE BIOLOGICAL CONTROL OF PLANT PARASITIC NEMATODES

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ABSTRACT

Environmental and health problems associated with nematicides have lead to the withdrawal of some products from the market and increased concern about those still in use. Such problems have focused attention on the development of other methods, including biological control for the management of nematode pests. In this paper some factors in the development of two organisms, *Verticillium chlamydosporium* and *Pasteuria penetrans* as biological control agents for cyst and root-knot nematodes are discussed.

INTRODUCTION

Two microorganisms, a bacterium *Pasteuria penetrans* and the fungus *Verticillium chlamydosporium*, have been studied at Rothamsted to determine their potential as biological control agents for cyst (*Globodera* & *Heterodera*) and root-knot (*Meloidogyne*) nematodes. *V. chlamydosporium* is one of the fungi involved in the natural control of cereal cyst nematodes in cereal monocultures in N. Europe, and is important in the suppression of several other cyst nematode pests. *P. penetrans* has been considered effective in controlling populations of root-knot nematodes in vineyards in S. Australia and in vegetables in W. Africa (see Kerry 1987). However, such natural control has developed fortuitously in crops grown in monocultures, is slow to establish in field soils and has so far proved difficult to manipulate. The future development of biological control for plant parasitic nematodes is likely, therefore, to depend on the development of techniques for introducing organisms into soil. This paper discusses some factors involved in the development of biological control agents for nematodes and contrasts the potential of *P. penetrans* with that of *V. chlamydosporium*.

HOST RANGE

V. chlamydosporium has been isolated from eggs of five species of cyst nematode, including those attacking soybeans and potatoes, and from eggs of *Meloidogyne incognita* and *M. arenaria*. Spores of *P. penetrans* adhere to the cuticles of a wide range of plant parasitic nematodes but individual populations of the bacterium are often highly specific; different populations of the same nematode species can vary considerably in their susceptibility. Such specificity would complicate commercial development and limit potential markets.

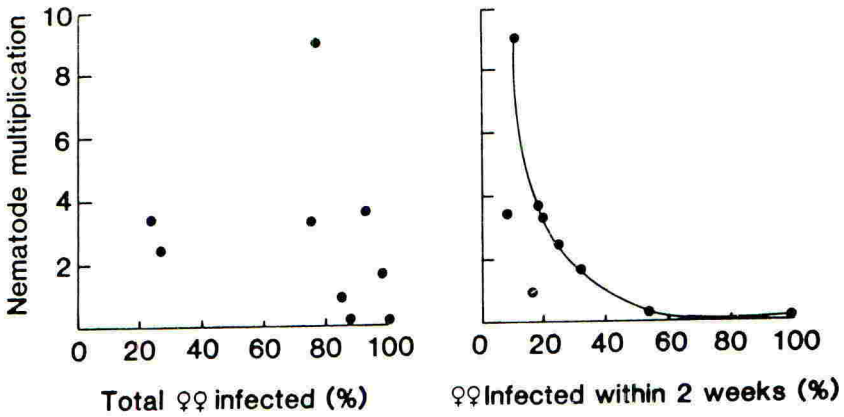
STAGE OF NEMATODE ATTACKED

V. chlamydosporium does not produce adhesive spores and only the sedentary stages (females and eggs) of cyst and root-knot nematodes are susceptible. Infection is by hyphal penetration and most parasitism occurs whilst the nematodes are still attached to the root.

A pot test was set up to compare the efficacy of nine isolates of the fungus mixed in soil as a 1% w/w sand-bran (1:1) inoculum for the control of the beet-cyst nematode, *Heterodera schachtii*. Multiplication of the nematodes was not related to the proportion of females colonised. However, there was a clear relationship between control and the proportion of young (<2 wk-old) females infected (Fig. 1). Early infection of females reduces fecundity and increases the proportion of eggs infected.

FIGURE 1.

Relationship between multiplication of *H. schachtii* on oilseed rape and the infection (%) of all nematode females, and those <2 wk old, by nine isolates of *V. chlamydosporium*.
(Nematode multiplication = $[P_f/P_i]$ where P_i is the number of eggs/g soil before planting and P_f is the number after harvest).



As *V. chlamydosporium* attacks nematodes after they have invaded the roots, applications of the organism are unlikely to affect crop yields significantly although nematode multiplication may be prevented. Therefore, the fungus acts in much the same way as a resistant cultivar and in heavily infested soil may need to be used with a nematicide to reduce yield losses.

P. penetrans produces spores that adhere to the cuticle of active nematodes including the infective second-stage juveniles of cyst and root-knot nematodes in soil. Most studies have been with *Meloidogyne* on which the spores germinate after the nematode has penetrated the root. However, spore-encumbered juveniles may be less able to invade roots (Table 1). In laboratory tests the invasion of tomato roots by *M. incognita* was significantly ($P < 0.05$) reduced if juveniles were encumbered by 15 or more spores and if 1000 or 3000 nematodes were added around the root system. Presumably, at these densities juveniles bearing spores were less able to compete for invasion sites than healthy ones. Infected females that establish in roots rarely produce any eggs. Hence, applications of *P. penetrans* may reduce nematode invasion and damage to crops and prevent nematode multiplication.

TABLE 1

Effect of nematode density, and *P. penetrans* spore burden on the invasion* of tomato roots by second-stage juveniles of *M. incognita*.

(*Figures given as % reduction in invasion compared with that for uninfected nematodes)

Number of juveniles added to soil around root system	*P. penetrans* spores/juvenile		
	5	15	25
500	-8	42	20
1000	22	72	80
3000	49	75	86

(From Davies *et al.* 1988)

V. chlamydosporium may affect the hatch of nematode eggs (Morgan-Jones *et al.* 1983), but neither this fungus nor *P. penetrans* appear to produce toxins during their parasitic phases. Isolates of each organism tend to differ greatly in their virulence and other important characteristics. It is therefore essential that they are carefully screened to ensure the selection of the most promising strains.

IN VITRO GROWTH

It is generally considered that the ability to grow and sporulate in submerged liquid cultures would be essential for the commercial development of these biological control agents. *V. chlamydosporium* grows readily on a range of standard solid and liquid media, although few chlamydospores are produced in the latter (Kerry *et al.* 1986). Up to 10^7 conidia/ml were produced in shaken cultures of Czapek Dox Broth.

P. penetrans has not been grown *in vitro* and currently spore inoculum can only be produced in infected nematodes. Approximately 10^8 spores/g air-dried root have been produced by adding infected *Meloidogyne* juveniles around tomato roots which are harvested when the bacterium has completed its development within the nematode female. This method, first described by Stirling & Wachtel (1980), may be used to produce sufficient inoculum for small plot tests but *in vitro* culture methods would have to be developed before *P. penetrans* could be used commercially.

COLONISATION AND SURVIVAL IN SOIL

V. chlamydosporium and *P. penetrans* have different strategies to ensure their survival in soil. The fungus must colonise soil and make contact with its sedentary nematode hosts. Hence, it has to compete with the indigenous soil microflora for scarce energy sources. To establish the fungus in soil a food source must be added to help it overcome such competition.

Recently, a selective medium has been developed which, for the first time, has enabled the proliferation and survival of *V. chlamydosporium* to be studied in soil (Kerry *et al.* 1989 in press). Hyphae and conidia of *V. chlamydosporium* grown in liquid culture were encapsulated in 1% (w/v)

sodium alginate containing 10% (w/v) kaolin or wheat bran and introduced into fallow soil at a 1% (w/w) dose rate. The fungus proliferated in soil only from granules containing bran as an energy source (Table 2). After only 1 wk, approximately 9 x 10^4 colony forming units (cfu) had developed, and, after 12 wk, 4 x 10^4 cfu still remained. These numbers are considerably in excess of those found in soils naturally suppressive to cyst nematodes, where approximately 10^3 cfu of *V. chlamydosporium* are present. However, it does not necessarily follow that if the application of alginate granules in the above test was reduced to a tenth that the numbers of cfu required for efficacy could still be established. Application rates can be reduced by applying the inoculum in rows rather than treating the entire soil volume and by reducing the size of the granule (1 mm). However, hyphal growth from alginate-bran granules in soil is limited (c. 1 cm in 4 wk) and different formulations might be more effective. The fungus has been successfully established in soil using inocula on sand bran, powdered grain, or aqueous suspensions of chlamydospores which presumably contain sufficient food reserves not to need additional sources.

TABLE 2

Numbers of cfu/g soil (x 10^3) of *V. chlamydosporium* established in soil from an application of alginate granules containing kaolin or wheat bran at a rate of 1% (w/w) soil

Application	Days after treatment						$\pm SE_D$
	0	7	21	35	56	84	
kaolin	0.1	0.9	0.8	0.9	4.0	7.4	8.2
wheat bran	0.3	89.1	66.1	83.7	53.4	42.3	

TABLE 3

Number (x10^3) of cfu/g soil of *V. chlomydosporium* in barley rhizosphere and non rhizosphere soil 5 and 11 weeks after application in alginate granules at a rate of 1% (w/w) soil

Soil	Time (wk)		$\pm SE_D$
	5	11	
non-rhizosphere	107	29	23
rhizosphere	89	91	

V. chlamydosporium is capable of colonising the rhizosphere of several important crops susceptible to cyst and root-knot nematode attack. The fungus does not cause lesions on the roots or affect their growth. The rhizosphere of barley plants grown in vermiculite was extensively colonised by *V. chlamydosporium* applied in 2% methyl cellulose to the seed. However, seed treatments were not successful in establishing the fungus on roots in

soil. Nevertheless, when *V. chlamydosporium* was applied to soil in alginate-bran granules the fungus survived longer in soil from the rhizosphere than in non-rhizosphere soil (Table 3). *V. chlamydosporium* is a successful saprophytic competitor and is able to survive in soil on nutrients leaching from roots.

P. penetrans is an obligate parasite and there is no vegetative growth in soil outside the nematode. The infective spores are highly resistant and may survive in soil for several years. As the bacterium does not grow in soil it would not need to be introduced with an energy source. However, as there is no proliferation in soil the number of spores required for nematode control would have to be supplied entirely in the inoculum. Spread in soil is also likely to be limited, so careful mixing of the inoculum is essential if large numbers of nematodes are to be infected. Nematode infection will depend on the density and distribution of the spores and on the period of activity of the nematode in soil. The lack of *in vitro* growth makes *P. penetrans* difficult to study and to manipulate. At present the number of spores/g soil required for control of any nematode is not known and the effect of environmental factors on efficacy has not been studied.

CONCLUDING REMARKS

Both *V. chlamydosporium* and *P. penetrans* can be added to soil to control the multiplication of cyst and root-knot nematodes. In small plot tests in which the spores of *P. penetrans* could be thoroughly mixed throughout the soil the bacterium was as effective at controlling *M. javanica* on tomatoes as a nematicide (Stirling 1984); *V. chlamydosporium* has not yet been tested in the field. The large application rates used in laboratory tests are often misleading, as the material applied tends to contain relatively low densities of the organism. Improved methods of production and formulation should result in significant reductions in the rates of application; such developments are essential if these organisms are to be used to protect arable crops.

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SESSION 7B

DEVELOPMENTS IN SOIL- AND SEED-BORNE PATHOGENS

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CROP LOSSES DUE TO DELETERIOUS RHIZOBACTERIA AND THEIR PREVENTION BY BACTERIZATION

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Deleterious rhizosphere bacteria (DRB) live on or in plant roots and inhibit plant development, often resulting in crop yield losses (Schippers et al., 1987; Suslow, 1982). DRB are predominantly saprophytes living from organic compounds released by plant root cells. This distinguishes them from true plant pathogens which parasitize root tissue thereby causing obvious disease symptoms.

