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Issues and Prospects

Chairman and Session Organiser:
P. R. DAY

PLANNED RELEASE OF GENETICALLY MANIPULATED PLANTS AND MICRO-ORGANISMS - SOME REGULATORY ASPECTS

B P AGER
Secretary, Advisory Committee on Genetic Manipulation, Medical Division, Health and Safety Executive, Baynards House, 1 Chepstow Place, London W2 4TF

SUMMARY

It has been recognised for some time that the use of genetically manipulated organisms is likely to bring considerable benefits in agriculture[1]. This type of application will necessarily involve the release of genetically manipulated organisms into the environment and proposals for such work have posed questions of potential hazard to human, animal and plant health and of environmental impact in general.

This paper describes the approach being taken by the Health and Safety Executive (HSE), as advised by the Advisory Committee on Genetic Manipulation (ACGM)* in collaboration with relevant government departments, on the need for regulations and guidance in this area. The outcome of this activity is that guidelines on planned release are expected soon to be issued by HSE. These guidelines essentially comprise a framework for the notification of individual projects for case by case consideration by ACGM, HSE and relevant government departments, together with a list of factors to assist initial local risk assessment by those undertaking such work.

International activities, notably an OECD study on safety and regulations in biotechnology, have been taken into account during the development of these guidelines. As a consequence, the approach being taken to this issue in the UK is expected to be broadly in line with that being adopted in other countries.

INTRODUCTION

The techniques of genetic manipulation or recombinant DNA have been with us now for over 10 years. The development of these techniques was accompanied by safety concerns as originally witnessed by the Gordon and Asilomar Conferences in the USA in the early 1970's and in the subsequent development of national advisory bodies such as the US Recombinant DNA Advisory Committee (RAC) and the UK's Genetic Manipulation Advisory Group (GMAG). In their respective countries these bodies established guidelines for the risk assessment of the conjectured hazards of genetic manipulation work together with guidance as to how such work should be physically contained within the laboratory. In setting up such guidelines, bodies such as RAC and GMAG facilitated the development of genetic manipulation by providing a framework within which to work that met the needs of scientists, the public, employers, employees and occupational health and safety agencies such as the Health and Safety Executive. As experience

*ACGM consists of an independent Chairman, representatives of employers and employees, together with scientific specialists. It was set up to replace GMAG in 1984 and it advises the Health and Safety Commission and the Health and Safety Executive and government departments where necessary.

accumulated and the conjectured laboratory hazards of genetic manipulation remained conjectured and not based on incident, so the guidelines were relaxed by RAC, GMAG and other bodies worldwide.

We are now reaching a "second phase" in that genetically manipulated micro-organisms are being grown on a large scale by industry for example to produce valuable protein molecules that could only be previously obtained by low yield traditional chemical means or by tissue extraction. Of more relevance to this symposium, there is also now considerable interest in releasing genetically manipulated plants and micro-organisms for agricultural and environmental purposes. This promises considerable benefit such as the improvement of crop yields by for example the use of genetically manipulated micro-organisms to introduce growth factors and nutrients or by the direct introduction into plants of herbicide and disease resistance genes and the use of manipulated micro-organisms to protect crops against pests and other environmental stresses.

Several proposals[2] involving the planned release in the USA of genetically manipulated micro-organisms have been widely reported as have the attendant legal struggles between the Foundation on Economic Trends and the National Institutes of Health[3] and the inter-agency debate[4] on biotechnology regulation. This movement from the laboratory bench to the large-scale fermenter and the open field has reopened the debate on genetic manipulation safety and as before, national expert advisory bodies and regulatory agencies are moving towards establishing frameworks which it is to be hoped, whilst not unduly hindering such applications, assure that due regard is paid to any potential undesirable effects.

There are however significant differences between the second phase and the original debate in the early 1970's especially as far as planned release is concerned. First, although to date we have an excellent track record for genetic manipulation with regard to occupational health and safety, in contrast to laboratory or large-scale work, it is not possible to underwrite concerns about the planned release of genetically manipulated organisms with physical containment. Whilst the containment of glasshouse facilities is a reality, the same is hard to envisage for a 50 acre field! Second, in order to ensure adequate survival of a released micro-organism it may prove unrealistic to use disabled strains of the sort that give a safety margin and often allow a reduction in physical containment at the laboratory level. Third, and again in contrast to the laboratory and the large-scale fermentation situations, risk assessment of planned release projects have to take the complexities of environmental interactions into account.

Against this background the UK Advisory Committee on Genetic Manipulation shortly after its establishment in 1984 advised that planned release should be given priority consideration and a specialist Working Group of ACGM was set up accordingly. This included representatives from relevant government departments, ie MAFF, DoE and DHSS and appropriate scientific expertise. ACGM has now completed its deliberations and subject to the endorsement of the Health and Safety Commission and relevant government departments, guidelines for planned release in the UK should be issued early in 1986.

UK REGULATORY POSITION WITH REGARD TO GENETIC MANIPULATION

Before considering planned release specifically and discussing the emerging guidelines, it may be helpful to outline the requirements relating to the health and safety aspects of genetic manipulation in general.

The current UK health and safety requirements applicable to genetic manipulation are based on general legislation, specific notification regulations, published guidelines and on-site inspection of health and safety standards by HSE. The requirements of the Health and Safety at Work etc Act 1974 cover genetic manipulation techniques and their application. The Act places a general duty on the employer requiring,

> "the provision and maintenance of a working environment for his employees that is, so far as is reasonably practicable, safe without risks to health and adequate as regards facilities and arrangements for their welfare at work."

The employer is also charged with a duty to avoid exposure of those not in his employment, including the general public, to risks.

It should be noted that these general duties are qualified by the phrase:

> "........ so far as is reasonably practicable ...."

In essence, the employer must make a cost-risk analysis and assess, on the one hand, the risk of the work and, on the other hand, the difficulty and expense involved in avoiding that risk. Thus greater risks require greater precautions. The theme of the Act is one of getting the right balance with a clear emphasis on self-regulation.

Aside from the general requirements of the Health and Safety at Work etc Act, the Health and Safety (Genetic Manipulation) Regulations 1978 (currently under review) require the notification to HSE of intention to carry out genetic manipulation as defined and the provision of details of individual experiments. Individual proposals submitted under these Regulations are considered by HSE and circulated to members of ACGM. Generally speaking, ACGM and its HSE Secretariat concentrates on the biological aspects of a particular proposal and HSE's specialist Inspectors deal with physical containment aspects during on-site inspection.

There are various guidelines published by HSE indicating how an employer may discharge his responsibilities under the Health and Safety at Work etc Act. For genetic manipulation detailed guidelines drawn up by the GMAG and its successor body ACGM are available. In effect, these indicate how to achieve the right balance between the level of risk and the cost of taking precautions to avoid that risk under the general requirements of the Health and Safety at Work etc Act. It should be noted that such guidelines are not themselves inflexibly enshrined in regulations.

RELEVANCE OF CURRENT POSITION TO PLANNED RELEASE

I mentioned earlier that notification to HSE is required of intention to carry out genetic manipulation as defined in the Health and Safety (Genetic Manipulation) Regulations 1978. The definition given in those Regulations is:

"Genetic manipulation means the formation of new combinations of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell into any virus, bacterial plasmid, or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation."

It is clear, therefore, that only the construction of recombinants within this definition requires notification to HSE under these Regulations. The use or application of such organisms, eg in large scale fermentation or planned release falls outside the scope. There is, however, a non-mandatory notification scheme for large scale fermentation but, other than a request by GMAG in its Third Report[1] to be kept informed at the earliest possible opportunity of any planned release proposals, there has been to date no notification requirement for such work. There are, however, notification requirements under the Pesticide Safety Precaution Scheme and the Plant Health Act 1967[5] that may be relevant for some release applications.

Lastly under this section it may be useful to focus briefly on HSE's role in the consideration of planned release. Broadly speaking, HSE is concerned with the oversight of hazards to humans that arise out of a work activity. This does not extend to animal or plant health or to the well being of the environment in general. Furthermore, it could be argued that animal, plant and environmental effects constitute the majority of the potential concerns associated with planned release. These areas are the responsibilty of government departments such as MAFF and DOE. However, HSE has a central role to play in the development of guidelines on planned release and this stems from the fact that the most appropriate national expert body to draw up guidelines and to consider release proposals is ACGM. This committee reports to the Health and Safety Commission and the Health and Safety Executive and has its Secretariat in HSE.

EMERGING ACGM GUIDELINES ON PLANNED RELEASE

So what will be the shape of ACGM's guidelines for planned release? The committee's view can be summarised as follows:

- the potential benefits of such applications are clear, but there needs to be a framework for determining whether there are likely to be any associated, potentially deleterious effects.

- although there is experience of the introduction of naturally occurring organisms to ecosystems where they are not native, there is little information directly relevant to the release of genetically manipulated organisms.

- although it is not yet possible to devise a single, broadly applicable risk assessment scheme, it is possible to identify factors that should be taken into account during risk assessment. Such assessments may in some cases be complex.

- after initial local risk assessment, proposals for such work must, for the present, be considered on a case by case basis by ACGM, HSE and relevant government departments before the work begins.

An important point to emphasise is that such guidance is intended to facilitate applications for planned release whilst ensuring proper regard is paid to potential concerns by providing a framework for consistent risk assessment and for consideration of individual proposals by an expert national body. This approach follows the pattern set for laboratory work with a notification requirement and flexible guidance on detailed procedure.

Some of the foreseeable benefits of planned release will be covered by other speakers at the symposium and others have been detailed elsewhere[6]. Potential problems associated with release have also been widely discussed[7] but in summary, the Committee's view was that although there is little evidence that the majority of organisms that have been bred purposely then introduced by man would be able to survive in the wild, eg cereals, domestic farm animals and the like, deliberate introduction of novel types to foreign habitats could disturb the natural equilibrium of those habitats. Furthermore, deleterious effects associated with the release of genetically manipulated organisms could conceivably occur if there were either unanticipated and undesirable characteristics in the novel organisms or, transfer of genetic material to other host organisms which then promoted such an effect. The release of any novel organism will involve introduction not of individuals, as with natural mutation, but of relatively large numbers. This must increase the chances of the manipulated nucleic acid being introduced into natural populations through gene exchange.

The definition and scope of the guidelines proved difficult. It was recognised that simply to adopt the UK definition as given earlier would exclude novel organisms constructed by other means that may carry similar conjectured concerns. ACGM believes that in relation to the release of organisms constructed with novel combinations of genes, the guidelines should be applied not only to those live organisms whose construction were to fall within the above definition, but also to organisms constructed by techniques such as cell fusion, conjugation, micro-injection, and micro-encapsulation which may otherwise fall outside the scope of the definition. The guidelines are also meant to be applied to the use of potentially infective nucleic acid molecules and to organisms that have undergone intentional gene deletion but not to organisms produced by classical methods of strain improvement. The scope of the guidelines is intended to cover not just release into the environment at large but any trial outside the laboratory in enclosed but non-contained facilities. In other words, a broadening of the UK definition of genetic manipulation for this specific purpose; the underlying view being that possession by an organism of intentionally constructed novel combinations of genes should warrant the application of the guidelines.

So what do the guidelines actually require from those who intend to release such organisms? The central requirement is that although there is at present no statutory obligation, notification should be made to HSE and thence ACGM for case by case consideration before release takes place. Such notification should include a local initial risk assessment. On receipt by HSE notifications will be passed to relevant government departments for their views. This does not affect the need to respond where necessary to other existing notification requirements, eg Pesticide Safety Precaution Scheme, Plant Health Act. In those circumstances the

notified agency will consult with HSE/ACGM. HSE will aim to give a swift response (within 30 days where there are no other notification requirements). There are arrangements for handling commercial-in-confidence information established by the Health and Safety Commission for Advisory Committees such as ACGM.

Because of lack of experience, the complexity of environmental interactions and the variety of likely applications it is not yet possible to devise a broadly applicable risk assessment scheme. However, there are a number of factors that can be identified to direct and assist the proposer in making his initial risk assessment on a case by case basis. The guidelines list a number of such factors although it is not expected that for any particular release proposed all will be relevant. Examples are:-

- the nature of the organism or the agent to be released, ie the species (or cultivar), its host range and pathogenicity (if any) to man, animals, plants or micro-organisms.
- the procedure used to introduce the genetic modification.
- the nature of any altered nucleic acid and its source, its intended function/purpose and the extent to which it has been characterised.
- genetic stability of the novel organism.
- details of any target biota (eg pest in the case of pest control agent).

INTERNATIONAL PERSPECTIVE

Biotechnology is an international industry and there are advantages in harmonisation of national approaches towards safety and regulations in this area. Such harmonisation should help to reduce barriers to scientific development and to trade in the field of biotechnology. It is therefore reassuring to note for example, that emerging guidelines in the USA[8] and Australia[9] seem to be on similar lines to the UK approach outlined in this paper. Furthermore, a major international study by the Organisation for Economic Co-operation and Development (OECD), set up in late 1983 and comprising government experts from 24 OECD Member Countries has recently been completed. The study focussed on safety concerns behind industrial, agricultural and environmental rDNA applications and the emerging UK position is in line with its conclusions and recommendations. Although the recommendations of the OECD are not binding on member countries, there is no doubt that the outcome of such a study will have considerable influence on the development of approaches to the planned release of genetically manipulated organisms in many nations.