Although they are saprophytes, DRB may penetrate deep into the healthy root tissue, in the intercellular spaces in particular. Their phytotoxic metabolites affect the plant by disturbing physiological processes. This may lead to impaired uptake of nutrients from the soil, an altered distribution of carbohydrates or other nutrients in the plant, or to a change in the susceptibility to true pathogens.

Whether DRB develop in the rhizosphere and release certain metabolites at phytotoxic levels seems to depend on particular environmental factors. These factors apparently are created by cultural practices such as cropping sequence, high cropping frequency, no tillage and others yet unknown. These features of DRB make it very difficult to identify the causal organism and their toxic metabolites.

Most experimental evidence for the effects of DRB on crop production has been obtained indirectly from increased production in a variety of crops following seed or tuber bacterization with plant growth-promoting rhizobacteria (PGPR) (Burr & Caesor, 1984; Hemming, 1985; Schippers et al., 1987; Schippers, 1988). Only recently, experimental data on the identity of DRB and on the mechanisms of their deleterious activity have become available.

In winter wheat, for example, a non-fluorescent Pseudomonas sp. isolated from the rhizosphere, which produces a low molecular weight polypeptide phytotoxin, is considered to be responsible for the inhibition of root growth and for bad crop development, particularly during cool, wet springs (Elliott & Lynch, 1984; Frederickson & Elliott, 1985; Cherrington & Elliott, 1987). If inoculated on non-sterile wheat straw, it maintains populations of approximately 10^6 cfu g^{-1} straw throughout the winter. It is able to colonize roots from straw residues and to negatively influence root growth (Stroo et al., 1988).

In another example, seedlings of canola (Brassica campestris "Candle") were inhibited in growth by strain rp2 of a non-fluorescent Pseudomonas sp. This Pseudomonas strain can only invade the roots during the first 4 to 5 days after seed germination. It penetrates the root tissue apparently by disruption of the middle lamella of cell walls by the enzyme polygalacturonic acid hydrolase (Campbell et al., 1986, 1987).

For potato, evidence increases that particular strains of Pseudo-

monas spp. inhibit root development and yields by their production of HCN, thereby disturbing the energy metabolism of root cells (A. W. Bakker & Schippers, 1987; Schippers et al., 1987). The counteraction of their deleterious activity by PGPR resulting in higher seed tuber yields is supposed to be mediated by competition for Fe^{3+}. Yield increases by PGPR were demonstrated to be linked to a high cropping frequency of potato (P.A.H.M. Bakker et al., 1986; Schippers et al., 1987; Schippers, 1988). Recently, evidence was obtained for increased seedling development in hydroponic systems by suppression of deleterious rhizosphere pseudomonads proliferating in the endorhizosphere of tomato, cucumber and lettuce (Van Peer & Schippers, 1989).

The DRB and their toxic metabolites, as well as the sites of their deleterious activity, seem to be diverse. This also holds for the environmental factors or cultural practices that trigger their development.

The deleterious activity of HCN-producing pseudomonads and its suppression by PGPR resulting in improved root functioning and growth and increased tuber yields in high frequency cropping of potato are based on the following processes.

More than 50% of the fluorescent Pseudomonas populations in potato rhizospheres possess the potential to produce HCN (A. W. Bakker & Schippers, 1987). HCN is toxic to root growth even at extremely low concentrations (0.5 µm) (A. W. Bakker, unpublished results). Whether phytotoxic concentrations of HCN are produced by DRB depends in the first place on the availability of Fe^{3+} in the rhizosphere soil (A. W. Bakker & Schippers, 1987), but also on the levels of glycine and proline in potato root exudate. Laboratory experiments revealed that the amount of HCN produced depends on the ratio of these two amino acids. If proline dominates, the glycine-induced HCN production decreases (A. W. Bakker et al., 1987). HCN production by rhizosphere pseudomonads therefore possibly differs between crops and crop cultivars depending on their root exudate composition.

The Fe^{3+} availability for microorganisms is low in neutral and alkaline soils (10^{-17}M, pH7). Potato tuber bacterizations with Pseudomonas putida strain WCS358 or P. fluorescens strain WCS374 increased potato root growth and tuber production significantly in high frequency potato cropping soil, both in pot and field experiments. There are good reasons to suppose that these increases are, at least partly, due to the suppression of HCN-production, mediated by the competition for Fe^{3+}.

Strain WCS358, which releases iron-chelating siderophores in soil low in Fe^{3+} to obtain iron for its metabolism, apparently competes successfully for Fe^{3+} with HCN-producing pseudomonads (Bakker & Schippers, 1987; Schippers, 1988). It was demonstrated indeed that the siderophore pseudobactin 358 of strain WCS358 could not be used by 99% of the HCN-producing Pseudomonas rhizosphere isolates tested. Moreover, WCS358 can make use of a wide variety of siderophores produced by other Pseudomonas isolates. That pseudobactin 358 plays a key role in the stimulation of root growth and yield in high frequency cropping of potato was demonstrated by making use of isogenic mutants of WCS358 that had

lost their ability to produce pseudobactin 358 by Tn5 transposon mutagenesis. These Sid$^-$ mutants were not able to increase potato seed tuber yields, while their wild type did so by 10-15% in the same experimental fields (P. A. H. M. Bakker et al., 1986).

Yield increases in the field obtained by seed or tuber bacterization with PGPR are variable (Geels et al., 1986). However, significant seed tuber yield increases of 5-15% were obtained by our group in three out of seven seasons in field experiments at the Experimental Farm "De Schreef" between 1981 and 1987. Several factors may be responsible for a failure of PGPR. In some cases it may be due to a failure of DRB to develop deleterious effects. This may be so if the environmental factors being a prerequisite for their deleterious activity do not occur. This is like a pesticide having no effect if the target pathogens do not occur.

Failure of PGPR to increase yield may also be due to a simultaneous development of a major disease which cannot be overcome by the PGPR strain used. For example, losses in potato crops caused by Verticillium dahliae, Rhizoctonia solani, Streptomyces spp. or others may obscure the potential yield increasing effects of PGPR in our field experiments at the Experimental Farm "De Schreef" in some seasons.

Another important reason for a failure of PGPR in field experiments seems to be an inadequate colonization of plant roots. The development of the PGPR on roots is often restricted to the 40 cm top layer of the soil, becoming irregular and diminishing during the season (Bahme & Schroth, 1987; P. A. H. M. Bakker et al., 1986). Root colonization by PGPR, however, can possibly be improved by manipulation of environmental factors, such as the availability of free water in soil, by irrigation or sprinkling. Another approach may be the development of a more effective delivery system of PGPR for colonizing roots in the field.

Finally, the prospect is now within reach to use genetic engineering to combine in one strain the attributes of antagonistic activity and abilities to aggressively colonize roots or to survive in soil. This will open new possibilities of developing bacterial products to control DRB, true pathogens and pests. The suppression of DRB and of major pathogens during seedling development in soil and during crop growth in hydroponic systems seems to be much less hampered by inadequate root colonization (Van Peer & Schippers, 1989).

The interest of governments for the development of crop production systems with lower input of pesticides harmful for the environment has now become serious. PGPR, therefore, deserve more attention.

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APPROACHES TO THE CONTROL OF FUNGAL VECTORS OF VIRUSES WITH SPECIAL REFERENCE TO RHIZOMANIA

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ABSTRACT

The soil-borne fungal parasites of plant roots that transmit viruses do so by means of motile zoospores that are very sensitive to fungitoxic compounds and adverse environmental conditions. However, these fungi survive as thick-walled resting spores which protect the virus particles for long periods and are extremely resistant to chemical and microbial action.

Chemical control measures have been most successfully exploited in hydroponic culture systems, where growing roots can be continually supplied with the active material. Some heavy metal ions and surfactants are effective under these conditions. In general, fungicides have been less successful when applied to soil. Even where glasshouse pot trials have identified potential candidates for controlling Polymyxa betae, the fungal vector of rhizomania disease, subsequent field trials have, in the main, proved disappointing. Partial soil sterilants with fumigant action are generally effective in disinfecting the upper layers of soil but are usually too costly for routine use against rhizomania.

Biological control of the fungal vector has been little explored to date and no promising agents have yet been identified. Of agronomic factors that can be manipulated, early sowing is advocated as an amelioratory measure in Europe whilst the mechanical transplanting of sugar beet is widely used in Japan.

Breeding for resistance to rhizomania is in its relatively early stages. The first sugar-beet cultivars with some resistance have only recently been released commercially. This is likely to be the most effective control measure in the forseeable future. The extent to which this resistance is to the fungal vector (rather than to the virus) and whether such a character can be deliberately selected, has not yet been extensively investigated.

INTRODUCTION

All the known fungal vectors of plant viruses are from two taxonomic classes of fungi, the Plasmodiophoromycetes and the Chytridiomycetes. They are characterized by their ability to form resilient, thick-walled and long-lived resting spores, by the absence of any hyphae and by the production of motile zoospores as their means of spread from plant to plant. Virus transmission occurs through the zoospores, the virus particles being carried either within the spore, as in the Plasmodiophoromycetes, or adhering externally.

Though all the species so far identified as vectors (Table 1) are obligate parasites of plant roots, only Spongospora subterranea is recognized as a pathogen in its own right, causing powdery scab of potatoes and crook root of watercress (Jones, 1988; Tomlinson, 1988). However some, eg Polymyxa spp., are closely related taxonomically to species that cause severe disease problems, such as Plasmodiophora brassicae, the cause of club root in crucifers. Hence many of the control measures devised for these damaging pathogens have been evaluated for their efficacy against the virus diseases transmitted by fungi.

TABLE 1

Soil-borne fungi associated with the transmission of plant viruses (see Cooper & Asher, 1988)

Fungus	Virus transmitted
Polymyxa graminis	Barley yellow mosaic Oat golden stripe Oat mosaic Peanut clump Rice necrosis mosaic Rice stripe necrosis Soil borne wheat mosaic Wheat spindle streak mosaic
Polymyxa betae	Beet necrotic yellow vein Beet soilborne
Spongospora subterranea	Potato mop top Watercress chlorotic leaf spot agent
Olpidium brassicae	Lettuce big vein agent Tobacco necrosis Tobacco stunt
Olpidium radicale	Cucumber necrosis Melon necrotic spot

Because of the worldwide distribution of these diseases and the number of different crop species affected, this review can do no more than touch on selected aspects of control. However, the rapid spread worldwide of the rhizomania disease of sugar beet (Richard-Molard, 1985) discovered for the first time in the UK in August 1987, has stimulated considerable research activity in the last few years and progress towards achieving effective control of this disease will be discussed in more detail.

CHEMICAL CONTROL

The fungal zoospore, which transmits the fungus-vectored viruses, appears to be extremely sensitive to changes in its chemical environment. Spores can readily be inactivated by low pH (eg <5.5 for S.subterranea, Cooper et al., 1976; and P.betae, Ivanovic, 1984) by surfactants (O.brassicae, Tomlinson & Faithfull, 1979; O.radicale, Tomlinson & Thomas, 1986), by heavy metal ions (eg zinc and copper; O.brassicae, Tomlinson & Faithfull, 1979; S.subterranea, Tomlinson, 1956) and by many more complex fungicidal compounds. As with most other soil-borne diseases, the problem of control is due not so much to a lack of active compounds as to the difficulty of adequately penetrating and protecting a continually expanding root zone.

By contrast, the resting-spore stages of these vector fungi are apparently very resistant to chemical action and to microbiological degradation, though they are sensitive to heat treatment (P.betae resting spores are killed at 60°C for 10 min; Abe, 1987) and to some soil sterilants. Virus particles survive within the fungal resting spores probably for as long as the spores remain viable. Relatively high populations of P.betae, the fungal vector of beet necrotic yellow vein virus, have been found in soils free of susceptible host species for 17 years (M J C Asher, unpublished data) and severe rhizomania has occurred after 15 years absence of sugar-beet crops (Schlosser, 1988).