CONCLUDING REMARKS

HSE, ACGM and relevant government departments are developing a framework for the notification of individual planned release projects prior to commencement of work. The details of such projects will be considered on a case by case basis by ACGM, HSE and relevant Government departments before a response is sent to the notifier. This notification requirement is not

at present legally enforceable. In some examples, eg pesticides, plant pathogens, there may be other existing notification requirements. This approach parallels that already taken for laboratory genetic manipulation and large scale growth of manipulated micro-organisms.

This requirement is set out in guidelines which are expected soon to be issued by HSE. They include a list of factors to be considered locally when initial risk assessment of each project is made prior to notification. Such guidelines will be subject to review by ACGM in due course. The establishment of such a framework is intended to strike the right balance between avoiding undue restraint on this important development in the application of genetic manipulation and ensuring that appropriate consideration is given to the potential concerns that may be associated with this type of work.

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PATENT ISSUES IN BIOTECHNOLOGY

R. S. CRESPI

British Technology group, London, United Kingdom.

ABSTRACT

Patenting in biotechnology is subject to wide international legal variation. It follows and extends precedents set in agrochemical patenting but struggles with problems of description of the more complex biologically active materials including micro-organisms. Deposition of cultures provides a legal solution but entails risks which are detrimental to commercial interests. These arise from the steps and typical timescales involved in patent procedure. The types of patent obtainable in classical biotechnology are well established. The more recently developed techniques of constructing new organisms offer even more variety of possible protection but the validity and permissible scope of such patents remains to be tested legally. Plant genetic manipulation focusses attention on the choice between patent or plant variety right as the most appropriate and effective form of legal protection.

INTRODUCTION

Most statements about patent law carry geographical limitations. This is especially true for inventions in biotechnology where the law is not uniform among the national patent systems of the world including those of industrialised countries as well as those of developing countries. For information in greater depth on this subject an OECD report(1) has recently issued which analyses and reviews the subject internationally and recommends greater harmonisation of law on the major issues affecting biotechnology inventions. In this simplified overview, I will attempt to throw light on some of these issues and various facets of European, United States and Japanese patent law relevant to the topics focussed at this symposium. This is a rather broad focus since innovation in this area embraces many disciplines.

CHEMICAL INVENTIONS

The patenting of chemical compounds having biological activity against insects and other crop pests, or as herbicides, or other useful agents in the field or greenhouse is now well established, allowing us to differentiate between countries which allow patents for new products *per se* and those which offer protection only for particular processes of manufacture. Where the products are not new materials as such patent protection can be obtained for pesticidal and other formulations of the active material or for methods of treatment of crops or soil utilising such materials. The principles of patent law for chemical compounds and chemical processes, which have been established mainly from court decisions, have shown how the legal criteria of novelty, inventiveness, and utility are to be applied in the relatively simple areas of inorganic and organic chemistry. There must be novelty of chemical structure or in the process of manufacture or use and the degree of difference from the prior art must be sufficient to dispose of the frequent charge of obviousness. Unexpected utility or level of activity are key factors in the typical arguments on these issues.

The experience derived from chemical patenting can be extrapolated to other useful biological agents of crop control and improvement having more complex constitutions but these entail the additional burden of the disclosure requirement in patent law, i.e. these products must be identified and defined in suitable physical, chemical, biological or other terms acceptable to patent law. The requirement for adequate description and characterisation, which can be met relatively easily in traditional agrochemical inventions, raises serious difficulties in the patenting of the more complex materials and these have become particularly troublesome where micro-organisms and higher organisms of the plant or animal kingdoms are involved. The informational purpose of the patent system is to leave on record a description of an invention which may be put into practice by skilled persons when the period of protection expires (the patent specification). With living material this cannot often be achieved from the written word alone and it has therefore been necessary to expand the law to cover biological inventions.

BIOLOGICAL INVENTIONS: DEPOSITION OF MICRO-ORGANISMS

It is in the field of microbiological invention that the difficulties of description and definition have first arisen most acutely. The fundamental problem from the legal standpoint has been overcome by the use of Culture Collections as patent depositories for micro-organisms and other incompletely describable biological materials. Indeed this practice has for some years been regulated by an international Convention (the Budapest Treaty) which allows a single depository to be chosen from among many recognised Culture Collections for the purposes of patenting in all member countries. The making of deposits of micro-organisms for patent purposes is now fairly straightforward in principle. However, in practice, local regulations and case law are different from one country to another and this has made the situation more complex than desirable. These differences mostly concern the question of public release of deposited cultures (see later). As regards date of deposit, a culture is usually deposited before the filing of a patent application (as required in European patent law) but a recent US court decision(2) has allowed it to be made subsequently. Furthermore after 5 years of operation of the Budapest Treaty a review of some of the practical difficulties experienced by depositors and depositories is timely.

If deposition provides a legal solution it does not necessarily provide a commercially acceptable solution. Deposition is required not merely to provide a reference material for Patent Office purposes but more importantly to enable samples to be made available to third parties. This requirement for availability of samples at the appropriate time cannot be resisted for it is of the essence of patent law that in return for legal protection the patentee must provide information and teaching which is of practical use to others skilled in the art. The controversy on this issue is therefore chiefly about what should be the appropriate time for release of the culture and what conditions should be attached to this release. The timing and other conditions of release of samples of deposited micro-organisms are far from satisfactory to industry and other applicants for patents and are seen to disfavour the patent route as compared with the alternative of industrial secrecy. These arguments cannot be fully appreciated without some familiarity with patent procedure and time-scale.

PATENT PROCEDURE

The time-scale of patent procedure is illustrated below. In Figure 1 the general plan of international patenting procedure is shown giving some idea of the stages and the typical times involved before the results are known. In Figure 2 the corresponding plan shows how the release of deposited strains fits into the general scheme.

An application for patent protection is normally first made in the country of residence or place of business of the applicant. This establishes a so-called priority date which will be recognised in most of the other countries of the world under the provisions of an international convention known as the Paris Convention. In practice this means that the major expense of a foreign patenting programme can be postponed until towards the end of one year after the initial filing date in the home country. For this purpose an application for a European (regional) patent is on the same footing as national applications in other countries filed under the Paris Convention. The value of this one-year interim period, both to industry and to other organisations which have the problem of assessing the potential industrial importance of new research results, is considerable. The other major advantage given by the Paris Convention is that the inventor can publish details of his invention at any time after his priority date without detriment to his patent prospects. The only provisos here are that the invention is clearly defined and well supported by data in the specification filed with the first application and that the foreign applications are filed no later than one year after the first application.

If the applicant decides that a deposit of the biological material is necessary, deposition will usually be recommended before the first patent application is filed so that the Culture Collection accession number can be quoted in the specification. This is the safest course in order to comply with requirements in all countries and to leave no doubt about the priority date of the invention.

In many countries the specification will be published by the Patent Office at 18 months after the priority date even before the applicant has requested full examination of the application. (USA is a notable exception in postponing publication until the patent is granted.) After such a request has been filed the application will be examined for patentability and finally a patent will be granted or refused. The term of patent is variable, usually 20 years from application in European countries and 17 years from grant in USA, subject to payment of renewal fees in most countries.

A micro-organism deposit under the Budapest Treaty must be stored by a Culture Collection for at least 5 years after the last request for a sample and, in any event, for not less than 25 years.

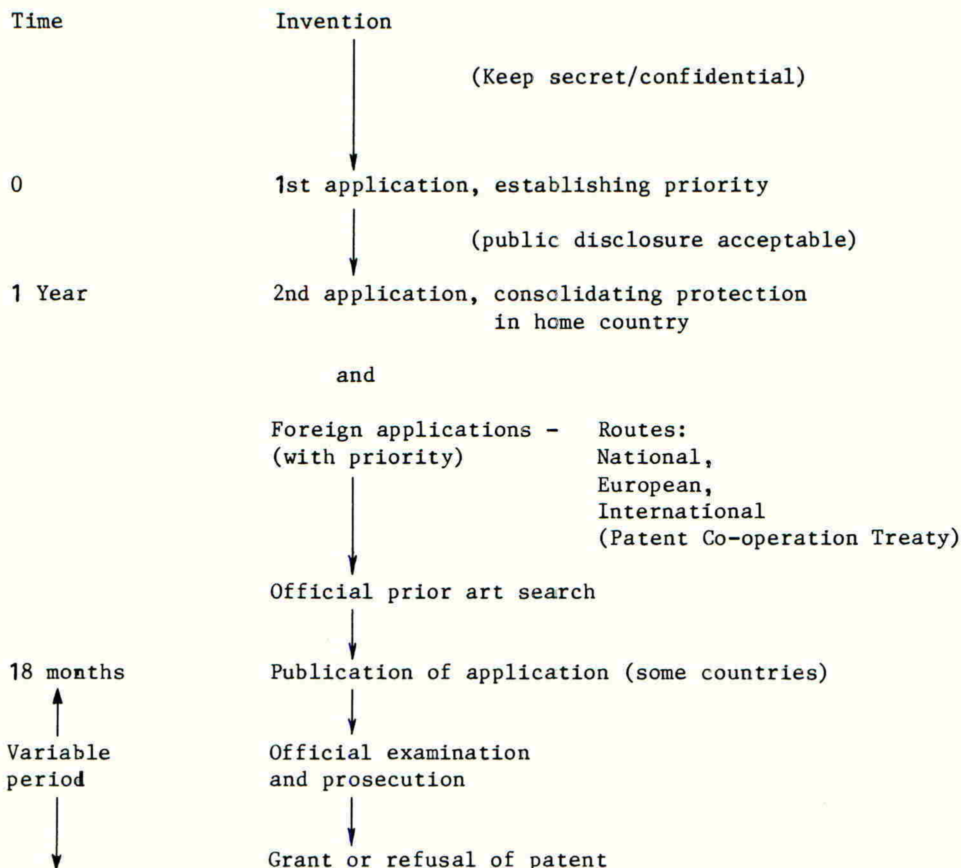

Figure 1 Patenting procedure.

Referring to Figure 2, in the most favourable systems e.g. those of USA and Japan no release of a deposited culture takes place until the applicant for the patent has an enforceable right. By contrast in the British and German national systems general release of the deposited strain takes place when the unexamined unaccepted application is published at the 18 month stage. Admittedly, release is subject to certain undertakings, e.g. not to transmit the culture to others and to use the culture for experimental purposes only. These undertakings remain so long as the application is pending but their value is questionable. An intermediate position is taken by the European Patent Office giving the applicant the right to specify that release of the strain at the 18 month stage must be through the intermediary of an independent expert who must not transmit the strain to the party for whom he acts. This situation obtains until the patent is granted whereupon the culture becomes generally available effectively without restriction. If however the application is refused or abandoned all restrictions on the supply of the culture to third parties are removed.

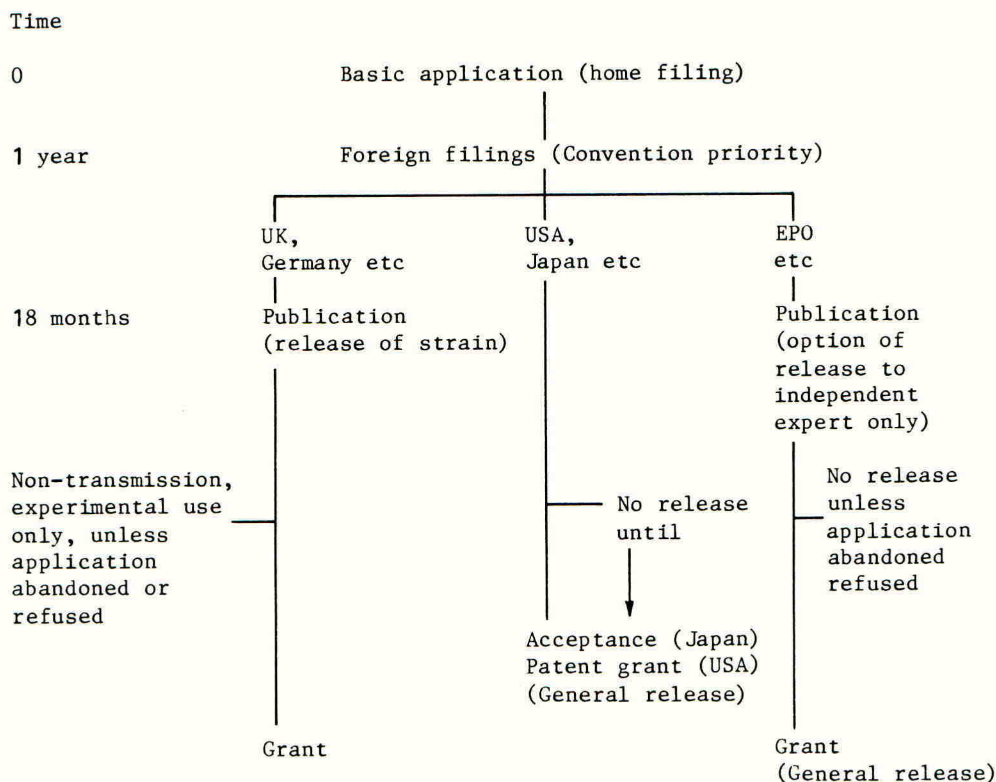

Figure 2. Release of deposited strains.