Fungicides

Surfactants and metallic ions

The sensitivity of these zoosporic fungi to low concentrations of surfactants and heavy metal ions has been exploited in the hydroponic cultivation of lettuce, cucumber and watercress. Control of lettuce big vein, transmitted by O.brassicae, and of melon necrotic spot virus in cucumbers, transmitted by O.radicale, has been achieved using the surfactant, alky phenol ethylene oxide (Agral) at 20 ug/ml in the circulating nutrient film (Tomlinson & Faithfull, 1979; Tomlinson & Thomas, 1986). The crook root disease and chlorotic leaf spot of watercress have both been successfully controlled by continuously supplying zinc sulphate to watercress beds at 0.3-0.5 ug/ml (Tomlinson & Hunt, 1987).

The use of zinc applied to soil has been less successful. Only small benefits were obtained against big vein infestation in field- grown lettuce (Marlatt, 1959). However zinc oxide applied at 1360 kg/ha reduced the incidence of symptoms of potato mop top virus (transmitted by S.subterranea) from 44% to 6% (Cooper et al., 1976). Zinc sulphate was ineffective in controlling P.betae in glasshouse pot tests (Figure 1a) and no yield benefits were obtained in trials on rhizomania infested land in France (Anon, 1979-85). Of the other metallic ions, copper in the form of copper oxychloride at 250 ppm was found to inhibit P.betae in pot tests but had no effect on rhizomania in field trials in France (Anon, 1979-85) or Italy (Canova et al., 1975).

Similar experiences were encountered when cuprous oxide was tested against S.subterranea in pot and field trails (Cooper et al., 1976). Mercury, usually in the form of mercurous chloride (calomel), has been widely tested following demonstrations of its effectiveness in controlling club root (eg Ann et al., 1983). Again, despite its total suppression of P.betae in pot tests (Schaufele, 1987), no benefits were obtained when it was applied at 1 kg/ha to a rhizomania infested site (Alghisi & D'Ambra, 1966).

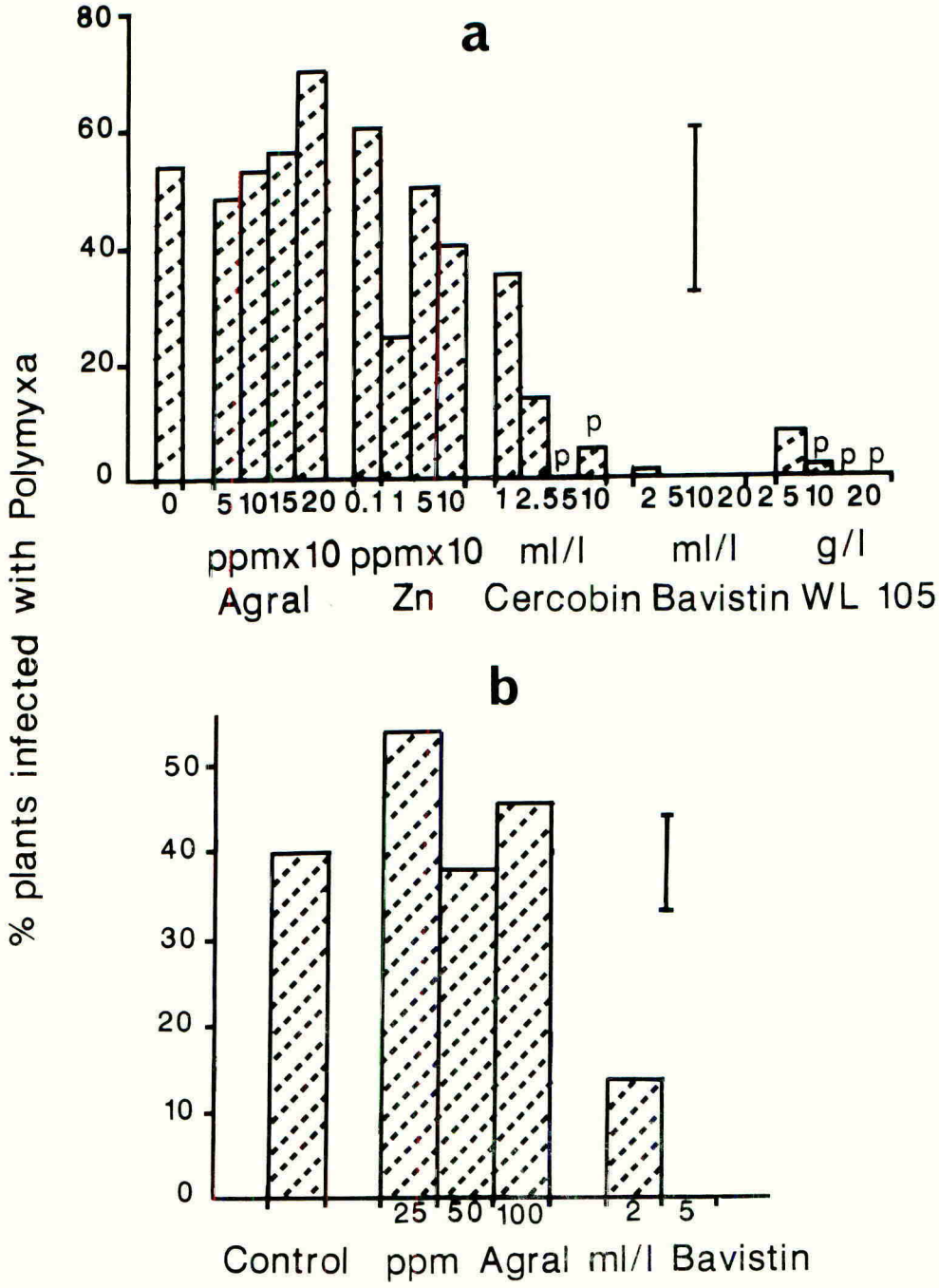

Fig.1. Control of Polymyxa betae by selected fungitoxic compounds in (a) glasshouse pot and (b) field plot trials. Chemicals watered onto soil at two leaf stage and plants sampled 5 weeks later. p = evidence of phytotoxicity.

Of the several surfactants found to be active by Tomlinson & Faithfull (1979) only ammonium lauryl sulphate and Agral have been reported as having been tested against P.betae in soil. Neither was effective in reducing infection at the concentrations examined (Schaufele, 1987; Figure 1).

Sulphur

The activity of sulphur applied to soil seems to be due mainly to its effect on pH. Applications to soil in field trials in Japan, to lower the pH to <6.0, inhibited infection of roots by P.betae (Abe & Tsuboki, 1978) and reduced the damage caused by rhizomania (Migawaki et al., 1983). This control measure, effective also in Italian trials (Canova et al., 1975; Casaroni, 1975), is now recommended, in conjunction with soil fumigation (see later), as the standard treatment for newly discovered, isolated patches of rhizomania infestation in fields in Japan (Abe, 1987). Similar effects of sulphur have also been observed on S.subterranea and potato mop top virus incidence in potatoes (Cooper et al., 1976).

However, low pH (<5.5), though inhibiting zoospore activity, does not affect resting spores. Hence if the pH of the soil rises, or is increased for subsequent crops by liming, the disease potential is restored. Furthermore sugar beet itself takes up nutrients most efficiently in neutral soils and allowing the pH to fall below 6.5 results in increasingly adverse affects on growth.

Organic fungicides

A large number of organic compounds with proven fungicidal or fungistatic activity have been tested for their ability to control P.betae in pot tests or to reduce the symptoms and yield loss due to rhizomania in field trials, or both (e.g., see Schaufele, 1987). Most had no detectable effect on fungal infection in glasshouse tests at rates that were not phytotoxic. However, several, such as the benzimidazole fungicides, quintozene, fenaminosulf and prothiocarb have demonstrated activity against this group of fungi (Bruin & Edgington, 1983).

Benomyl and thiophanate methyl both reduced infection of sugar-beet seedlings by P.betae when applied to soil in glasshouse pot tests (Schaufele, 1987; Figure 1a) but, when applied to rhizomania infested field trials in France and Germany, the latter failed to increase yields (Schaufele, 1987). Carbendazim, formulated as Bavistin (BASF), was also very effective in preventing or delaying infection by P.betae in both glasshouse and small plot trials (Figure 1). However Tomlinson & Faithfull (1979) have suggested that the toxicity of Bavistin to zoospores of O.brassicae was due to the surfactants in the formulation. Following this, Hein (1987) examined each of four constituents of the 50% WP formulation separately for their effectiveness in preventing infection of beet seedlings grown in rhizomania infested soil in pots in the glasshouse. The surfactant HM2 prevented virus infection whereas carbendazim alone did not. Similar conclusions were reached with a 25% EC formulation of tolclophos methyl (Risolex); activity appeared to be due to a component that was not the designated active ingredient. However, in field trials HM2 applied at up to 450 kg/ha to a depth of 10 cm prior to drilling had no effect on the development of the disease (Hein, 1987).

The only fungicide reportedly tested as a seed treatment to date is prothiocarb. At rates of 1 mg/seed, improvements in seedling growth were obtained in glasshouse trials using soils heavily infested with P.betae and

O.brassicae. Soil incorporation also reduced infection by both species in pot tests (Horak & Schlosser, 1978). The closely related substance, propamocarb is not effective (Hein, 1987; Schaufele, 1987). Other fungicides that have exhibited some activity against P.betae in soil in pot tests are fosetyl-aluminium (Hillman, 1984; Hein, 1987; Schaufele, 1987) tricyclazole (Schaufele, 1987) and the experimental fungicide WL 105305 (syn. NK483, DSC 33520F; Hein, 1987; Schaufele, 1987; Figure 1a). This latter compound, a salicylamide derivative, has been reported to be effective in controlling Plasmodiophora brassicae (Buczacki, 1983; Dixon & Wilson, 1984).

In field trials in France, fenaminosulf improved sugar yields in trials on rhizomania infested land (Anon, 1979-85) but quintozene, despite its reported ability to control S.subterranea in Queensland, Australia (Hughes, 1980), was ineffective (Alghisi et al., 1964; Anon, 1979-85). Salicylamide, when sprayed into the open seed furrow at 30 kg/ha, did not improve yields (Ahrens, 1986). It appears that even the most promising compounds emerging from glasshouse screening tests to date have failed to give effective control of rhizomania in the field. This is perhaps surprising given the yield benefits that can be obtained by simply delaying the time at which seedlings become infected with P.betae in the field (eg by using transplanting techniques, see below). Clearly, the problem of protecting the root system for a sufficient length of time by chemical means has yet to be overcome and awaits the development of effective symplastically translocated fungicides.

Soil partial sterilants

All partial soil sterilants tested, when applied in the autumn to rhizomania infested fields, have given considerable yield benefits in subsequent sugar-beet crops. They include methyl bromide (Alghisi et al., 1964; Richard- Molard, 1985; Martin & Whitney, 1988), metham sodium (Alghisi et al., 1964; Anon, 1979-85; Horak, 1970), chloropicrin (Martin & Whitney, 1988) and the nematicides, dazomet (Bongiovanni, 1973; Abe, 1987), dichloropropene (Telone: Hess & Schlosser, 1984; Martin & Whitney, 1988) and the mixture of dichloropropene with dichloropropane (D-D: Abe & Tsuboki, 1978; Schaufele, 1987). Some of these have also been found to be effective in controlling soil-borne wheat mosaic virus (McKinney et al., 1957) and wheat spindle streak mosaic virus (Slykhuis, 1970) transmitted by Polymyxa graminis, as well as the club root disease of brassicas (White & Buczacki, 1977). However, environmental and economic considerations have tended to restrict the use of soil sterilants. Methyl bromide, probably the most effective (Table 2) but also the most toxic and expensive, is likely to be used only as an exceptional sanitation measure, such as in those fields first infected with rhizomania in England (S A Hill, personal communication). In Japan, D-D is recommended for the partial eradication of the disease from discrete patches only, within lightly infested fields (Abe, 1987). In California, however, Telone is applied routinely to infested fields to a depth of 17 cm approx 2 weeks before drilling. This compound was the most cost effective of those tested by Whitney & Martin (1988), increasing root weight by an average 137% and sugar content by 45% (Table 2). Such treatments may be economically worthwhile where the disease is widespread and severe and no other control measure is available. However, they are only partially effective because, although resting spores in the upper layers of the soil may be killed, re-infestation from lower layers occurs during the growing season and treatments must be repeated each time a crop is grown.

TABLE 2

The effect of soil partial sterilants applied prior to sowing on the yield of sugar beet on rhizomania infested sites in California (Whitney & Martin, 1988).

Fumigant	% plants infected*	Root weight (t/ha)**	Sugar concn. (%)**	Sugar yield (t/ha)**
Methyl bromide	0	69.8	11.3	7.9
Dichloropropene (Telone II)	0	43.4	10.6	4.6
Untreated control	100	18.3	7.3	1.3

* as detected by ELISA, 12 weeks after sowing
** at harvest
Chemicals applied by injection to 17cm depth; soil surface sealed by compaction or covered.

AGRONOMIC PRACTICES

The association between a wide range of agronomic factors and cultural practices (such as soil type and pH, rotation, drainage, fertilizer and herbicide usage, irrigation and sowing date) and rhizomania has been examined in large scale surveys in Germany (Hillman, 1984). In general the disease appeared to be favoured by late sowing, short rotations and high soil pH. Poor drainage and excessive irrigation have also been found to exacerbate the disease (Anon, 1983) by favouring the motile stages of the fungus. Few of these factors can be manipulated to give even small benefits once rhizomania has become established but a policy of wide rotations and good soil management is advocated in England to prevent or slow development of the disease (Anon, 1988).

Early sowing is, however, recommended in most European countries as a means of reducing yield losses on rhizomania infested land (Anon, 1983). Observations at Broom's Barn Experimental Station by S J Blunt (personal communication) illustrate the effects on the fungal vector (Figure 2). In naturally infested field plots sown deliberately late (5 May), infection by P.betae was already well established when the first samples were taken at about the two-leaf-stage, 36 days later. The incidence of infection continued to increase until all plants were infected. In contrast, with early sowings (eg 24 March) no infection was detectable until 56 days later, when plants were already at the four leaf stage. Subsequently the incidence increased to about 50% of plants infected but, because plants were already well established, and had become more resistant, further infection was halted. In rhizomania infested soils, the more extensively and the earlier the virus is introduced into the plant by the fungal vector, the more it multiplies during the growing season and the greater the damage. Ahrens (1986) has reported yield increases of up to 70% from sowings at the end of March, compared with late April, in infested soils in Germany.

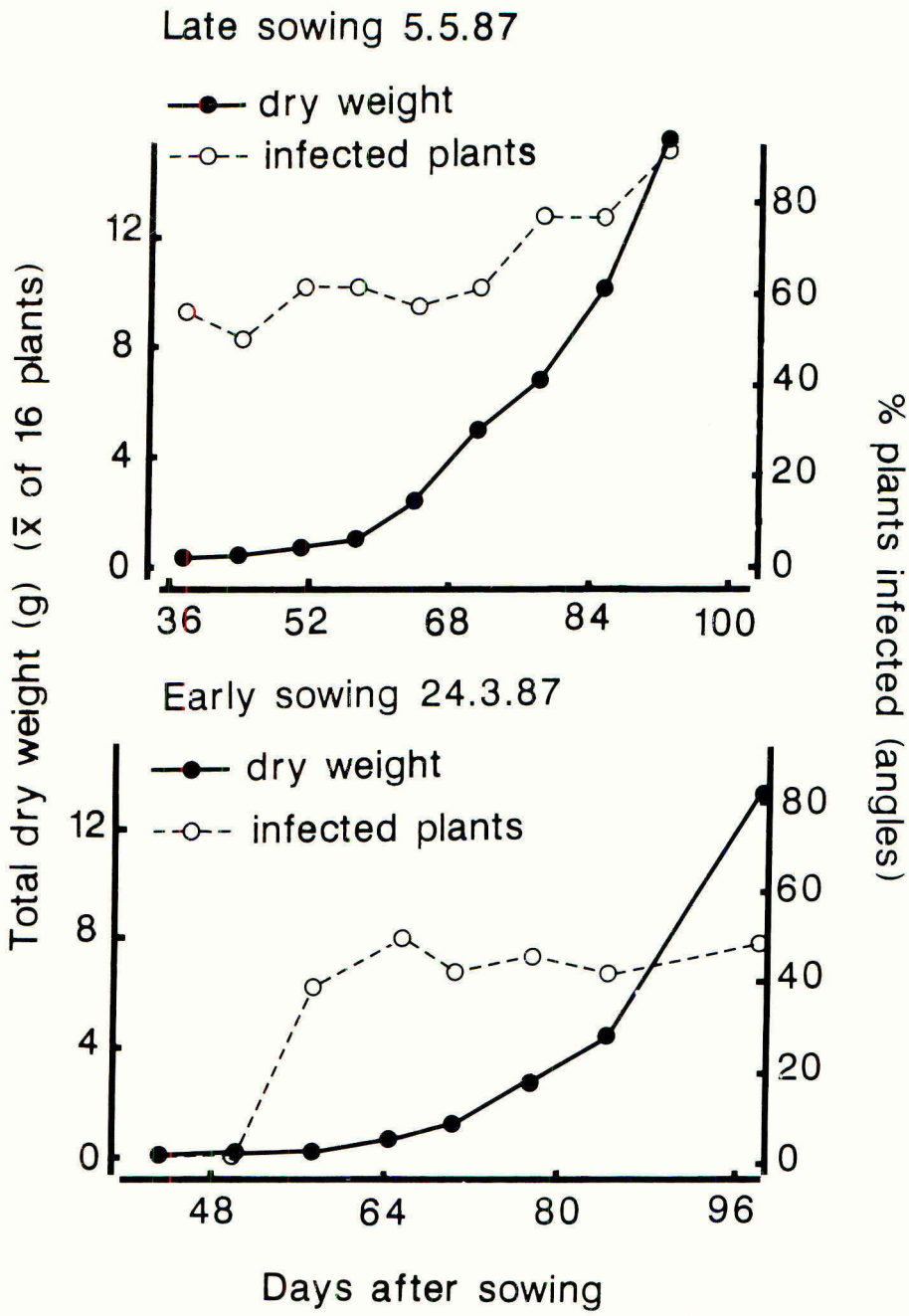

Fig.2. Effect of sowing date on rate of infection of sugar-beet seedlings by Polymyxa betae (S.J. Blunt, unpublished data).

This principle is also exploited in the use of the transplanting technique, in which sugar-beet seedlings raised in partially sterilized soil in paper pots are mechanically transplanted into the field. In trials in France, yield benefits of over 100% have been obtained (Richard-Molard, 1985). For general use however the technique is not cost effective and has not been adopted in Europe or the USA. In Japan, by contrast, high guaranteed sugar prices enable the use of transplanting throughout the beet growing area (74,000 ha) and considerable benefits have accrued from its use on the 23% of fields infested with rhizomania (Abe, 1987).

BIOLOGICAL CONTROL

The prospects for biological control have only recently begun to be evaluated. While Trichoderma harzianum has been shown to parasitize the resting spores of P.betae under laboratory conditions (D'Ambra & Mutto, 1986), it was not successful as a control agent in field trials (E Schlosser, personal communication). Similarly, an inoculum of Pseudomonas fluorescens was ineffective in French trials (Anon, 1979-85). The longevity of survival of resting spores of the vector fungi suggests that they are remarkably resistant to microbial degradation. Preliminary results (S J Blunt, unpublished data) indicate that inoculum levels of P.betae are slightly increased by continuous (10 years) cultivation of sugar beet, compared with normal rotations, implying the absence of any pronounced build up of natural biological control agents. However, the possibility that the germination of resting spores, and the activity of the zoospores released, might be influenced by other soil micro-organisms and thus be amenable to some biological control should not be dismissed.

HOST RESISTANCE

Genetic resistance to diseases caused by soil-borne viruses with fungal vectors has been quite extensively exploited, particularly in the cereals. Cultivars more or less resistant to soil borne wheat mosaic (Brakke & Langenberg, 1988), barley yellow mosaic (Friedt, 1988), wheat spindle streak mosaic (Jackson et al., 1976), rice necrosis mosaic (Usugi, 1988) and also potato mop top (Solomon & Wastie, 1988) are available to farmers in various countries. Generally, the basis of the resistance has not been explored but in the case of barley yellow mosaic it is quite clearly resistance to the virus rather than the vector, P.graminis (Adams et al., 1986; Friedt, 1988). By contrast, field resistance to soil borne wheat mosaic is thought to involve resistance to infection by viruliferous zoospores of P.graminis (Brakke & Langenberg, 1988).

In sugar beet, the selection of lines resistant to rhizomania has become a major objective of breeding programmes in Europe, the USA and Japan. Rizor, the most resistant cultivar yet to be released commercially in France (Richard-Molard, 1987) was developed as a result of many years' selection on rhizomania infested land in Northern Italy. Annual improvements in its performance, both on rhizomania infested and disease free trial sites, are still being made in response to repeated selection (Figure 3). Its resistance is expressed both as a reduced titre of virus in the root and in a reduced rate of multiplication of P.betae (De Biaggi, 1987). Other cultivars with useful levels of resistance have been identified in Southern Germany (Gunther, 1984). In the USA, a breeding programme begun in 1984, a year after the disease was first discovered there, has utilized resistance derived from various sources, including the

wild beet, Beta maritima (Lewellen et al., 1987). In most cases resistance is identified as a reduced virus content as well as improved yields in infested trials. However the extent to which resistance to the fungal vector might contribute to, or be responsible for, this has not been examined extensively to date.

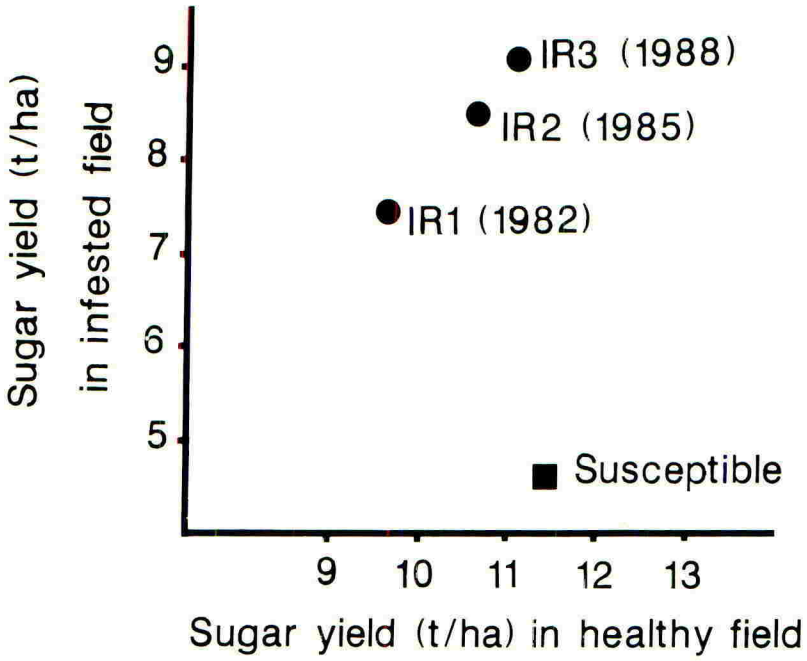

Fig.3. The response to repeated annual selection of a rhizomania resistant breeding line (IR) of sugar beet compared with a susceptible cultivar. Mean of trials carried out on infested and uninfested sites in France in 1986. ( ) = year of commercial introduction. (Anon, 1987).