The problems described above apply to typical microbiological inventions but they have had hardly any impact so far on plant biotechnology inventions.

PATENTABILITY IN BIOTECHNOLOGY

In classical biotechnology inventions fall into the following main categories (classified by means of typical patent claims):-

1. Process of producing a new micro-organism.
2. The new micro-organism as produced by the defined process.
3. The new micro-organism *per se*.
4. Process of cultivating or otherwise using a defined micro-organism to produce an end-product which may be:
   (a) a form of the multiplied micro-organism itself, e.g. a vaccine or edible biomass;
   (b) a by-product of microbial growth, e.g. an antibiotic, enzyme, toxin, or an otherwise useful industrial product (even if inactive biologically); or

(c) some other product or substrate which is produced or improved by the culturing process, e.g. a purified industrial product or effluent.

5. The products of any of the processes defined in (4) - defined by a per se claim or product-by-process claim as appropriate.

6. Particular formulations of new strains or cultures thereof, including combinations with other substances, designed to utilise and exploit their special properties, e.g. in human or animal foods or for industrial uses.

For the new biotechnology, the reply to the oft-repeated question 'What can be patented in genetic engineering?' is 'Practically anything' provided it is new, inventive, and has an industrial or other useful capability. Many of the categories listed above will have their counterpart in the newer techniques of micro-organism construction such as by gene splicing into plasmids and other vectors or by cell fusion to produce hybridomas and other combinations. In plant genetic manipulation there will be corresponding possibilities of protection. Genetic manipulation offers a host of possible patent types although it appears to this writer that many of them will be for laboratory techniques rather than production processes in the ordinary sense. The question will very often be whether they are worth patenting and how they are to be exploited, i.e. can they be handled by normal patent licensing techniques? Stanford University have pointed a way here in the licensing of the Cohen Boyer patents. It is too early to list categories, as if these were established certainties, but some areas for consideration are the following:-

1. Novel genetic engineering strategies.
2. Isolation of genes.
3. Modification of genes.
4. Synthesis of natural or modified genes.
5. Construction of gene inserts.
6. Vector systems.
7. Methods of transforming cells.
8. Transformed organisms.
9. Manipulating protoplasts and the like.
10. Methods of regenerating whole plants.
11. The new plants.

In the development of patent practice in this field the major question will be not about the categories as such but about the scope of patent claims allowable. Already there are indications, particularly in Europe and Japan, that patents may be rather narrow, e.g. limited to specific DNA sequences or specifically described and deposited plasmids and transformed cells.

INVENTIONS IN PLANT SCIENCE AND TECHNOLOGY

Plant cell and tissue culture methods and the use of plant cells, e.g. in immobilised or other form, for the production of valuable chemical products are considered by patent examining authorities to be within the general category of microbiological process inventions. These are accordingly patentable as processes and, up to a point, the new products of such processes may also be patented. The point at which difficulty is encountered is when the attempt is made to patent a new plant as such. In Europe this difficulty stems from the European Patent Convention which in Article 53(b) states that:-

> European patents shall not be granted in respect of:
>
> (a) . . .
>
> (b) plant or animal varieties or essentially biological processes for the production of plants or animals; this provision does not apply to microbiological processes or the products thereof.

In attempting to draw a distinction between a process which is 'essentially biological' and one which is 'microbiological' the legal draftsman was presumably aiming at excluding traditional plant breeding methods and there can be no quarrel with that objective. But the exception for microbiological processes leaves open the patentability of producing a new plant by means of such a process, e.g. genetic manipulation. So at least one question mark attaches to the interpretation of this law. Also with regard to product patents the related question arises: Are new plants produced by genetic manipulation to be considered as 'plant varieties' and therefore unpatentable as products? This writer argues for a narrow interpretation which would only exclude patents for plants bred by traditional methods. This raises important and controversial issues which impinge upon the other legal system for protection, namely, that of plant variety rights (plant breeders' rights, certificates of variety protection etc.).

PLANT VARIETY RIGHTS

The intention of the plant variety right system was to give to the breeder broadly similar incentives and opportunities for reward as were available to inventors under the patent system. However, because a plant is a self-reproducing mechanism which can give rise to an indefinite number of descendants and quantity of consumption material, the legislators deliberately restricted the scope of plant variety protection. Thus the line was drawn by reference to propagation and the intention of the propagator of the new variety. The activities covered by this form of right are limited to (a) the production for sale and (b) the sale of reproductive material with the intention that it be used as such, e.g. seed for sowing. The UPOV Convention states the nature of the right as covering:

> production for purposes of commercial marketing, offer for sale, and marketing of the reproductive or vegetative propagating material as such.

The right does not extend to the saving of seed from a current crop for sowing in a later season. Also, it does not cover the production and sale of consumption material of the new variety e.g. fruit or grain. Finally, the right does not prevent use of the protected variety as source material for the addition of further variation in order to create yet another variety unless commercial production of the latter requires the repeated use of the protected variety.

THE IMPACT OF PLANT GENETIC MANIPULATION

What legal system will accommodate innovation in these techniques and the new plants generated from their use? Presumably such plants will not come straight off the laboratory bench. The DNA manipulation step may be only the first part of the whole process to be followed by conventional breeding methods although with a reduced time-scale. If so, then the licensing of plant variety rights on the end-product may be the predominant mode of exploitation of the technology by the breeder. If, however, the genetic manipulation expertise comes from someone other than the breeder it is not immediately obvious how a sharing of the ultimate benefit could be structured. Would the situation be clearer if patent protection was available for plant genetic manipulation techniques and products?

For European patent law we can begin from the standpoint that genetic manipulation techniques of the kinds under discussion are patentable as microbiological processes. Accordingly it seems to be possible to claim the methods and intermediate products up to and including the stage of transformed plant cells. However, claims to plants regenerated from such cells meet with objection under Article 53(b). This official vigilance against attempts to patent plants has even been applied to a European patent application(3) claiming chemically treated propagating material, e.g. seed treated with certain oxime derivatives in order to confer resistance to agricultural chemicals. Fortunately the Appeal Board has overruled this objection. Such a product cannot be described as a 'plant variety'. Again, to take another (hypothetical?) example, suppose it became possible to transfer a gene responsible for a certain pathogen-resistance in one plant species to a distant plant species or different plant genus, a patentee might well want to claim:

> 'Plants of the species (or genus) X having resistance to pathogens of the type Y by virtue of the transferred gene Z.'

This claim is not directed to a plant variety; the Plant Variety Rights Office would not recognise this as defining anything for which they could grant rights. Nevertheless it would be a crucial test case in the European Patent Office.

In US patent law there are no such difficulties from the written statute. Indeed some important patents have been granted in the past for procedures that would be classed as essentially biological in our terms, e.g. well known patents(4) for hybrid maize based on utilising cytoplasmic or genetic male sterility. The Agrigenetics patent(5) which drew a public protest from plant breeders a few years ago also comes close to this category. (The corresponding European patent for this

method of producing hybrids has not yet been granted.) Under US law the possibility of specific variety protection as well as more general patent protection has hitherto not been seen as a problem. More recently the US Patent Office has attempted to refuse a patent(6) on a maize seed having a specified minimum endogenous free tryptophan content achieved by biotechnology techniques on the ground that this subject matter was only protectable under the Plant Variety Protection Act of 1970. However the Patent Office has been overruled on appeal. The US law remains therefore the most open and flexible system for protection in plant biotechnology.

Japan also has the two legal systems (patent law and seeds and seedlings law) and the choice between them has usually been fairly straightforward. With the new possibilities offered by genetic manipulation there will be concern in Japan and also in some European countries that this demarcation may be less clearcut in future.

FUTURE PROSPECTS

The advance of this technology compels attention to the European legal problems outlined above. If, for example, the products of micropropagation/tissue culture techniques become a viable alternative to seed it has already been recognised that plant variety protection would be inadequate. In any case patents will almost certainly be granted for these processes and products and the consequences of this will require examination. Again, if gene sequences can be patented and usefully transferred to plants those who invest in this research will expect a legal mechanism for protection which enables a reasonable return on the investment to be achieved. Representatives of the international plant variety right system UPOV have already expressed uneasiness over the possible consequences of patents covering genetic material which is inserted into the plant genome. Of particular concern is the extent to which the owner of a patent for a DNA sequence, for example, could pursue his rights through to the finished variety and whatever further commercial use or research activity might be made of it. This is a topic of considerable legal difficulty and should not be the subject of theoretical speculation long in advance of an actual test case.

It would be outside this writer's competence to graft an extension on to the present plant variety law which broadens the scope of such protection whilst still falling short of the protection given by patents. If at all possible this can best be done by specialists in plant variety law. However, such an expedient would not stop the issuance of patents on what are clearly microbiological inventions in plant biotechnology. We might as well accept the possibility of overlapping protection as permitted under US law and not assume that the innovators in this technology will as a consequence use their rights in such a way as to ruin the industry which they serve and on which they depend.

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INDUSTRIAL FUNDING - PROBLEM OR OPPORTUNITY

A.J. COLEMAN

Wellcome Diagnostics, Temple Hill, Dartford, England.

Academia's quest for industrial funding has heated up considerably over recent years as the flow of Government money for basic research has progressively diminished. This is not solely a British phenomenon, it is an inevitable response from any financial resource which is put under increasing pressure to critically justify every penny of its expenditure. Nor is the phenomenon limited to academia - the R & D departments of most companies have already been through the management exercise of rationalising projects, consolidating expertise into fewer areas and reducing business diversification. Any untargetted research is a luxury which only the largest of companies can indulge in and then only to a very limited extent. All companies have cost versus benefit envelopes for their research where both internal and external projects are subject to stringent financial risk/benefit and scientific feasibility analysis. Many people in industry (jokingly!) state that companies are run by accountants and it is certainly an up hill struggle to convince them that your research programme will bear fruit in 10 years time - apply a discount cash flow to that and any financial benefit instantly vanishes.

Perhaps it is no wonder therefore that the academic, unable to get his accustomed Government grant for an alpha graded project throws up his hands in horror and frustration at the difficulty of extracting funds from industry. In fact the picture is not that bleak, but I would like to paint this picture from the viewpoint of at least one industrialist. Before doing so let us go back to the basic problem of dwindling Government funding.

Why should the Government fund any basic research in the U.K.?

What would be lost if all U.K. universities were turned into applied research institutes whose aims were directed by joint academic/industrial panels? Already as a result of Government cut backs a drift from basic to strategic or applied research is becoming evident in academia.

With the academic freedom of information, large multinational companies could use other countries (e.g. U.S.A.) to obtain any basic research innovations. Small companies would be only too happy to expand their research base applied to generating a few more products.

Can the U.K. economy afford to fund the quest for pure knowledge that individual U.K. companies consider too much of a luxury?

One case put forward to support the present system is that our strong academic base attracts investment from abroad - certainly there are many foreign multinationals with major R & D establishments in the U.K. but one might question their exploitation of that knowledge base by looking at where their manufacturing facilities and major reinvestments are being placed.

If the U.K. tax payer's money is put into basic research should he be assured that any commercial applications will be offered first to U.K. companies and not be auctioned off worldwide to the highest bidder (usually not British!)?

Several attempts have been made to increase the efficiency of transfer of technology from U.K. academia into industry - Celltech and AGC resulted from this as did a growth in the number of pre-competitive research 'clubs' of companies. In the end we are faced with the conservative policies of the average U.K. company versus the seeming ability of American companies to gamble large amounts of funding on uncertain research programmes.

What factors motivate industry to fund external academic research and what do we look for in a research collaboration?

- there is the 'look see' at the relevance of a new technology without the commitment of employing full-time staff 'in house'.

- the academic group will have built up an indepth expertise in a specific field with a critical mass of people, equipment and space to tackle a research topic effectively.

- the 'seed' funding of leading groups keeps an early alert open to possible step jump innovators.

Other factors will be discussed and how they relate to the alternative mechanisms of industrial funding - from industrial clubs funding pre-competitive research through CASE students and on to major research collaboration between a company and an academic department.

The potential problems faced by companies funding research externally rather than in-house will be outlined. Difficult aspects such as confidentiality, rights to patents and knowhow, control of the programme and technology transfer will be discussed - these all have solutions if both the company and the academics are willing and able to see each other's point of view.

Although I cannot draw up a universal DIY guide to getting industrial funding, a number of aspects will be outlined which the academic ignores at his peril when starting the quest for industrial support.

The cry of many academics is - "But industry won't tell us what it wants us to do" - often this is because in new and rapidly developing areas there are few people in the company who actually know what is scientifically feasible which might lead to commercial applications. It is only by bridging this gap that academia will get industrial funding and industry will get innovative products.