CONCLUSIONS

In general, chemical control measures have only proved effective against fungal vectors of plant viruses where it has been possible to ensure a continuing supply of the active ingredient to potential infection sites on the developing root system, ie in hydroponic systems. Fungicides applied to soil have so far failed to protect developing root systems for long enough under field conditions.

Host plant resistance has been more successful as a control measure, particularly against soil-borne cereal viruses, though there is increasing evidence of strain specificity (eg in barley mosaic virus; M J Adams, personal communication) and corresponding concern for the durability of such resistance. Resistance specifically attributable to inhibition of the fungal vectors has not been widely explored, probably because of the difficulty of assessing such resistance on a large scale. Certainly there are reports of differences among lines of wild Beta species in their

susceptibility to P.betae (Fujisawa & Sugimoto, 1979). Whether this character can be usefully exploited as a control measure for rhizomania will depend on the threshold level of resistance necessary to prevent virus entry and multiplication in the root. In addition, its future potential will be dictated by the ease with which such resistance can be assessed and manipulated in a breeding programme. Clearly, however, genetic resistance offers the most promise as a control measure for rhizomania in the long term.

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EXPERIMENTAL ANALYSIS OF THE FOOT-ROT COMPLEX OF PEAS.

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ABSTRACT

Pea plants (cv. Puget) from eight Lincolnshire fields were infected by foot rot. In non-compacted soil, Fusarium solani f. sp. pisi was isolated predominantly from root tissues and Phoma medicaginis var. pinodella from the stem; Mycosphaerella pinodes occupied a median position. In compacted soil (wheelings), increased levels of Fusarium occurred in stems and Phoma in roots. Mixed spore inocula in glasshouse conditions reproduced the colonization pattern seen with non-compacted soil. Root exudates induced more rapid germination of chlamydospores of Fusarium than of the other two fungi.

INTRODUCTION

Pathogenic fungi are the most likely cause of pea foot-rot (Gane et al., 1984). Predominant species implicated in the U.K. include Fusarium solani f. sp. pisi and two of the Ascochyta group fungi (Lawyer, 1984) namely Mycosphaerella pinodes and Phoma medicaginis var. pinodella (Biddle, 1983). Pathogenicity tests indicate that each species may induce symptoms (Mabey, 1987). Under field conditions, at least one pathogen is usually present (Biddle, 1983).

Initially in these studies the relative distribution of F. solani f. sp. pisi, M. pinodes and P. medicaginis var. pinodella in diseased crops was examined. Following earlier work (Buxton & Perry, 1959; Kerr, 1963) regarding a possible interaction between fusarial pathogens in the plant, experiments were performed using mixed culture inocula. Studies of chlamydospore germination were also made.

MATERIALS AND METHODS

Sampling of naturally infected plants

Eight fields, with a long history of pea cultivation, in Lincolnshire U.K. were surveyed (July 1985). All plants (cv. Puget), sown at approximately the same time (1 - 7 March) carried small pods in the lowermost trusses. Two lots of 20 plants per field were sampled at random from non-compacted soil and wheelings, respectively. In all plants, discolorations above the cotyledon scar were dark-brown to black, while those of the root were either light-brown or orange.

Isolation of fungi from naturally infected plants

After rinsing, axes were trimmed and cut to 10cm (5cm above and 5cm below the cotyledon scar). Resulting portions were surface

sterilized for 2min (25% v/v NaOCl) and cut into sequential 1 cm sections for plating on PDA (Oxoid). Incubation was for 7d at 25°C in darkness. The relative distrubutions of *E. solani* f. sp. *pisi*, *M. pinodes* and *P. medicaginis* var. *pinodella* in pea tissues were compared, following bulking of all results for plants collected from non-compacted soil and, separately, those from wheelings (Fig. 1).

Mixed inoculum experiment.

One highly pathogenic single-spore culture of each species was selected by the tube culture technique (Whalley, 1984): F (*E. solani* f. sp. *pisi*), M (*M. pinodes*) and P (*P. medicaginis* var. *pinodella*) . Spore suspensions (macroconidia of F; pycniospores of M and P) were prepared in distilled water (Mabey, 1987) before mixing to give a series of inocula (Table 1).

Plants (cv Puget), were derived from surface sterilized pea seeds (25% NaOCl for 3min) and grown in trays of Levington's Potting Compost (Fisons Ltd) for 12d. Root-dipping inoculation was chosen, prior to transplantation into Levington's Compost, four plants per 13 cm pot (Wells *et al*, 1949). Controls were dipped in distilled water.

Plants were maintained at 25°C (12h daylength regime in Gallenkamp growth cabinets). A total of 160 plants was used per host-inoculum mixture, plus 80 controls. At each of 3, 7, 14 and 21 days, 20 plants per treatment were selected and plated out on PDA. Controls were sampled at 21d to determine the extent of any background contamination. Mean disease index scores (Biddle, 1983, 1984) were calculated for the 21d plants. The stem base and root system of each plant were separately assigned a score between 0 and 5; 0 = no discoloration and 5 = complete decay of root or stem cortical tissues. In addition the value 6 was given to each region when a plant was dead (Clarkson, 1978). Assessments were combined as follows: total score for stem base + total score for root system / 2 X number of plants examined = mean disease index

TABLE 1: Effects of mixed inoculum of foot rot pathogens on incidence of foot-rot in a glasshouse assay.

	Treatment			
Isolate	1	2	3	4
F	5×10^5	5×10^5	–	3×10^5
M	5×10^5	–	5×10^5	3×10^5
P	–	5×10^5	5×10^5	3×10^5
Total Spore Concentration /ml	10^6	10^6	10^6	9×10^5
Mean disease Index (21 days)	5.2	5.9	5.4	5.9

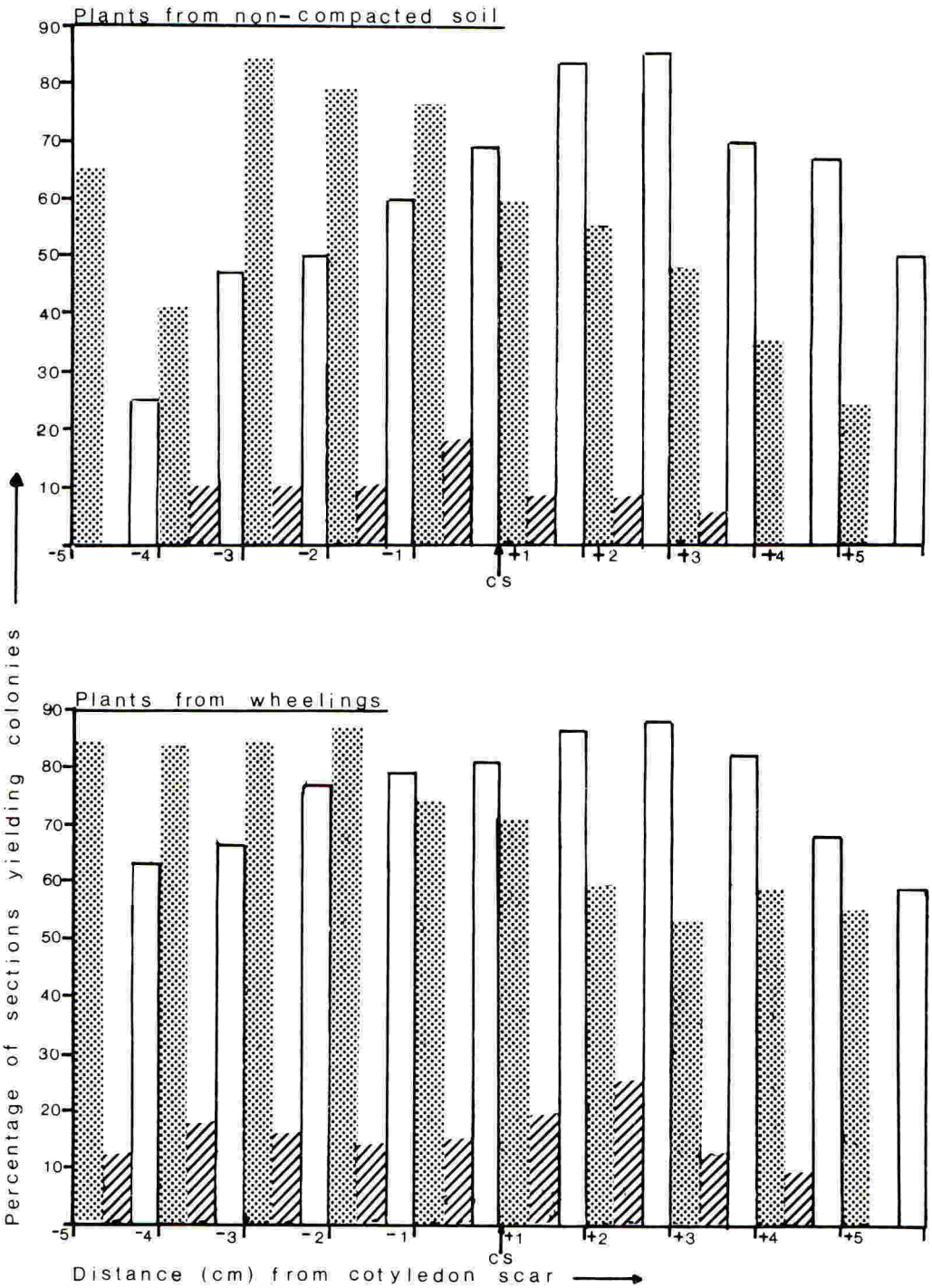

Fig 1.

The isolation of three foot-rot pathogens (F. solani f. sp. pisi ⁘ , M. pinodes ⁒ and P. medicaginis var. pinodella ▯) from naturally infected pea plants (cv Puget) from two field situations.

Chlamydospore germination experiment

Separate populations of chlamydospores of isolates F, M and P were prepared in samples of a sandy loam soil with no previous history of pea foot-rot disease. The method of Whalley & Taylor (1973) in which mycelial mats were incorporated into air-dry soil was found to be satisfactory for this purpose; abundant chlamydospores were detected within 4 wk when soil-smears were stained with lactophenol acid fuchsin (Nash et al, 1961).

Root exudates of cv. Puget (equivalent to 10ml solution per plant) were collected in distilled water (Whalley & Taylor, 1973) and pipetted into sterile exudate diffusion units (0.2ml/unit); control units were filled with distilled water. A unit, which could be autoclaved, consisted of a short length of glass tubing fixed by means of Araldite resin to a piece of porous ceramic tile (1.0 X 1.0 X 0.5cm). The top of the tube was covered with a removable cap of aluminium foil. The tile portion was buried to a depth of 1cm in chlamydospore-supplemented soil, wetted to field capacity (14% water) in a glass Petri dish base (Whalley & Taylor, 1973) at 25°C. Two units were removed at each sampling time (6, 12, 24 and 36h) and soil-smear slides prepared from soil crumbs adhering to the bases. Germination was assessed from counts of 400 propagules per treatment.

RESULTS

Distribution of fungi in naturally infected plants.