SCIENCE AND THE MEDIA

C. TUDGE

Dulwich, London

There's a band of people in this world who call themselves science writers. They are a multifarious bunch. Some are former scientists, too idle to do research, and some just feel that writing about science is a very nice way of indulging their interest in it; a way of turning work into a hobby and back into work again. Others have degrees in English and Psychology and other such pleasant pursuits, but found belatedly that the science they'd been talked out of doing at school really is the most prolific source of exciting new ideas they could have dreamed on. Some are journalists, raised on the stone or whatever the expression is, who discovered the same thing. Some are very clever and original, and contribute to the body of science; and some get things wrong, ever so slightly but ever so consistently, and are rather an embarrassment. Whether people are brilliant or embarrassing is not directly related to their paper qualifications. Some of the very best and cleverest people have arts degrees or no formal qualifications at all; and there are some with excellent credentials who can never put two sentences together without the first being equivocal and the second a non-sequitur. Some of the very best are women, who write exceedingly good prose - though that's merely an empirical observation; I'm not offering it as a deep sociobiological insight. In short, science writing is quite a nice little microcosm of what is generally known as "life".

It's a very pleasant occupation - like being an undergraduate through all the days of your life, except that you arrange your own seminars. I also think, however, that it's important. In fact, I think it's become one of the most important occupations that there is. I'd like to spend the next 10 minutes saying why it's important, and the 10 after that asking whether those of us who do it do it well enough and if not, why not - and what else needs doing.

The reason science writing is important is that science is important - but it is also both flawed and hampered. For science is not accepted as a proper and respectable part of culture - or at least, certainly not in Britain. The fact that it isn't has serious, far reaching consequences. It will be a long uphill struggle to establish science as a part of culture, but it is necessary. Science writing, in the press, in popular books, and through radio and television, is perhaps the single most powerful force that can bring about the necessary change.

C.P. Snow drew attention to the schism between science and other cultural pursuits in the late 1950s. In "The Two Cultures" he pointed out that educated people included two distinct species; those that were trained in the sciences, and those who were not. What he suggested was true then is just as true today. The evidence is obvious at every turn. The heavy Sunday papers are a quite a good guide to what "educated" people are supposed to be thinking about. In the various magazine sections that go with them you will find pages devoted to the newly-discovered posthumous letters of Vita Sackville West, or the annotated laundry lists of D.H. Lawrence; but you won't find any science. The book reviews will include the latest novels and biographies, and the latest anthology of posthumous letters from various members of the Bloomsbury set, to various

other members, plus laundry lists. But you'll find very few books about science. Sunday papers don't have to be like that. One of many pleasures I discovered in India last year was The Sunday Statesman, published in Delhi, where the lead review, after the chess and bridge and cricket, was of Gwyn Macfarlane's excellent biography of Alexander Fleming. You'd be shocked if you found such a piece on an English breakfast table.

And just the other day I listened to a Radio 4 programme about Milton Keynes. Has it got shops? the interviewer asked. Yes, it has shops. Are the schools good, is there a social centre? Not too bad, not too bad. And the arts, what about the arts? Ah, there are bags of arts: John Dankworth and Cleo Laine live there, or thereabouts. But it never occurred to the interviewer or the producer to ask, is there any science? Are there ponds? Is there a museum? What's the local geology? How do people farm? But then, as Mandy Rice Davies said in a slightly different context, they wouldn't, would they? Well no, they wouldn't; and the slightly shocking fact is that nobody, I would guess, apart from myself, actually noticed that they didn't.

In fact, it remains the case that to be informed about science, and to express an interest in it, is still regarded as somewhat infra dig. If you were in polite society and it transpired that you had never heard of Samuel Beckett, you would be gently frozen out, and properly ashamed to be thus exposed. But I discovered the other day that two of my old friends, one doing rather well in publishing and one in the Foreign Office, had never heard of Peter Medawar. Neither, it transpired, had they heard of immunology. They were not at all ashamed. Why should they know such things? What's it got to do with anything?

I won't labour the point. Everyone here who is British knows that this is the case: scientists are considered somewhat odd, and what they do is not necessary for respectable and educated people to know about. In fact what they do is really rather disreputable: morally suspect, and indeed responsible for much that's cheap and evil in society.

Does it matter that this is the case? I believe that society's indifference to science not only matters, but is one of the serious flaws in society.

A trivial point is that it really is a shame that so many people go to their graves with no inkling of the pleasure that's to be derived from the puzzles of maths or biology, and without ever realising that the ideas of Darwin and of Mendel, and of Crick and Monod and Maynard Smith, really are among the most intriguing and exciting that can be contemplated. It's like going to the grave without ever having listened to Schubert - or being given the opportunity to listen; a sad, personal, cultural loss.

The larger point is that so long as science remains outside the domain of public discussion, then it will never on the one hand be able to function and flourish as it should; and will never, on the other hand, be properly harnessed to the needs of society. It is in everybody's interests that there should be a continuing, uninterrupted dialogue between scientists and the rest of society, and that this dialogue should be as informed and subtle as possible. The dialogue just does not occur.

The fact that science isn't properly encouraged hardly needs to be stated here, in these times. But then it never has been. Certainly, there have been many times in recent history when science has not only been

encouraged, but positively flaunted. American presidents have won votes by promising to "lick cancer", or land men on the moon. Harold Wilson spoke of the "white heat" of technology. In both cases - though, to be fair, particularly the former - science wasn't so much being encouraged, as used: presented as a talisman, a slogan, just as the present government employes the slogan of "law and order". Certainly, during periods of euphoria it can be absurdly easy to obtain grants, provided you write in the appropriate buzz-word; it's amazing how many arcane reaches of biology and chemistry and even particle physics can be shown to have some deep and ineluctable association with cancer, provided you keep your fingers crossed. Isaac Asimov even managed to prove once - without irony - that going to the moon could throw light on cancer; something to do with the possibility of finding long-lost organic molecules.

Certainly, times of ease are better than present times, when even self-evident, and self-justifying excellence may often go unsupported. But neither situation is satisfactory. It just isn't good enough that support for research is used as a political expedient, for the convenience of politicians to fly kites at some periods or save a bit of cash at others. It is just possible that governments would not find it quite so easy to finance scientific research on such a stop-go basis if people at large were more aware of what was going on, and what is implied. Despite everything, I still have a naive belief in democracy: or at least, the slightly less naive belief that politicians are not so cavalier when the public eye is upon them than when it is not.

But it is also the case that science is not well deployed. It's very difficult on the one hand to give scientists the freedom they need to follow their noses, if they're truly to find out about the world - and on the other to ensure that the lines they pursue are truly for the common good. In fact, no nation on Earth has ever managed to control science properly or well. No nation has ever gathered all the fruits that are there to be gathered, or avoided the undesirable side-effects. The human species cannot survive in this world in present numbers without a very high input of very high tech; and if we want to save even a sizeable minority of our fellow species from extinction while we ensure our own survival, then that becomes doubly true. So it's very important that the world deploys science well; and very serious that ways of controlling the things it produces and determining the problems it tackles, are so crude.

And they really are crude. In the 1960s Mao Tse Tung sent scientists into the fields so they could learn to look at the world through peasants' eyes, and focus their minds on peasant problems. The exercise in practice was merely punitive; and China lost a generation of excellent scientists. On a different level, but with comparable intent, Lord Rothschild in this country introduced the customer contractor principle; again an attempt to see that scientists did what the country needed. The best I've heard anyone say of that particular exercise was that most of what had happened before Rothschild, continued afterwards as before, albeit with more paperwork.

But again, I'd like to re-invoke the notion of democracy. In democratic societies - or indeed in socialist countries that aspire to be communist - what happens in society should express the will of the people at large. Science and the high tech that it generates are the biggest single agents of change in the world. Ideas and ideologies change societies, of course; but it's amazing the extent to which ideas and

ideologies are products of material circumstance, which in turn depends largely on technology. Just to take a trivial example; people take their clothes off on the beach now, whereas a hundred years ago they used bathing machines. That has something to do with a change in public morality - but more to do with the development of the Jumbo jet which has taken millions of people to the hottest beaches and the most liberal countries on Earth. And the Jumbo in turn is a piece of spin-off from basic research on aerodynamics, and the behaviour of alloys at high temperatures, and the chemistry of combustibles. But although science is so powerful, it really isn't under the control of the people at large, because people at large have virtually no say in what it does or why. One very important reason why they have no say is that they hardly know of it's existence. All that most people see of science is the down-stream product, and by that time it's too late to influence events. But the only way in which science can be wielded democratically, within democratic societies, is if its affairs and its notions become part of public consciousness; in other words, if it becomes a part of culture, along with theatre, and music, and football.

So how can it become a part of culture? Not easily, is the short answer. The roots of the schism between science and the rest run deep, and they go back a long way. The 17th century was notable for its Renaissance men - they were all men in those days - who were equally versed in the sciences, and the arts, and in theology: but the roots of the schism undoubtedly began in the 17th century, grew during the 18th, and were well established by the beginning of the 19th. I know of no scholarly analysis of the reasons for it; presumably it has to do in part with social class - science was associated with the rise of the technological merchant classes - and with the antipathy expressed by Rousseau and by Blake to factories and dark Satanic mills. Coleridge dabbled in chemistry and Gothic novels crackled with electricity. Perhaps if the coin had flipped the other way, science might have been carried along by the romantic movement instead of in the end being shuffled off - there's plenty of romance in science, after all. But of course that's not how things turned out. In the great public schools of the 19th century it was hard to learn science at all. Indeed, one of the best science writers that I ever knew, a former deputy editor at New Scientist, used to recall how difficult it was to persuade the mandarins of his public school that he should be allowed to read geology. And he was at school in the early 1950s.

So there's a lot of leeway to make up: several centuries of non-communication, and the prejudice and suspicion that that leads to. The task has to be tackled at many levels. The deepest of those levels is that of formal education, and that will take several generations, because it isn't my impression that many teachers are particularly sympathetic to science - and certainly not in many primary schools, where formal education begins. The Royal Society has of course addressed this issue of late, with several cogent reports. The idea isn't to produce young scientists, though that would be one consequence, but to produce Renaissance children, used to thinking about the world in several different ways, and assessing it by many different criteria.

But informal approaches to learning are at least as important as the formal routes. In addition to schooling in science, it is necessary to create the ambience of science. And it's here that the science writers

come in, because unless there is good science in newspapers and general magazines, and on radio and television, and unless there are good popular books, then it will be very hard for people at large ever to find out about it.

What's needed, however, isn't more of what we have at present, but a different approach. There is good science writing in accessible places; there are good programmes on television and radio. But much of what's written and presented simply reinforces all the present aversions and prejudices; the prejudice that says that science is dour, boring, and in essence repellent; the one that says that the people who do it are slightly touched - amusing in small doses, possibly, but not to be allowed out by themselves.

For instance, well aware of the kinds of problems I'm talking about, the Ciba Foundation recently launched the Media Resources Service, to further the cause of science reporting. In the publication that accompanied the launch last summer it struck a note of optimism, for it said that "A survey of all the items in two national daily papers during 1985 revealed that 5 - 10 per cent potentially have a major medical, scientific, or technological component." On the face of it, five to ten per cent doesn't sound too bad. That's a couple of pages worth in the average Sunday paper.

But then it goes on to give examples of items of interest in 1985: "research on early human embryos, Legionnaire's Disease, AIDS, acid rain, the psychology of terrorists and the stress on hostages and the Strategic Defence Initiative (Star Wars)".

So where is there a story that's actually <u>about</u> science? Where are the articles that tell you about pieces of research - what's entailed, who's doing them, to what ends, and with what ideas in mind? When critics write about the theatre, they describe the play, and the actors, and the ideas. They can enhance your enjoyment, entice you to go and see for yourself. The play's the thing. Science isn't written about in newspapers, as theatre and football are written about. It's just used to add another twist to the standard round of news stories - the usual round of war, sex, crime, and money. If people see science only in the context of vile diseases, megadollars, and despicable weaponry then of course they'll continue to believe that it's something nasty - at best a necessary evil, to be locked in the woodshed.

Perhaps you'll suggest that it just isn't possible to write about science in the same way that Kenneth Tynan used to write about theatre and Julie Welch writes about football; fluently and entertainingly, wittily and informatively. To which I can only say, "nonsense". Some scientists write beautifully, in ways that anyone could understand: Peter Medawar and Stephen Jay Gould, are obvious examples. Some science writers, too, qualify as commentators rather than as mere reporters - enhancing what they write about, and adding context: Bernard Dixon and Martin Gardner are two who come to mind. There are many others. Of course, some science is difficult in a way that theatre and football generally are not. Some is complex, and some is esoteric - so that even if you understand the details, or feel you do, you still can't see what the thing is actually <u>about</u>. But not all science is inaccessible - I come back to my point that some of the best science producers and editors I know are people with arts degrees, who just happen to be interested. Good writing carries people

along. Even if you don't quite understand, you can generally get the gist. And people learn a remarkable amount by osmosis, provided exposure is prolonged. The first time you read about particle physics it is indeed esoteric (more esoteric than complicated). But if you read or are exposed to six different articles or broadcasts that even so much as mention particles, then after a time you at least have the illusion of understanding. It's rather like the way people have come to "understand" gravity over the past 300 years. In fact gravity is almost as mysterious now as it was when Newton first wrote about it; but over the years we've grown used to it. It's still mysterious, but it's no longer strange.

The thing that is abundantly obvious to scientists is that science is a human activity. Scientists do their best to de-humanise it so as to purge it of the human defects, of sloppiness, wishful thinking, ambiguity and indeed mendacity. But in fact the creative act in science is not distinguishable from creativeness in the arts; and for all their attempts to keep their act clean scientists mercifully do not succeed in obliterating their own personalities, some of the qualities they have come through in the work, as is true of all human activities. The point isn't simply that the people who do science are human, but that their ideas are human. The scientific paper presents those ideas in a formal way for reference but the whole edifice of ideas at any one time is invariably rickety, full of uncertainties and speculations - just as is true of ideas in all disciplines. Science would be less repellent to people at large if that particular reality, the essential human-ness of the structure, were allowed to be more visible.

Sometimes it is allowed to be. On Radio 3 and 4 it's customary to allow scientists to speak for themselves - and they do indeed emerge as human beings. On television, there have been some marvellous straight-talking-to-camera pieces of late; I remember Sidney Brenner having half an hour to tell the story of the genetic code, as mesmerically as A.J.P. Taylor discusses the various notions that come into his head. But many directors are cowardly, when it comes to the point. They are afraid to let the subject and the people speak for themselves, and instead employ presenters who go out of their way to reinforce the standard cliche of the mad scientist, whith whirling arms and ill-fitting suits. Such eccentricity is not what's required. It merely confirms people in the belief that scientists really ought to be kept under lock and key.

There are some hopeful signs. There are good things on radio and television as I've said - on children's television, too. But there is something of a vicious circle. Most editors seem fairly convinced that people are not particularly interested in science, and so long as that is the case they will not give writers the opportunity to write about it in the way that Frank Keating writes about cricket. But so long as writers of such calibre are not employed in newspapers, or given the freedom to expand when they are employed, then science is bound to remain in its essentially repellent niche, among the AIDS vaccines and the hyper-destructive lasers. It is in the nature of vicious circles to spring rapidly apart once they are broken; and once editors realise that science is interesting, that there are people who can show this to be so, and that people at large could be interested, then one thing may lead to another. Science could begin to become part of our culture.

However, it is obviously the case that people who enjoy reading about theatre or football don't simply want to read about it. They also go to

the theatre, or even act or put up the lights. There is a game called football that's actually played, with a very palpable ball. If science is truly to become a part of our culture, then it's essential that the more active characters should actually do science, and not just read about it. So how do people do science?

In lots of ways, is the answer. It's still possible, and perhaps increasingly so, for amateurs to do serious things. Halley's comet has brought a remarkable array of amateur astronomers out of their respective wood-sheds, and many make observations of considerable value to the pros. Ornithology depends to a large extent on the labours of amateurs; archaeology to a lesser extent. And there are entire cabals of people who breed reptiles and rare plants. P.R. Raven commented a decade ago that one of the best ways to save a rare plant was to feed it into the horticultural trade, whereupon it rises phoenix-like in a thousand suburban greenhouses; and the director of London Zoo told me the other day that in his opinion the best way to conserve endangered fish was through the circuit of amateur aquarists - rather than in the zoo. Science isn't a proper part of our culture; but it does exist in a myriad sub-cultures, and the fact that it does shows that non-professionals really can do science, just as theatre buffs can do theatre.

Admittedly, it's hard to be an amateur biotechnologist - though there are plenty of home brewers and not a few cheese-makers, and plant tissue culture is already practiced in schools and could soon transfer to the amateur greenhouses. If people can keep delicate species of tropical marine fish in their front rooms - as they can - then they can regenerate orchids from single cells, with a little help from the pros. The majority - thank goodness - are not so active. Plato suggested, after all, that the only truly respectable members of society were the spectators. But again the majority needs something else, beside reading. Well, there's the Molecule Club, which inter alia has turned science into theatre; and the Royal Institution, with its Christmas lectures. Museums have become active places - or inter-active, as the expression is. You can go too far; there's a lot to be said for the mere presentation of beautiful objects, fish and skulls and insects; but I'm looking forward nonetheless to Richard Gregory's proposed Exploratorium, in Bristol - hands-on involvement in the ideas of basic science.

And then there are the museums that grade into real life. At one end of the spectrum there are all those reactivated water mills, that use an ancient technology but still make flour or yarn or whatever. At the other there are real-life factories and plants, involved in serious industry, that nonetheless find time for visitors. The hydro-electric plant at Blenau Ffestiniog really does make electricity (or at least it stores it); and to take the children round it is no longer considered a totally ludicrous way to spend an afternoon.

In fact the time is coming when very little will be done in this world, or at least in the developed world, simply for its own sake. There will be plenty of leisure, voluntary or enforced. Everyone will be a voyeur. The things that will be a respectable part of culture will be the things that are visible, which indeed entertain; but, with luck, the boundaries between entertainment and education will be increasingly blurred, such that the acquisition of information might be seen to be as pleasurable as watching a play or listening to music. The people that now shuffle

around the hydro-electric plants will be wanting stronger fare. They'll want to see how protoplasts are fused, how transformation can be used and stand by while new atomic particles leave their traces on the X-ray screen.

I think you should anticipate that day. Open the doors of the research institutes and invite all the local schools and the Rotary Club, the fire-station and the Women's Institute, and, indeed, the less destructive kind of tourist. If they come in small groups and don't do the complete tour it needn't be too disruptive. Then we'll have science as entertainment, which is necessary if we're ever to have science as culture. And science as culture is beyond question necessary. With luck, the 21st century could be the new Renaissance, or indeed the new and proper Age of Enlightenment. The present - well, late Mediaeval, perhaps; with noises off from the Inquisition.

6.
Poster Papers

Organisers:
S. G. LISANSKY
B. J. MIFLIN

PROSPECTS FOR THE COMMERCIAL DEVELOPMENT OF CODLING MOTH (*CYDIA POMONELLA*) GRANULOSIS VIRUS

J. BALLARD, C.C. PAYNE, N.E. CROOK

Glasshouse Crops Research Institute, Littlehampton, W.Sussex, BN17 6LP, UK

ABSTRACT

ABSTRACT

Codling moth granulosis virus (CpGV) is a promising selective agent for the control of codling moth (*Cydia pomonella*) on apple and other fruit crops. It is highly infectious and effective in suppressing codling moth populations, and in reducing fruit damage. However, present virus production techniques are labour-intensive and involve rearing in whole insects. The virus has no harmful effects on the beneficial fauna in the orchard. The prospects for the commercial development of the virus are discussed with respect to improving rearing methods, reducing virus application rates, increasing virus efficiency by improving the speed of kill though genetic manipulation, and developing a complete integrated pest management system for orchards based on CpGV.

INTRODUCTION

Codling moth (*Cydia pomonella*) is a key pest of apple and other fruit crops throughout the world. Conventional control of codling moth employs broad-spectrum insecticides which are detrimental to beneficial arthropods and lead to outbreaks of 'secondary' pests including the fruit tree red spider mite, *Panonychus ulmi* (Collyer 1953, Solomon 1982). There is a clear need to develop a selective control agent for codling moth to preserve natural enemies such as the predatory mite, *Typhlodromus pyri*, and prevent damaging outbreaks of *P. ulmi*. A granulosis virus (CpGV) specific for codling moth has been successfully field tested in the UK and elswhere over a number of seasons (Falcon *et al*. 1968, Huber & Dickler 1977, Glen & Payne 1984). The virus has proven virulence against codling moth larvae and has no adverse effects on beneficial arthropods. This paper considers the factors which influence the potential commercial development of CpGV and its use within integrated pest management (IPM) programmes in orchards.

The development of effective virus mass-production methods

At present CpGV must be produced in larvae. This is labour-intensive and costs are potentially high. Although labour costs can be reduced by mass-rearing larvae on a semi-synthetic diet (Guennelon *et al*. 1981) it is essential to prevent any contamination of the insect stock culture with virus or other pathogens by rearing them in isolation some distance from the site of virus production.

Codling larvae are small in comparison with many lepidopterous larvae (average final instar weight = 40 mg (Glen & Payne 1984)) and normally yield only 10^{10} virus capsules per larva (= one 'larval equivalent'). Attempts to find larger alternative hosts for the virus have not been successful. However, addition to the diet of 2 ppm methoprene (Altosid®) a juvenile hormone analogue, increases larval size and virus yield by 70% (Glen & Payne 1984).

Although codling moth cell cultures which support replication of CpGV have been demonstrated (Naser *et al.* 1984), the technology for virus mass production *in vitro* has not yet been established and costs are prohibitively expensive. Further developments in virus production must come, initially, from improving the economics of rearing the virus in larvae.

Virus application and codling moth control

Although the median lethal dose of CpGV in neonate larvae is extremely low (2-3 capsules; Crook *et al.* 1985), the period of larval exposure to virus in the field is brief, since larvae enter the fruit shortly after hatching and are thereafter largely protected from applied virus. Ingestion is required for infection to proceed and this is most likely to occur while the neonate larvae browse on the leaves before entering the fruit (Glen & Clark 1985). To ensure adequate control, virus applications must be timed to coincide with larval hatch; good spray coverage of the leaf and fruit surface is essential, and persistence of the virus is important. Unfortunately, virus is rapidly inactivated by the ultraviolet component of sunlight, and virus "half-lives" of only 2-3 days are common (Payne *et al.* 1983). Nonetheless, in field trials conducted in the UK since 1978, well-timed virus applications have given good control of codling moth populations and substantial reductions in fruit damage (Table 1). One drawback is that the virus takes several days to kill the codling moth larvae, and this can allow a certain amount of superficial damage to the fruit. However, more than 90% control of severe (deep entry) fruit damage can be obtained by doses as low as 265 larval equivalents per ha (Glen & Payne 1984).

TABLE 1

Reduction in codling moth deep-entry apple damage by CpGV: UK field trials 1978-1985 (Glen & Payne 1984*, Richards 1984**, Ballard, unpublished data***)

Year	Virus concentration at 600-1000 l/ha	% suppression of deep+ entry damage
*1978	7×10^{10}/l	77
*1979	7×10^{10}/l	80
*1980	2×10^{10}/l	97
**1981	2×10^{10}/l	88
**1982	2×10^{10}/l	89
***1985	1×10^{10}/l	79

+ Compared to unsprayed plots

Improving virus efficacy by modifying formulation and application methods

The incorporation of ultraviolet protectants into virus formulations have achieved only small improvements in virus persistence (Richards 1984). Further research is needed in this area. The problem of short persistence might be overcome by the application of several low-dose virus treatments at regular intervals throughout the critical and often extended period of

larval hatch. Thus, Dickler & Huber (1985) obtained as good control of codling moth with nine applications of 5×10^{12} capsules/ha as with the more conventional treatment of four applications of 5×10^{13} capsules/ha. The anticipated reluctance of growers to increase the number of spray applications could be overcome if it proves possible to tank-mix CpGV with fungicides and apply the virus with the regular fungicide spray programme. Such a system would have particular value when, as in 1985, the summer is cool and there is a protracted codling moth adult emergence. In 1985 this resulted in eggs hatching over a period of 6-8 weeks.

Improving virus efficiency by increasing speed of kill

It would be ideal if the virus-infected larva died before entering the apple so as to avoid cosmetic damage to the fruit. Possible advances in genetic manipulation may provide ways for improving the speed-of-kill of CpGV and other related baculoviruses. A physical map of the CpGV genome has been produced (Crook *et al.* 1985) and this provides the basis for future genetic studies to increase CpGV pathogenicity by incorporating genes which code for toxins or other products which will inhibit insect feeding soon after infection.

The selective use of CpGV within an IPM programme

There is no evidence of damage to beneficial arthropod populations resulting from CpGV application. By contrast, comparative trials of CpGV with chemicals have shown that chemical insecticides have deleterious effects on beneficial arthropods. For example diflubenzuron was shown to be damaging to earwigs (predators of woolly apple aphid (*Eriosoma lanigerum*), and codling moth (Glen & Phillips 1984) whilst organophosphate insecticides (such as azinphos methyl) kill the predatory mite *T. pyri* and cause outbreaks of *P. ulmi* (Glen *et al.* 1984). Similarly, Fig. 1 illustrates the massive rise in *P. ulmi* numbers resulting from deltamethrin application compared with the low populations in CpGV-treated plots: deltamethrin significantly decreased the *T. pyri* populations. A significant increase was also observed in rust mite (Eriophyidae) numbers (Ballard, unpublished data).