Colonies of all three fungi developed on PDA. In plants from non-compacted soil, higher counts of F. solani f.sp. pisi were obtained from roots than from stems, whereas P. medicaginis var. pinodella was more common on stems than roots (Fig. 1). In plants obtained from wheelings, which invariably yielded a higher total number of colonies, appreciable counts of Fusarium were also recorded from the stem and likewise increased levels of Phoma from the root. M. pinodes was isolated consistently less frequently than either Fusarium or Phoma and with no obvious difference between roots and stems (Fig 1).

Mixed inoculum experiment

All inoculated plants developed foot-rot, irrespectve of the inoculum combinations used (Table 1). The distribution of the three fungi (Fig. 2) corresponded closely to that noted for non-compacted soil in field conditions (Fig. 1). The respective association of F with the root and of P with the stem was observed in both the 7 and 14 day samples, although by day 21 both pathogens had spread considerably to opposite parts of the axis. M. pinodes was isolated most often from the uppermost root section and the frequency of isolation declined on either side of this. Control plants remained free of symptoms.

Fig 2. The re-isolation over a 21 day period of three foot-rot pathogens (*F. solani* f. sp. *pisi* ⁘ , *M. pinodes* ⁒ and *P. medicaginis* var. *pinodella* ▯) inoculated as spore mixtures to plants of cv Puget. Top row, F + P; second row, F + M; third row, M + P; bottom row, F + M + P

Chlamydospore germination

Spores of all three pathogens germinated in the presence of root exudates, although a differential response, both in terms of time and amplitude, was noted between isolate F and the Ascochyta group fungi (Fig. 3). Maximum germination of F occurred at 12h, as opposed to 36h for M and P. In all cases, germlings consisted of a single unbranched hypha, rarely longer than eight times spore diameter. In later samples, following peak germination values, germ tube lysis, together with an associated stimulation of bacteria and other fungi was apparent. Germination in the controls was consistently low, occurring sporadically but never exceeding 5% of propagules present.

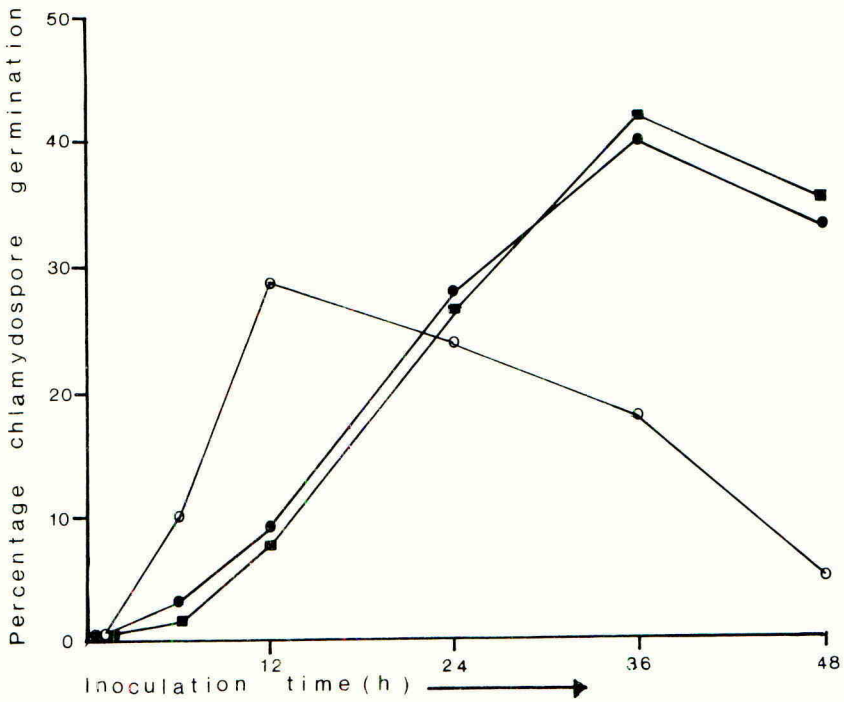

Fig 3.

The response of soil populations of chlamydospores of three foot-rot pathogens (F. solani f. sp. pisi -o- , M. pinodes -●- and P. medicaginis var. pinodella -■-) incubated in the presence of pea root exudates suppied via diffusion units.

DISCUSSION

Studies of naturally infected plants and of plants artificially inoculated with the three foot rot fungi indicate a clear predilection of *F. solani* f. sp. *pisi* for the root system, *P. medicaginis* var. *pinodella* for the stem base and *M. pinodes* for tissues at or near the cotyledon scar in the early stages of disease development. In view of the apparently greater importance of the former two fungi, as opposed to the latter, in recent field surveys in the UK (Biddle, 1983; Mabey 1987), it seems reasonable to assume that the typical black wedge-shaped lesion of the stem base and light-brown to orange discoloration of the root system, characteristic of British conditions, can be ascribed to infection by *P. medicaginis* var. *pinodella* and *F solani* f. sp. *pisi*, respectively (Gilchrist, 1926).

Sequential samples of artificially inoculated plants (Fig 2) reveal, however, that all of these fungi are able to spread from their inital sites of establishment, from roots to stem bases or vice-versa. In this respect, soil compaction seems both to favour disease development and to facilitate invasion of stems by *F. solani* f. sp. *pisi* and of the root system by *P. medicaginis* var. *pinodella*, with little apparent effect on the distribution of *M. pinodes*.

It is not clear from these studies whether the effect of compaction is 'physiological', for example by weakening the host as a result of an increase in soil bulk density (Vigier & Raghavan, 1980), or results from a restriction of the plant root system to areas of the soil with a higher population of foot-rot pathogens than that found in the subsoil layers (Burke *et al*, 1970).

The results of mixed inoculations showed no evidence of antagonism between the pathogens, nor of facilitating development of one pathogen as a result of the activities of others (Fig 2). It seems likely that the three foot rot fungi act synergistically to cause disease, a situation recorded elsewhere for mixed infections of pea roots involving *F. solani* f. sp. *pisi* and *Pythium* spp (Escobar *et al*, 1967).

Finally the components of pea root exudate were shown to fulfil the requirements for chlamydospore germination in soil by all three fungi. The more rapid germination response of *F. solani* f. sp. *pisi* is interesting in view of the preferential invasion of the root system by this fungus, the root being the first part of the plant to emerge from the seed.

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WATER RELATIONS IN PHYTOPHTHORA ROOT ROT OF RASPBERRIES

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ABSTRACT

A number of Phytophthora species have been isolated from raspberries badly affected by root and crown rot. The most commonly occurring of them has close affinities with P. megasperma and other non-papillate species, and causes severe root and crown rot of all major raspberry cultivars. Most of the other species, which came largely from plants growing in badly drained sites, caused little or no damage in conventional pathogenicity tests, but some caused severe damage when the host plant had been exposed to long periods of waterlogging. Ridomil plus (metalaxyl + copper) controlled root rot in waterlogged and non-waterlogged plants.

INTRODUCTION

Root and crown rot of red raspberry is a serious problem in most important raspberry-growing areas of the world. It has been recorded in Germany (Seemuller et al., 1986), United Kingdom and Eire (Duncan et al., 1987), France (Nourrisseau & Baudry, 1987), United States (Converse & Schwartze, 1968) and Australia (Washington, 1988). There are also unconfirmed reports from a number of other European countries of severe outbreaks of root rot on raspberries.

A number of Phytophthora species have been implicated as the cause of root and crown rot, but one in particular is thought to be responsible for most of the more serious outbreaks. It has close affinities with several non-papillate species and has probably been given a variety of names, but in this paper will be referred to as the pathogenic P. megasperma (Duncan et al., 1987). The other species occur less frequently on raspberry and less is known about their pathogenicity. They may however be important partly because of the damage that they might do, but principally because their occurrence in nursery stocks could confound the diagnosis of infection with the pathogenic P. megasperma. Since the major spread of root rot appears to be by way of planting infected nursery plants, accurate diagnosis is important.

Many of the less frequently occurring species have been isolated from raspberries growing in badly drained or waterlogged sites, and this could be a factor in determining the levels of damage caused by them. In this paper the frequency of occurrence of the various species on red raspberry, and the effect of flooding on the damage that they produce is examined.

MATERIALS AND METHODS

Phytophthora spp. were isolated from raspberry material showing symptoms of root and crown rot using a selective medium (Montgomerie & Kennedy, 1983). Single-zoospore cultures were established from the

isolates and sporulated in an extract of soilless compost (Kennedy et al., 1986). The resultant zoospore suspensions were used to inoculate established root cuttings of various raspberry cultivars, but principally Glen Moy. Plants were inoculated with 50 ml of suspension (1000 zoospores ml^{-1}), and then kept in a controlled environment cabinet at 15°C (Duncan et al., 1987). For flooding experiments, plants were immersed in pails of tap water for varying periods immediately after inoculation. Where fungicides were used, they were applied as drenches to rooted cuttings before inoculation with the fungus.

The effects of the various inoculations/treatments on plants were measured through percentage height increase, percentage wilted leaves, fresh weight of roots, and root rot (either as percentage roots rotted, or as a score on a 0-5 scale).

RESULTS

Phytophthora spp. isolated from red raspberry

Details of the relative occurrence of various Phytophthora species on raspberry in UK in recent years are given in Table 1.

TABLE 1

Number of isolates of Phytophthora species recovered from raspberries affected by root rot in British Isles (1984-1988).

Species	Number of times isolated
Total number of samples	62
Pathogenic P. megasperma	46
P. megasperma v. megasperma	8
P. cambivora	5
P. cactorum	3
P. citricola	3
Phytophthora sp. (P. cactorum ?)*	3
P. drechsleri	2
P. syringae	1
unidentified species	2

*there were another 15 samples in which sporangia and oospores typical of this fungus were observed on or in roots, but from which the fungus was not isolated.

The principal species isolated was the pathogenic P. megasperma. This is a slow-growing species which can readily be distinguished from P. megasperma v. megasperma by a number of characteristics, including growth rate, morphology, and oospore and zoospore size (Duncan et al., 1987). P. cambivora and P. megasperma v. megasperma also occurred fairly regularly, and there was one other species the sporangia and oospores of

which were often seen on roots but which was rarely isolated. This species has been identified tentatively as P. cactorum, although the few isolates that have been recovered (see P. cactorum ? in Table 1) are somewhat atypical of this species, and grow very slowly in culture. Most of the other isolates came from plants from badly drained sites.

The pathogenicities of Phytophthora spp. to raspberry and the effects of flooding

Plants of the susceptible cv. Glen Moy were inoculated with zoospore suspensions of a number of Phytophthora species. To test the effect of flooding on their pathogenicities, half of the plants were flooded for four days thereafter. Assessments of disease were made three weeks later and are presented in Table 2.

TABLE 2

Percentage leaf wilt, fresh weight of roots and root rot scores* of Glen Moy raspberry plants 3 weeks after inoculation with Phytophtora spp. and 4 days flooding.

	Non-flooded			Flooded		
	%	root		%	root	
Inoculum	leaves wilted	fresh wt.(g)	rot index	leaves wilted	fresh wt.(g)	rot index
Uninoculated	33	16.7	0	35	14.4	0
P. megasperma v. megasperma	35	21.2	0.2	31	14.5	0.8
P. cactorum	39	15.9	1.0	36	12.7	2.0
P. syringae	37	16.9	1.0	38	9.9[b]	2.6
P. erythroseptica	35	13.9	1.2	34	6.4[a]	3.0
P. drechsleri	33	17.1	0.6	100[ab]	1.5[a]	5.0
P. cambivora	42	4.7[a]	3.2	100[ab]	2.6[a]	5.0
Pathogenic P. megasperma	100[a]	2.2[a]	5.0	100[a]	2.9	5.0

*Root rot score on a 0-5 scale: 0 = no root rot; 5 = 76-100%.
a = significantly different from equivalent uninoculated treatment (non-flooded or flooded as appropriate); b = significantly different from the equivalent non-flooded inoculated treatment.