Although the virus preserves the beneficial arthropods, a successful IPM system requires the development of suitable low cost, highly specific agents to control other orchard pests e.g. winter moth (*Operophtera brumata*), aphids, tortrix moths (leafrollers), apple sawfly (*Hoplocampa testudinea*) and mussel scale (*Lepidosaphes ulmi*). Such control measures could include insect growth regulators and pheromone disruption techniques which have given encouraging results in trials in the Netherlands, France, Switzerland and Germany. *P. ulmi*, rust mites and woolly aphids should normally be kept below economically damaging levels by allowing the build-up of natural enemies. However, in orchards with a long record of chemical pesticide treatments, predatory mites may not, at first, be numerous enough for sufficient control of *P. ulmi* in the first year of an integrated programme; it may in such instances be necessary to introduce predatory mites or incorporate an additional pest-specific control measure during the first 1-2 years of CpGV application.

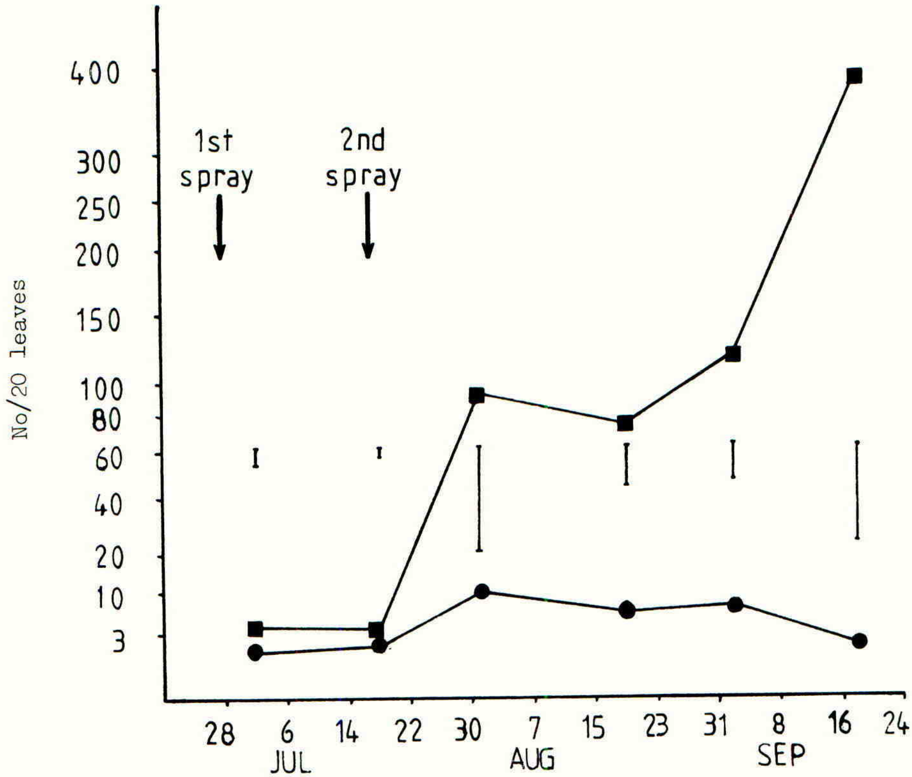

Fig. 1. Numbers of Panonychus ulmi recorded in 1985 season; S.E. England orchard trial. ■ deltamethrin (2 sprays); ● CpGV at 6 x 10^{13} capsules ha^{-1} (2 sprays). Numbers are shown on transformed (square root + 1) scale. Bars represent standard errors of difference of means.

CONCLUSION

CpGV has shown considerable potential as a selective control agent for codling moth. Prospects for the commercial development of the virus will be enhanced by improvements in the economics of virus use, by establishing cheaper methods of virus production and by reducing the doses of virus required to give satisfactory codling moth control. The development of a genetically manipulated virus with improved speed of kill could provide a patentable product and enhance commercial interest. Nonetheless, the full potential of the virus can only be realised by the careful development of a fully integrated pest management programme in which CpGV plays a key role.

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THE POTENTIAL OF ENTOMOGENOUS FUNGI AS CONTROL AGENTS FOR ONION THRIPS, THRIPS TABACI

A.T. GILLESPIE

Glasshouse Crops Research Institute, Littlehampton, W.Sussex, BN17 6LP, UK

ABSTRACT

In laboratory tests, Thrips tabaci proved susceptible to isolates of Beauveria bassiana, Metarhizium anisopliae, Paecilomyces fumosoroseus and Verticillium lecanii. The most pathogenic isolates, M. anisopliae (ME2) and B. bassiana (31) killed all treated insects within 4 days, while V. lecanii isolates killed only a maximum of 85% in the same time. A glasshouse experiment indicated that V. lecanii was able to reduce thrips populations on cucumbers.

INTRODUCTION

The widespread use of biological control agents has reduced pesticide use on cucumber. Currently, the predatory mite Phytoseiulus persimilis is used to control the red spider mite (Tetranychus urticae) and the parasite Encarsia formosa to control the whitefly (Trialeurodes vaporariorum). As a consequence, formerly minor pests have become important, e.g. Thrips tabaci. Although these pests are susceptible to chemical insecticides, chemicals cannot be used without adversely affecting the predator and parasite. Clearly, there is a requirement for a specific control agent for T. tabaci.

There are several records of fungi infecting T. tabaci, e.g. Zoopthora radicans (Bourne & Shaw 1934), Neozygites parvispora (MacLeod et al. 1976), Entomophthora thripidum (Samson et al. 1979), Beauveria bassiana (Dyadechko 1964) and Verticillium lecanii (Binns et al. 1982). However, there have been few serious attempts to develop fungi as biological control agents for this pest.

The present paper describes studies of four pathogenic fungal species on T. tabaci.

MATERIALS AND METHODS

Fungi

Fungi were maintained on Sabouraud dextrose agar (SDA) at 4°C. Conidiospores for pathogenicity tests were produced by spreading conidia onto SDA in Petri plates, incubating at 25°C for 10 days, harvesting spores by flooding plates with 0.01% Triton X-100 solution, agitating with a glass rod, filtering spore suspensions through 2 layers of muslin, centrifuging (3000 rev/min, 20 min) and resuspending in 0.01% Triton solution. Spore viabilities were determined by placing single drops of diluted suspensions (10^5 ml^{-1}) onto SDA coated on microscope slides, incubating in a moist chamber for 12-24 h and examining using phase contrast microscopy.

Hyphal bodies were produced in 1.6 litre fermenters using a glucose (4%) and yeast extract (2%) medium with agitation of 450 rev/min, aeration of 50 l/h^{-1} and temperature of 25°C.

Fungi tested for pathogenicity to T. tabaci are detailed in Table 1.

TABLE 1

Fungi tested for pathogenicity to Thrips tabaci

Fungus	Isolate	Host
Beauveria bassiana	31	Leptinotarsa decemlineata
" "	32	" "
" "	63	Mythimna unipuncta
Metarhizium anisopliae	ME2	Mahanarva postica
" "	Pemphigus	Pemphigus treherni
Paecilomyces fumosoroseus	21	Melolontha melolontha
Verticillium lecanii	1-72	Macrosiphoniella sanborni
" "	19-79	Trialeurodes vaporariorum*
" "	28-79	Hauptidia maroccana
" "	53-81	Thrips tabaci*
" "	Tt	" "

* Not used in pathogenicity test, method 1

Pathogenicity testing

Method 1

Groups of 20 adult T. tabaci were immersed in 0.01% Triton X-100 or in the same solution containing 5×10^7 conidia ml^{-1} of the fungi detailed in Table 1. The liquid was removed in a Buchner funnel (4.7 cm diameter) lined with filter paper (GFA, Whatman, England), then the insects were placed with a fine camel-hair brush on cucumber leaf discs (0.6 cm diameter), supported on agar contained in trays normally used for Enzyme Linked Immunosorbent Assay. Insects were contained with Clingfilm and maintained at 23 $\pm$ 1°C. Three replicates were used for each treatment.

Method 2

Cucumber plants, variety Telegraph, were sprayed to run-off with suspensions (2.5 g/l^{-1}) of the commercial V. lecanii products, Mycotal® (strain 19-79) or Thriptal® (strain 53-81). The plants were then incubated at a nominal 100% r.h. and 20°C for 4 days, when leaf discs (1.6 cm diameter) were removed and placed on agar contained in 25-compartment, square, Petri plates. Adult T. tabaci were anaesthetised with CO_2, individually placed on each of 20 leaf discs $treatment^{-1}$ and maintained at 20°C. Unsprayed leaf discs were used to maintain insects as controls. The experiment was repeated once.

Glasshouse experiment

Cucumber plants, cultivar Farbiola, were established in peat bags in a glasshouse (12 x 12 m; max. height 4 m). The previous crop had been infested with T. tabaci, which rapidly re-established on the fresh plants. Three groups of eight plants were sprayed with the treatments detailed in Table 2, using a hydraulic sprayer (CP3, Cooper Pegler Ltd., England; 250 ml $plant^{-1}$).

TABLE 2

Treatments applied to cucumber plants for control of *Thrips tabaci*

Treatment	Spore concentration (ml^{-1})	Viability
Autoclaved Mycotal®	-	0
Mycotal® (2.5 g $litre^{-1}$)	6.3×10^5	-
V. lecanii hyphal bodies (19-79) + 2g l^{-1} nutrients	10^7	99.2
V. lecanii hyphal bodies (53-81) + 2g l^{-1} nutrients	10^7	98.7

Mature thrips nymphs fall to the soil where they pupate. Thrips populations were assessed by trapping nymphs on Petri plates (9 cm diameter), coated with Boltac grease (Pan Britannica Ind., England), positioned on alternate peat bags throughout the glasshouse (Bassett, pers. comm.). Petri plates were replaced every 7 days. Direct counts of thrips were made 17 days after treatment, by examining 5 cm^2-areas on 10 randomly selected leaves $replicate^{-1}$. The glasshouse was maintained at a minimum of 20°C.

RESULTS

Laboratory experiments

All fungal treatments by method 1 caused significantly higher mortality (P = <0.05) of thrips after 4 days than treatment with 0.01% Triton X-100 (Fig. 1). Control mortality was 20% after 4 days, but exceeded 50% 5 days after treatment, probably due to exhaustion of food supply. The most pathogenic isolate was *M. anisopliae* (ME2) which killed some 55% of treated insects after 2 days. By 4 days after treatment, *B. bassiana* (31) had killed all treated insects and this mortality was similar (P = >0.05) to that recorded for *M. anisopliae* (ME2, Pemphigus), *B. bassiana* (63), *P. fumosoroseus* (21) and *V. lecanii* (1-72).

Using method 2, placement of thrips on leaf discs bearing sporulating mycelia of *V. lecanii* strain 19-79 (Mycotal®) killed more insects than *V. lecanii* strain 53-81 (Thriptal®, Fig. 2).

Glasshouse experiment

Mean thrips populations, as indicated by trapping, were similar with all treatments, during the period from 4 to 11 days after treatment (Fig. 3). During the next 7 days, thrips populations were significantly reduced on plants treated with Mycotal® or fresh *V. lecanii* (19-79) hyphal bodies (P = <0.05). For the next 21 days, all fungus treatments significantly reduced thrips populations compared to numbers on plants treated with autoclaved Mycotal®.

Direct counts of thrips on control plants showed appreciable infection by *V. lecanii* and *Neozygites parvispora* (Table 3). *V. lecanii* had probably spread from treated plants. *N. parvispora* commonly causes epizootics on

T. tabaci on cucumber (Samson et al. 1979, Gillespie, unpub. obs.) but cannot be cultured in vitro. Until N. parvispora can be effectively produced it is unlikely to be used as a mycoinsecticide.

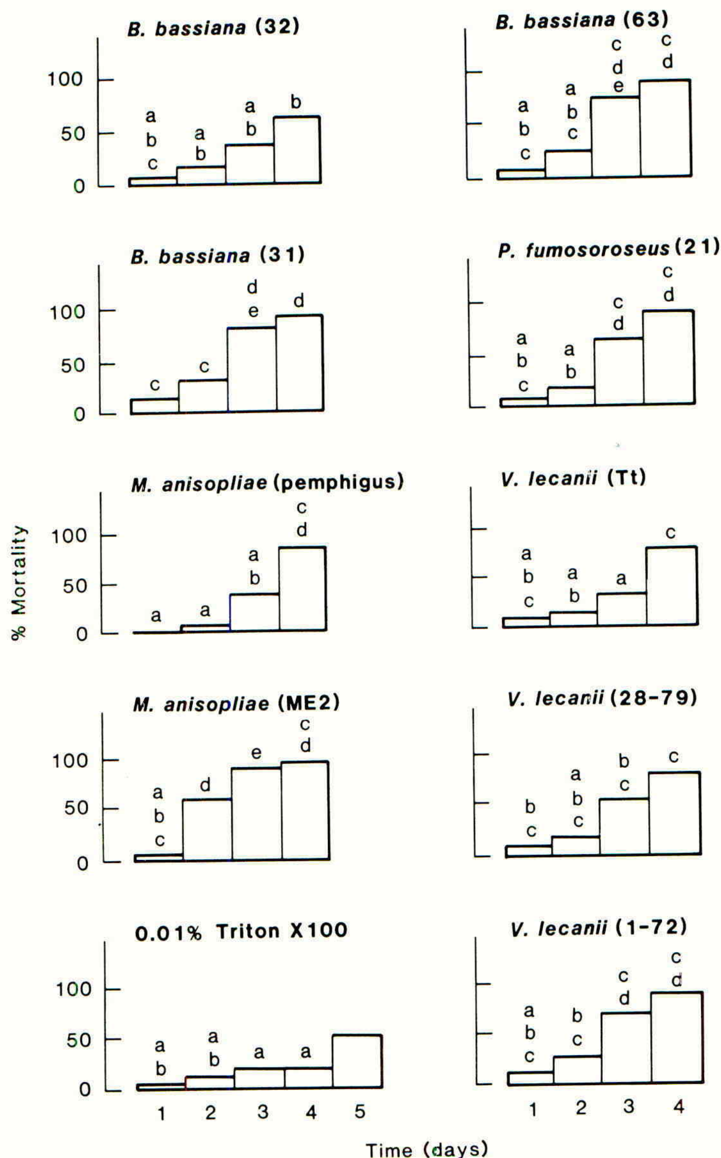

Fig. 1. Mortality of adult Thrips tabaci after immersion in suspensions of various fungi (5×10^7 conidia ml^{-1}) and maintenance on leaf discs at 100% relative humidity and 23°C. Histogram shows mean mortalities of three replicates of 20 insects. For each sampling date, different letters denote significant difference between treatments ($P = <0.05$).

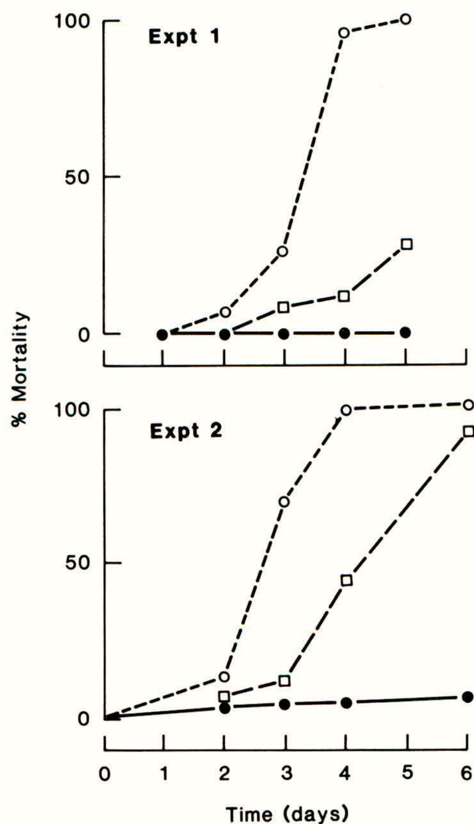

Fig. 2. Mortality of adult *Thrips tabaci* maintained on leaf discs (●———●) or on leaf discs previously sprayed with Mycotal® (o-----o) or Thriptal® (□——□) and incubated at 100% relative humidity and 25°C for 4 days. Twenty insects were used for each treatment.

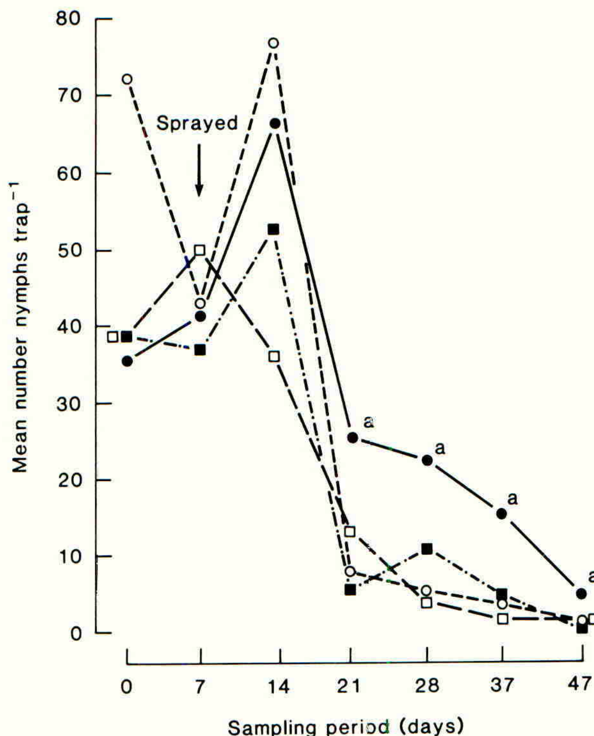

Fig. 3. Mean numbers of T. tabaci caught per trap after treatment of cucumbers with Mycotal® (o), or Verticillium lecanii hyphal bodies (19-79 (□) or 53-81 (■)) or autoclaved Mycotal® (●). 'a' indicates only values significantly different from others on same day ($P = <0.05$).

TABLE 3

Mean numbers of thrips, with mortality due to Verticillium lecanii and Neozygites parvispora, 17 days after spraying cucumbers with V. lecanii

Leaf surface	Treatment	Mean no. of thrips[a]			Percentage infected by	
		Live	Dead	% dead	V. lecanii	N. parvispora
Upper	Autoclaved Mycotal®	2.0	1.8	44.3	47.3	52.7
	Mycotal®	1.2	2.3	66.2	86.3	13.7
	V. lecanii (19-79)	1.2	3.3	76.0	89.9	10.1
	V. lecanii (53-81)	1.9	4.2	68.9	89.8	10.2
Lower	Autoclaved Mycotal®	1.5	2.6	61.4	60.3	39.7
	Mycotal®	0.45	3.1	87.5	98.4	1.6
	V. lecanii (19-79)	0.2	4.1	95.2	100	0
	V. lecanii (53-81)	0.3	4.1	94.8	99	1.0

a Mean number of thrips computed from direct counts on 10, 5 cm^2-areas $replicate^{-1}$

DISCUSSION

Laboratory experiments showed that isolates of M. anisopliae and B. bassiana were very pathogenic to T. tabaci. However, these isolates are not effective pathogens in the glasshouse environment, probably because they germinate slowly under glasshouse conditions (Gillespie 1984). By contrast, V. lecanii effectively controls aphids and whiteflies in the glasshouse (Hall 1982; Hall & Burges 1979).

In the laboratory, Mycotal® was more effective than Thriptal®, while in the glasshouse, both performed similarly. Possibly, the Thriptal® formulation tested contained a low number of V. lecanii spores.

Mycotal® is also pathogenic to Tetranychus urticae (Gillespie 1984) and thus potentially could be used to control all the major pests of cucumber. It is recommended that further trials are conducted to fully assess the potential of Mycotal® to control T. tabaci and T. urticae on cucumbers.

ACKNOWLEDGEMENT

Mycotal® and Thriptal® are registered trade marks of Microbial Resources Ltd., Theale, Reading, United Kingdom.

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EFFECTS OF ENTOMOGENOUS FUNGI ON THE BROWN PLANTHOPPER OF RICE *NILAPARVATA LUGENS*

A.T. GILLESPIE

Glasshouse Crops Research Institute, Littlehampton, W.Sussex, BN17 6LP, UK

ABSTRACT

Four strains of *Metarhizium anisopliae* and one of *Paecilomyces farinosus* were highly pathogenic to *Nilaparvata lugens*. In contrast, strains of *Beauveria bassiana* and *Verticillium lecanii* were of low to moderate pathogenicity. One strain of *M. anisopliae* was tested against *N. lugens* in a glasshouse but failed to achieve control. The potential of fungi as control agents for *N. lugens* and the requirement for further research is discussed.

INTRODUCTION

There are numerous reports of fungi infecting *N. lugens*. Pathogens recorded include Entomophthoraceae e.g. *Conidiobolus coronatus* (Gabriel 1968), *Entomophthora delphacis* (Shimazu 1976) and Deuteromycetes e.g. *Beauveria bassiana* (Srivastava & Nayak 1978), *Hirsutella citriformis* (MacQuillan 1974), *Metarhizium anisopliae* (Daoust & Roberts 1982) and *Paecilomyces farinosus* (Aoki 1957, Kuruvilla & Jacob 1980).

Some 90% of rice is grown in flooded soil. The relative humidity within the rice canopy is generally high and approaches saturation at night (Gillespie unpub. obs.), conditions very suitable for fungi. This study was undertaken to assess the potential of fungi as rice pest mycoinsecticides.

MATERIALS AND METHODS

Insects

Cultures of *N. lugens* were established on rice, variety TN1 and maintained at 25°C and a 16 h photoperiod. Under these conditions adult insects were produced in 28 days.

Fungi

Fungi were maintained on Sabouraud dextrose agar (SDA) at 4°C. Conidia for pathogenicity tests were produced by uniformly spreading conidia onto SDA Petri plates, incubating at 25°C for 10 days, then harvesting spores by flooding plates with 0.025% Triton X-100 solution, agitating with a glass rod, filtering spore suspensions through 2 layers of muslin, centrifuging (3000 rpm, 20 min) and resuspending in 0.025% Triton solution. Spore concentrations were then determined using an improved Neubauer haemocytometer and solutions standardised at 10^7 conidia ml^{-1}. Spore viabilities were determined by placing single drops of diluted suspensions (10^5 ml^{-1}) onto SDA, coated on microscope slides, incubating in a moist chamber for 12-24 h and examining under phase contrast microscopy. Suspensions were used immediately or stored at 4°C for a maximum of 1 week.

Fungi tested for pathogenicity to *N. lugens* are detailed in Table 1.

TABLE 1

Fungi tested for pathogenicity to Nilaparvata lugens

Fungus	Isolate	Host
Beauveria bassiana	43-81	Mythimna unipuncta
" "	94-82	Leptinotarsa decelineata
" "	95-82	" "
" "	110-82	Nephotettix sp.
" "	111-82	Nilaparvata lugens
" "	258-85	Coleoptera
Beauveria brongniartii	98-82	Melolontha melolontha
Metarhizium anisopliae	83-82	Mahanarva posticata
" "	99-82	Pemphigus treherni
" "	100-82	Melolontha melolontha
" "	101-82	" "
Paecilomyces farinosus	104-82	" "
Paecilomyces fumosoroseus	50-81	" "
Verticillium lecanii	53-81	Thrips tabaci
" "	102-82	Trialeurodes vaporariorum

Insect treatment

Experiment 1

Groups of 20 adult, brachypterous N. lugens were immersed in a cold (10°C) 0.025% solution of Triton X-100 or the same solution containing 10^7 conidia ml^{-1} of the fungi detailed in Table 1. The liquid was removed in a Buchner funnel (4.7 cm diameter) lined with filter paper (GCA, Whatman, England), then the insects were placed with a fine camel-hair brush on 6-week-old rice plants and contained in a propagator (Stewart Plastics, England). Insects were then maintained at 25°C and mortality recorded daily.

Experiment 2

Three replicate groups of 20, adult, brachypterous N. lugens were treated as above with suspensions of 5×10^7 conidia ml^{-1} of B. bassiana (110-82), M. anisopliae (83-82, 100-82, 101-82) or P. fumosoroseus (50-81).

Multiple dose assays

Groups of 20 adult, brachypterous N. lugens were immersed in surfactant or Triton X-100 solutions containing M. anisopliae (83-82) conidia (10^4, 10^5, 10^6, 10^7 ml^{-1}). Insects were maintained as in earlier experiments and mortality recorded after 4 days. The experiment was repeated 3 times with different batches of insects. Data were analysed by probit analysis using maximum-likelihood analysis (Ross 1970).

Glasshouse experiment

Rice seedlings, variety TN1, were transplanted (65 per bed) to two polythene-lined beds (2.0 x 0.6 x 0.18 m) in each of two glasshouses (3.6 x 2.7 m; maximum height 3.2 m) when 3 weeks old. After 17 days, 50 adult N. lugens were introduced into each bed. Six days later beds were sprayed with water suspensions (200 ml per bed) containing M. anisopliae (83-82) conidia (10^7 spores ml^{-1}), 1.5 g l^{-1} barley flour and 0.5 g l^{-1} skimmed

milk (Oxoid, England). Two adjacent beds in one chamber were sprayed with viable conidia (98.7%), while the remainder were sprayed with autoclaved spores. Further similar treatments were made 14 days later. Insects on 20 random plants per bed were counted before the first spray and again after 14 and 28 days. Throughout the experiment air temperature was maintained at 25°C and the beds and surrounding soil kept flooded in an attempt to maximise humidity.

RESULTS

In the initial screening experiment, M. anisopliae (83-82, 99-82, 100-82, 101-82) and P. farinosus (104-82) were highly pathogenic. All treated insects died within 7 days (Fig. 1). B. bassiana, P. fumosoroseus and V. lecanii were of low to moderate pathogenicity and killed from 20-80% of insects in the same time (cf. control mortality 15%).

In the replicated experiment, M. anisopliae (83-82, 100-82, 101-82) were of similar activity and were significantly (P = 0.05) more pathogenic than B. bassiana (110-82) or P. fumosoroseus (50-81) (Fig. 2). The mean LC_{50} value for M. anisopliae (83-82) was 1.86 x 10^6 conidia ml^{-1}, after 4 days incubation at 25°C (Table 2). Assays were read after only 4 days as the planthoppers' feeding damaged the rice plants and control mortality increased after this time.