The pathogenic P. megasperma caused severe root and crown rot in non-flooded conditions with symptoms consistent with those observed in the field. Under the same conditions only P. cambivora among the other species caused severe enough root rot to produce mild aerial symptoms. Flooding exacerbated the amount of root rot in all cases except the pathogenic

P. megasperma. The increases were mostly small, but there was a particularly marked effect with P. drechsleri and to a lesser extent with P. cambivora. In non-flooded conditions plants inoculated with P. drechsleri were virtually indistinguishable from the uninoculated controls, but flooded plants were killed within three weeks of inoculation. Flooding of uninoculated plants had only very small effects on their growth.

Extending the period of waterlogging increased the severity of disease produced by the less pathogenic species such as P. megasperma v. megasperma (Table 3), which then symptoms similar to those produced by the pathogenic P. megasperma in non-flooded conditions. As the period of flooding was extended much beyond four days the root systems of all plants, whether inoculated or not, were affected to some degree with a bluish-black rot.

TABLE 3

Effect of length of flooding period on the % leaves wilted and root rot on Glen Moy plants uninoculated or inoculated with an isolate of P. megasperma v. megasperma or the pathogenic P. megasperma.

	Uninoculated			P. megasperma v. megasperma			Pathogenic P. megasperma		
Days of flooding	% leaves wilted	root fresh wt.	root rot %	% leaves wilted	root fresh wt.(g)	root rot %	% leaves wilted	root fresh wt.(g)	root rot %
0	18	18.0	0	21	23.9	0	62[a]	4.7[a]	100[a]
2	19	19.1	0	23	19.8	0	61[a]	5.1[a]	100[a]
4	22	16.5	0	18	18.0	17[ab]	53[a]	5.4[a]	100[a]
8	20	12.9	0	44[ab]	3.5[ab]	97[ab]	63[a]	8.2[a]	100[a]

a = significantly different from equivalent uninoculated treatment;
b = significantly different from equivalent non-flooded treatment (0 days).

Control of root rot by fungicides

A number of fungicides have been tested in glasshouse trials for control of root rot by the pathogenic P. megasperma (Table 4). Many of them were formulated for the control of late blight of potato (P. infestans), and most gave effective control of raspberry root rot.

The tests detailed in Table 4 were done under non-flooded conditions, and to test the effects in flooded conditions plants of cv Glen Moy were treated with metalaxyl + copper oxychloride (treatment 6 in Table 4), inoculated with several Phytophthora spp., and then flooded for two days. The results are given in Table 5. Again the fungicide was effective in controlling root rot by all three species used in the experiment, in non-flooded and flooded conditions.

TABLE 4

Effect of various fungicides applied as drenches to pot grown raspberry plants inoculated with the pathogenic P. megasperma.

Material	Equivalent product rate* kg per hectare	% height increase	% wilted leaves	% root rot
1) Nil (Uninoculated)	-	61[a]	31[a]	0[a]
2) Nil (Inoculated)	-	45	68	98
3) Chlorothalonil (50% ai w/v)	12.5	57	41[a]	90
4) Cymoxanil (5.25% ai) + MnZn dithiocarbamate (71.6%)	23.0	51	23[a]	42[a]
5) Ofurace (5.75% ai) + MnZn dithiocarbamate (67% ai)	21.0	62[a]	25[a]	11[a]
6) Metalaxyl (15% ai) + copper oxychloride (35%)	8.0	53	23[a]	6[a]
7) Etridiazole (35% ai)	12.5	57	24[a]	0[a]
8) Oxadixyl (10% ai) + MnZn dithiocarbamate (56%)	12.0	60[a]	29[a]	0[a]
9) Oxadixyl (25% ai)	4.8	61[a]	34[a]	0[a]
10) Fosetyl Al (80% ai) drench	3.8	64[a]	35[a]	0[a]
11) Fosetyl Al (80% ai) foliar≠	3.8	65[a]	31[a]	0

*for fungicides containing phenylamides the rates were based on a standard weight of the phenylamide component of 1.25 kg per hectare

≠applied as foliar sprays

a = significantly different from inoculated controls.

TABLE 5

Effect of treatment with metalaxyl + copper fungicide* on root rotting caused by three Phytophthora species on cv Glen Moy raspberry plants subjected to 2 days flooding.

Treatment		Non-flooded %			Flooded %		
Inoculum	Fungicide	height increase	leaves wilted	root rot index	height increase	leaves wilted	root rot index
uninoculated	-	43	28	0	50	27	0
	+	72	26	0	45	30	0
P. drechsleri	-	70	25	1.5^{a}	22	30	3.0^{ac}
	+	64	27	0.3^{b}	56	33	0.3^{b}
P. cambivora	-	11	56^{a}	4.0^{a}	10	62^{a}	4.8^{ac}
	+	63	22^{b}	0^{b}	39	33^{b}	1.8^{b}
Pathogenic P. megasperma	-	9	81^{a}	5.0^{a}	11	65^{a}	5.0^{a}
	+	71	29^{b}	0.3^{b}	61	33^{b}	1.3^{ab}

* metalaxyl + copper (15% + 35% ai) at the same rate as in Table 4.
a = significantly different from equivalent uninoculated control:
b = significantly different from equivalent treatment without fungicide:
c = significantly from equivalent non-flooded treatment.

DISCUSSION

The survey of affected raspberry stocks and the pathogenicity tests show that of all the species of Phytophthora isolated from raspberry, the pathogenic P. megasperma and, to a lesser extent P. cambivora, pose the most serious threat to the crop. Nevertheless the other species can cause damage like that caused by the pathogenic P. megasperma, especially in badly drained or flooded conditions.

Most of these species, such as P. cactorum and P. drechsleri, cause disease on other crops, so they probably occur widely in soils and their occurrence on raspberry is not surprising. Their importance in the context of raspberry root rot may lie mainly in obscuring the diagnosis of infection by the pathogenic P. megasperma. Any damage that they themselves cause, may be limited to small areas of fields, and could be controlled by fungicides.

The pathogenic P. megasperma is now widespread in British raspberry plantations, almost certainly because infected planting material has been distributed from diseased nursery beds. Ensuring that stocks are free of it will require the development of rapid and reliable detection techniques. Microscopic examination of roots together with isolation onto selective media has been used to detect its presence but this is time-consuming,

requires some expertise and small amounts of tissue only can be handled. A possible alternative is an enzyme-linked immunosorbent assay (ELISA), such as that developed for P. fragariae (Mohan, 1988). This has been used to detect infection of raspberries by several Phytophthora species (unpublished). However the problem of specificity still remains since polyclonal antisera are generally unable to discriminate between different Phytophthora species. New systems based on monoclonals and/or antigens produced only in plants infected with the pathogenic P. megasperma are being developed.

The effect of flooding in exacerbating damage has been noted for other diseases caused by Phytophthora spp. (Wilcox & Mircetich, 1985). It could operate by reducing the resistance of the host or by promoting sporulation and growth of the pathogen, but probably by a combination of these factors. Both merit further attention in relation to raspberry root rot.

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DELIVERY OF MICROBIAL INOCULANTS INTO THE ROOT ZONE OF TRANSPLANT CROPS

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ABSTRACT

A simple method is described for applying alginate gel to the roots of transplant crops or to the bases of cuttings. The gel provides a delivery system for microbial inoculants or chemicals and can also protect wounds by physical exclusion of pathogens. Applications of the method for inoculation of tree seedlings with ectomycorrhizal fungi are described. Other potential applications, including control of crown gall and of disease caused or transmitted by zoosporic fungi, are discussed.

INTRODUCTION

Microbial inoculants have the potential to enhance plant growth, either directly (e.g. Rhizobium and mycorrhizal fungi) or indirectly by controlling deleterious organisms. But few inoculants have been exploited commercially. One reason for this is the difficulty of delivering them into the root zone or to other plant surfaces such that they can establish and grow in competition with indigenous organisms.

Recent work has involved the use of alginate gels or other polymers for delivery of microbial inoculants (Kierstan & Bucke, 1977; Walker & Connick, 1983; Fravel et al., 1985; Le Tacon et al., 1985; Bashan, 1986; Lewis & Papavizas, 1987). For example, microbes can be incorporated into a solution of sodium alginate, then gel beads are produced by dripping this into a solution of a calcium salt. Most microorganisms survive entrapment and at least temporary storage in the gel matrix (references as above). Fungal spores are reported to germinate readily when gel beads are incorporated into soil, producing a dense mycelial ball around each bead.

A simple variation on this procedure is described here, whereby the gel is created directly on a plant surface. The use of the method is illustrated by studies on inoculation of tree seedlings with mycorrhizal fungi. In separate studies, the gel alone was found to protect chrysanthemum and tomato cuttings from infection by the crown gall bacterium, Agrobacterium tumefaciens. The technique has many potential applications for protection of wounds against pathogenic invasion, and for delivery of both microbial inoculants and chemicals into the root zone of transplanted crops.

BASIC METHOD

Parts of plants to be treated are dipped into a 1% (w/v) solution of sodium alginate, then into a 1% solution of calcium chloride, which results in the deposition of a firm gel of calcium alginate on the plant surface. Microbial inocula or appropriate chemicals are added to the sodium alginate solution so that they are incorporated into the gel.

The gel can easily be applied to the bases of cuttings, to bare roots of seedlings and potentially to the intact peat or soil block of module-raised plants. The concentration of sodium alginate can be reduced to 0.5% or less and that of calcium chloride to 0.1 or 0.2% depending on the degree of firmness required of the gel.

In the following experimental studies, laboratory grade sodium alginate (Sigma Chemical Co. Ltd., Poole) was used, but several trials have shown that a cheap commercial source of alginate ("Manutex KPR", Kelco International Ltd., London) is equally effective.

APPLICATIONS FOR ECTOMYCORRHIZAL FUNGI

Field comparison of two gel-delivery systems

One-year old seedlings of Sitka spruce were lifted from a commercial seedbed in a tree nursery (Economic Forestry Group, Maelor, Shropshire) on 25 March 1986, with a minimum of soil adhering to the roots. The beds had been fumigated with "Basamid" before the seed was sown and the plants had become infected naturally with mycorrhizal fungi typical of nursery conditions. Of the mean 268 root tips per seedling, 76% were uninfected, 12% bore mycorrhizas of Thelephora terrestris, 9% had an unidentified mycorrhizal type and 3% were moribund.

The seedlings were transplanted to a "lining-out" bed, for a further season's growth, which is normal nursery practice. Treatments, applied immediately before transplanting, included inoculation with two mycorrhizal fungi, Hebeloma subsaponaceum and Thelephora terrestris, grown for 10 wk at 25°C in sterile vermiculite-peat mixture moistened with modified Melin & Norkrans solution (Marx, 1969). Inocula were applied by dipping root systems in two gels: Laponite (Fluid Drilling Ltd., Leamington Spa) (500 ml inoculum mixed in 500 ml water with 9.3 g Laponite) or alginate (500 ml inoculum in 400 ml water with 4 g sodium alginate, followed by dipping in 0.6 g $CaCl_2$ in 200 ml water). Controls consisted of (1) sterile vermiculite-peat in Laponite or alginate, (2) unamended Laponite or alginate and (3) no treatment. The experiment was of randomized block design, with 96 replicate seedlings distributed in 8 blocks, the seedlings being spaced 20 cm apart. The soil was a sandy loam and fertilizer was applied by the nursery staff as in normal practice.

Twenty-four plants per treatment were sampled after 6 months (25 September 1986) and the whole root system was examined microscopically for occurrence of mycorrhizas. Plants from all controls had a predominance of Thelephora mycorrhizas, with mycorrhizas of an ascomycete being sub-dominant, and a small proportion of mycorrhizas had been formed by Hebeloma sp. or were of an unidentified type (designated "T") (Table 1). All these mycorrhizas developed from naturally occurring inocula and they are typical of mycorrhizas that occur in the nursery.

TABLE 1

Percentage (as arcsine) of total root tips bearing mycorrhizas and percentage of all mycorrhizas attributable to different fungi, on Sitka spruce seedlings 6 months after inoculation by root dipping in alginate (Alg) or Laponite (Lap) gels and transplanting of seedlings to a nursery bed*

	Gel type	Inoculation treatment**					SED ($t_{0.05}$ =1.96)
		Heb	Thel	Gel	Gel+vp	None	
Tips with mycorrhizas	Alg	64.1	62.9	56.4	56.4	60.5	3.08
	Lap	59.9	64.0	56.6	62.6	60.5	
Mycorrhizas of different types							
Hebeloma	Alg	60.7	1.5	5.0	0	5.7	4.56
	Lap	76.6	0	3.5	5.3	5.7	
Thelephora	Alg	8.8	76.8	58.1	45.1	58.4	7.69
	Lap	9.7	86.6	63.9	62.7	58.4	
Ascomycete	Alg	18.0	11.8	24.2	15.6	20.8	7.11
	Lap	4.5	3.4	17.8	14.6	20.8	
Type "T"	Alg	3.1	0	4.9	33.0	4.4	3.97
	Lap	0.7	0	8.6	7.9	4.4	

* Means of 24 replicate plants
** Heb = Hebeloma subsaponaceum; Thel = Thelephora terrestris; Gel = gel alone; Gel+vp = gel + sterile vermiculite-peat; None = no treatment

Plants inoculated with Thelephora had a high percentage of Thelephora mycorrhizas, though not always significantly different from that of controls, a low percentage of ascomycete mycorrhizas and virtually no other mycorrhizal types on their roots. In contrast, plants inoculated with H. subsaponaceum had a high percentage of Hebeloma mycorrhizas (significantly different from all controls) and a low percentage of Thelephora mycorrhizas (again significantly different from all controls). Both carrier gels were equally effective in establishing the inoculants.

Compared with all controls, there was a slight increase in total mycorrhizal status in some inoculant-gel combinations. There was no significant increase in plant growth following inoculation, but this was expected in view of the early sampling of the experiment.

Glasshouse comparison of two gel-delivery systems

An identical experiment to that above was done in a glasshouse at Edinburgh, using seedlings from the fumigated nursery beds. They were planted on 11 April 1986 in 9 cm diam pots containing a brown earth soil of low fertility from a tree-less hill pasture (Castlelaw). The plants were incubated at 14-22°C beneath 400 W mercury lamps supplying 10 W m^{-2}, 16 h daylength; they received no fertilizer. Of the original 16 replicates, 8 were sampled on 26 August 1986 (20 weeks); the others were incubated for longer and monitored for shoot growth before being used in further experiments.

TABLE 2

Percentage (as arcsine) of all mycorrhizas attributable to different fungi, on Sitka spruce seedlings 20 weeks after inoculation by root dipping in alginate (Alg) or Laponite (Lap) gels and transplanting of seedlings to soil in a glasshouse*

Mycorrhizal type	Gel type	Inoculation treatment**					SED ($t_{0.05}$=1.96)
		Heb	Thel	Gel	Gel+vp	None	
Hebeloma	Alg	58.0	0	0	0	0	2.5
	Lap	6.1	0	0	0	0	
Thelephora	Alg	18.6	49.6	42.7	46.7	34.8	3.2
	Lap	33.1	47.0	28.5	50.2	34.8	
Ascomycete	Alg	0	0	0	2.6	0	9.2
	Lap	5.0	6.5	0	0	0	
Type "T"	Alg	0	5.1	6.8	2.2	8.5	8.5
	Lap	20.6	5.6	18.6	4.4	8.5	
Trichoderma	Alg	22.9	38.9	44.5	40.2	46.8	9.2
	Lap	34.4	36.9	46.1	37.3	46.8	

* Means of 8 replicate plants
** Heb = Hebeloma subsaponaceum; Thel = Thelephora terrestris; Gel = gel alone; Gel+vp = gel + sterile vermiculite-peat; None = no treatment

The results for alginate-based inocula were similar to those from the field trial. Hebeloma mycorrhizas established well from inoculum of H. subsaponaceum, and the development of other mycorrhizal types was correspondingly reduced in this treatment (Table 2). However, Hebeloma mycorrhizas did not establish to any large degree from Laponite-based inocula.

There was no significant effect of inoculation treatment on seedling growth after 20 weeks. But after 31 weeks (14 November 1986) there was a significant growth response to inoculation with H. subsaponaceum in alginate gel (Table 3).

TABLE 3

Heights (mm) of seedlings (originally 86 ± 1.7 mm) 31 weeks after inoculation and transplanting into pots of brown earth soil in a glasshouse (means of 7 replicates)

Carrier gel	Inoculation treatment					SED ($t_{0.05}$=2.0)
	Heb	Thel	Gel	Gel+vp	None	
Alginate	192	142	134	153	138	9.4
Laponite	144	139	143	142	139	

Field trial with alginate gel in commercial conditions

A second field trial, planted 7 May 1986, again used one-year old seedlings lifted from nursery beds, but stored in a cold room for one week. They were randomized into four batches: control (no treatment), alginate gel treatment (no inoculum), alginate gel containing H. subsaponaceum and alginate gel containing T. terrestris. The inocula had been grown for 20 weeks and were used at twice the previous concentration (500 ml in 200 ml water); both sodium alginate and calcium chloride were used at 1% concentration. Immediately after treatment, the seedlings were planted by nursery workers, using a commercial lining-out machine, into a non-fumigated bed of clay-loam soil. The trial comprised four blocks, each an 8 m length of lining-out bed (120 cm wide) with 6 rows of seedlings along each bed, the rows being 20 cm apart. Each treatment was planted as a row of seedlings (20 plants m^{-1} length of bed) in each block.

On sampling in April 1987, after 11 months, the untreated control and gel-control plants bore a range of mycorrhizal types (Table 4) predominantly formed by ascomycetes, one of which was distinctive and was associated with ascocarps of Amphimena in parallel glasshouse experiments. Thelephora mycorrhizas were uncommon. Inoculation with T. terrestris had no significant effect on mycorrhizal status of the plants, presumably because the soil type in this part of the nursery was unsuitable for the development of Thelephora mycorrhizas. Inoculation with H. subsaponaceum resulted in a dominance of Hebeloma mycorrhizas, a marked reduction in numbers of ascomycete mycorrhizas, and the complete suppression of development of other mycorrhizal fungi (Table 4).

Inoculation with H. subsaponaceum led also to an increase in total number of mycorrhizas on the plants and to significant increases in plant height, shoot dry weight and root dry weight compared with that in all other treatments (Table 4).

TABLE 4

Percentage (as arcsine) of all root tips of Sitka spruce seedlings with different mycorrhizal types (or uninfected), and assessments of seedling growth, 11 months after inoculation with alginate gel and machine-transplanting of seedlings to a nursery bed*

Mycorrhizal type	Inoculation treatment**				SED ($t_{0.05}$=1.96)
	Heb	Thel	Gel	None	
Hebeloma	54.1	10.4	12.5	7.4	3.8
Thelephora	0	4.3	1.2	1.2	2.0
Amphimena	0	8.8	14.6	9.7	4.1
Ascomycetes (various)	14.8	34.5	30.8	37.0	3.3
Uninfected tips	10.6	12.4	11.0	19.5	2.6
New (uninfected) root tips**	25.3	37.4	36.8	35.0	2.5
Shoot height (mm)	327	276	278	278	11.0
Shoot dry wt (g)	6.57	4.38	4.65	4.22	0.46
Root dry wt (g)	3.31	2.66	2.60	2.35	0.26
Total no. of mycorrhizas	306	200	210	212	34.4

* Means of 30 replicate plants
** Heb = Hebeloma subsaponaceum; Thel = Thelephora terrestris; Gel = gel alone; None = no treatment
*** New tips that were too young to have mycorrhizal sheaths

Discussion

In all three experiments, and in numerous smaller tests, the alginate delivery system was effective in establishing mycorrhizas of inoculant fungi, provided that the experimental or site conditions were suitable for development of these. It is well known that different mycorrhizal fungi have different soil or site preferences (Trappe, 1977); this probably explains why Thelephora failed to establish mycorrhizas in one of the field trials, where the level of establishment from indigenous inoculum of this fungus was also low.

It is notable that in all experiments the seedlings were originally mycorrhizal with fungi indigenous to the nursery and that the inoculants, particularly H. subsaponaceum, established in competition with the existing mycorrhizal fungi and with inoculum naturally present in the lining-out beds. This contrasts with the results of Le Tacon et al. (1983) who added alginate beads containing mycelium of Hebeloma cylindrosporum to soil: their inoculum was effective in establishing mycorrhizas on newly-emerging seedlings in fumigated seedbeds but not in non-fumigated beds. Almost all of the mycorrhizas recorded in the present work would have been formed on root tips that developed after transplantation. The success of the inoculation method probably lies in the fact that the new roots grew through the gel containing the inoculum, ensuring early, massive establishment of the inoculant fungus.

Alginate may not necessarily be better than other gels such as Laponite, but the failure of Laponite in one experiment (Table 2) led us to abandon this treatment. Moreover, alginate has the practical advantage that it immediately forms a firm gel on treatment with calcium, whereas Laponite formed a viscous fluid from which inoculum particles were easily dislodged during handling.

APPLICATIONS FOR WOUND PROTECTION AGAINST PATHOGENS

In a series of experiments described in Deacon et al. (1988) the bases of stem cuttings of chrysanthemum and tomato were treated by successive dipping in solutions of sodium alginate and calcium chloride (with no supplements) and then immersed in suspensions of pathogenic A. tumefaciens before being planted in soil. Carrot disks were similarly treated before being inoculated with A. tumefaciens.

In all instances, the gel coating of wounds gave significant protection against infection by crown gall. The protection was sometimes as good as that provided by the biocontrol strain K84 of A. radiobacter, and sometimes less effective but still significant in comparison with untreated control plants. The gel also protected plants against infection by a strain of the pathogen insensitive to control by strain K84. Sodium alginate alone (without calcium treatment) was ineffective, showing that the main effect of the gel was physically to exclude the pathogen from wound surfaces.

FURTHER POTENTIAL APPLICATIONS

The simplicity and cheapness of the method described here suggests that alginate coating of roots or stem bases could prove valuable in many nursery operations. In limited tests with tomato and chrysanthemum

cuttings, there was no evidence that alginate gel delayed root initiation or adversely affected plant growth in any way (Deacon et al., 1988).

The gel matrix is retained on the treated plant surface and can give significant protection against invasion of wounds by pathogens as well as being a vehicle for the delivery of microbial inoculants. It could potentially be used to place fungicides, pesticides, plant hormones and other compounds in immediate contact with roots, reducing losses of the compounds during handling and also minimising hazards to workers.

Another potential application is to coat peat blocks of module-raised plants when these are transplanted to field sites - by dipping of modules or by spraying the components of the gel. This merits attention for control of diseases caused or transmitted by zoosporic fungi (e.g. clubroot, soil-borne viruses), where it is known that delayed infection improves the yield of crops in infested soils (e.g. Asher, 1988). Alginate induces encystment of the zoospores of Phytophthora spp. (Irving & Grant, 1984) and Pythium aphanidermatum (S. W. Jones, personal communication) which might be killed by toxicants incorporated in the gel. These and other prospects raised by the availability of gel technology warrant further study.

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