TABLE 2

Pathogenicity of Metarhizium anisopliae (83-82) to adult Nilaparvata lugens

Assay	LC_{50} with 95% fiducial limits	Slope ± SE[a]	Chi[2b]	d.f.[cb]	p
1	1.86 (.95 - 4.11)	1.5 ± 0.6	2.0	2	>0.05
2	2.41 (.38 - 6.01)	1.4 ± 0.5	1.2	2	>0.05
3	1.33 (.55 - 3.15)	0.9 ± 0.2	1.9	2	>0.05

a Standard error
b Heterogeneity about regression line
c Degrees of freedom

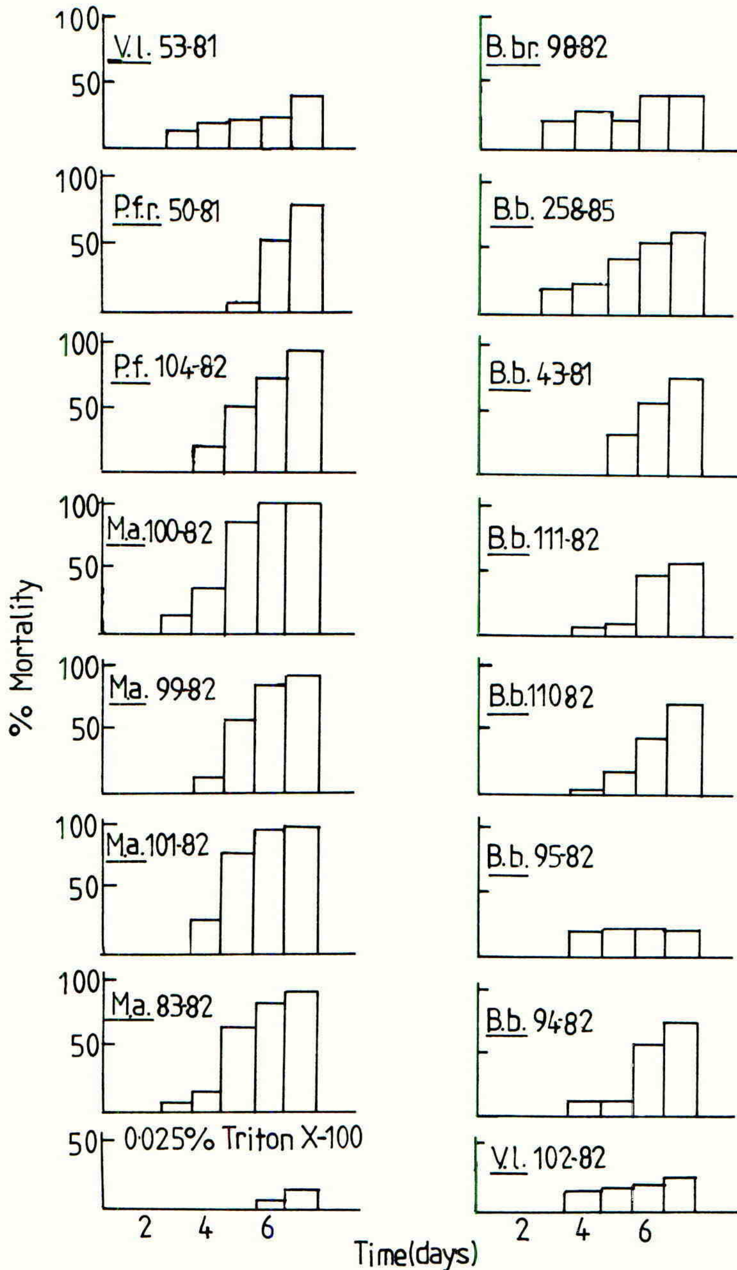

Fig. 1. Mortality of adult brachypterous *Nilaparvata lugens* after immersion in 0.025% Triton X-100 or similar solutions containing 10^7 conidia ml^{-1} of *Beauveria bassiana* (B.b.), *B. brongniartii* (B.br.), *Metarhizium anisopliae* (M.a.), *Paecilomyces farinosus* (P.f.), *P. fumosoroseus* (P.f.r.) or *Verticillium lecanii* (V.l.) and maintenance on rice plants at 25°C. Histogram shows percentage computed from 20 insects $treatment^{-1}$.

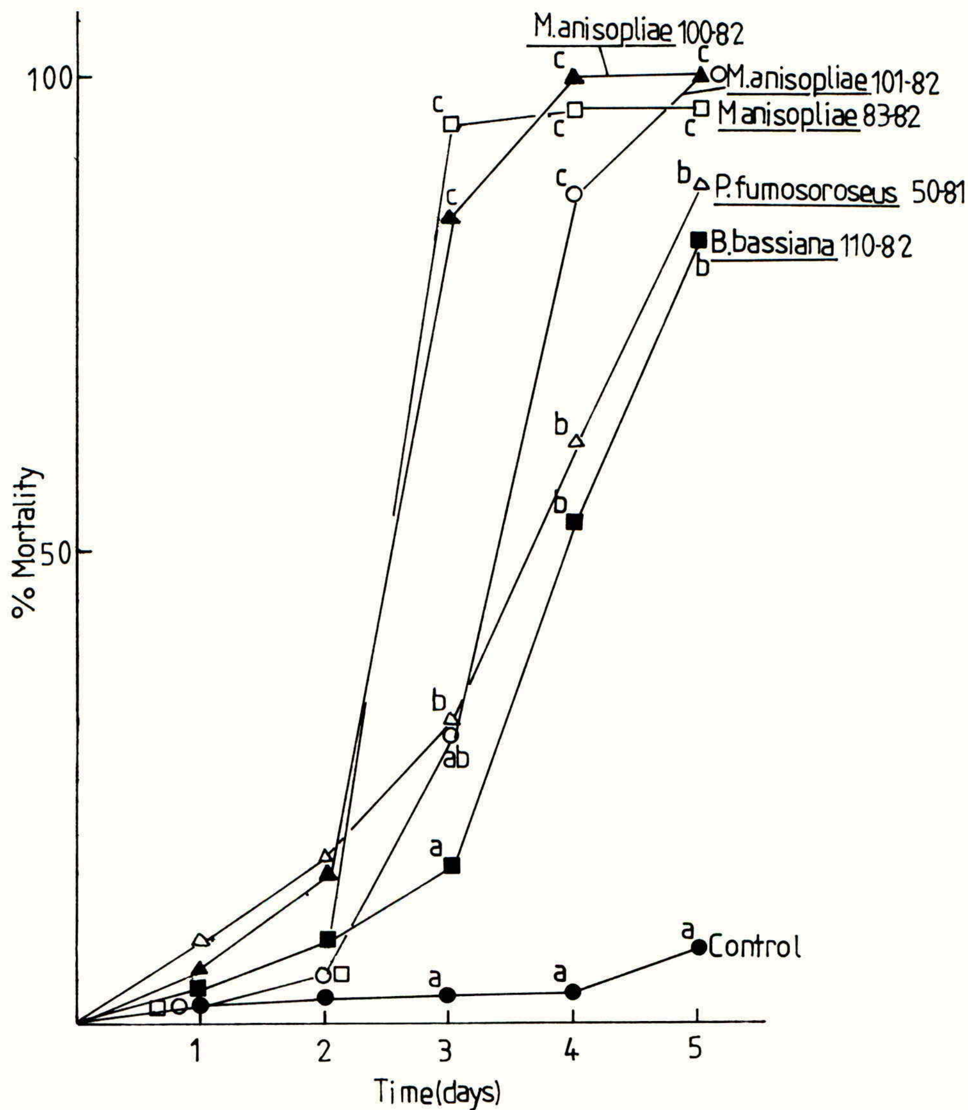

Fig. 2. Mortality of adult, brachypterous Nilaparvata lugens after immersion in 0.025% Triton X-100 or similar solutions containing 5×10^7 conidia ml^{-1} of various fungi and maintenance on rice plants at 25°C. Figure shows mean mortalities computed from 3 replicate groups of 20 insects per treatment. For each sampling date, different letters indicate significant difference (P = 0.05).

Glasshouse experiment

Fourteen days after the first spore application adult numbers declined, while their progeny (nymphs) reached a mean number of about 10 per plant (Fig. 3). After a further 14 days plants treated with viable conidia had more adults per plant (mean 10) than on control rice treated with autoclaved spores (mean 7.4). Coinciding with the increase in adults, populations of nymphs declined, the mean number on viable conidia-treated plants being 3.4 and on controls 5.4. Dead planthoppers fell from rice plants and made mortality assessments on single plants impossible. By 28 days after the first spore application, some 7% of M. anisopliae-treated adults and 1% of nymphs were found infected with M. anisopliae. The fungus did not control planthoppers and 5 weeks after the first spore application most plants were dead, irrespective of treatment.

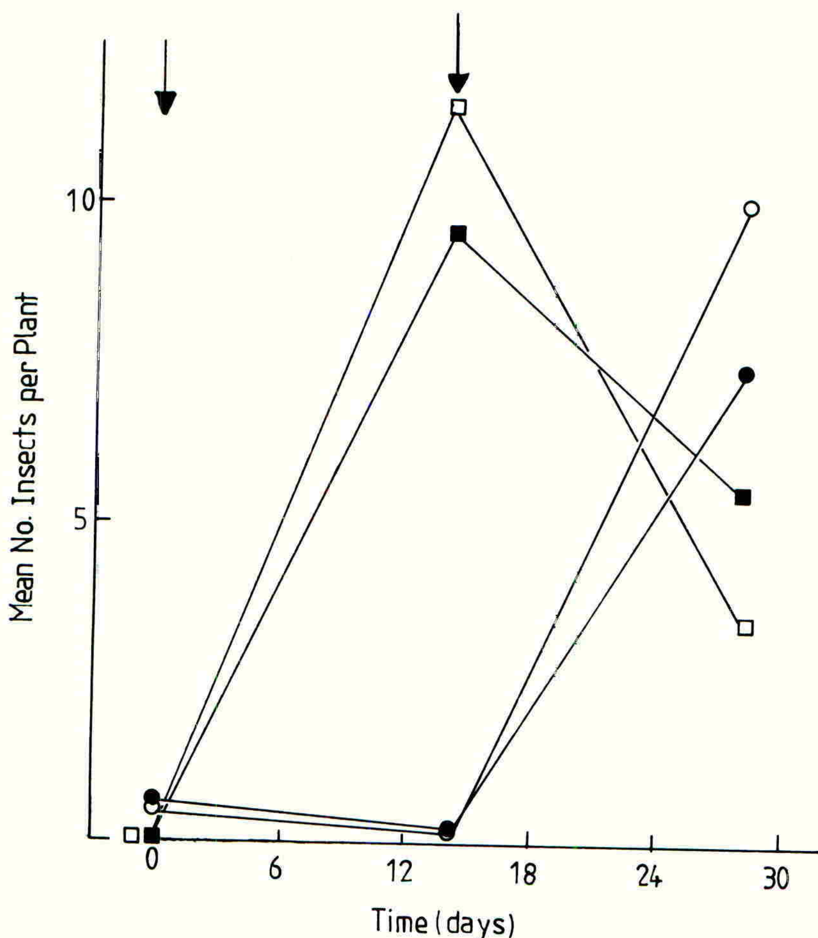

Fig. 3. Mean numbers of Nilaparvata lugens adults (● O) and nymphs (■ □) on rice plants before and 14 and 28 days after application of Metarhizium anisopliae (83-82) and nutrients (open symbols) or autoclaved spores + nutrients (closed symbols).

DISCUSSION

M. anisopliae proved pathogenic to N. lugens under optimum conditions of temperature and humidity in the laboratory, but failed to achieve significant mortality in the glasshouse. The glasshouse was maintained at a minimum 25°C which necessitated a considerable input of heat with concomitant lowering of relative humidities to about 80%. Probably, micro-climate humidities were too low to permit adequate germination and mycelial growth of the fungus. M. anisopliae requires a r.h. of 95% for spore germination (Gillespie 1984). The r.h. in the rice canopy in the tropics does not fall below 90% (Gillespie unpub. obs.), thus the glasshouse simulation is atypical of conditions in a rice paddy. It is recommended that further modifications to the glasshouse environment should be tried and that experiments be conducted in the tropics.

Presently, N. lugens is not regarded as a major pest of rice in areas where it is largely controlled by the use of hopper-resistant varieties. However, where resistant varieties are not used, it can still cause hopperburn over large areas. The author observed hopperburn in Thailand during November 1985. To be accepted by the grower, mycoinsecticides for use on rice must efficiently and reliably control a range of rice delphacids and jassids. Thus it is imperative that field experiments are undertaken to study the effect of fungi on N. lugens, Sogatella furcifera (white-backed planthopper) and Nephotettix spp. (green leafhopper), and that the search for new isolates of fungi continues.

ACKNOWLEDGEMENT

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UNDERSTANDING THE MOLECULAR BASIS OF PATHOGENICITY IN *FULVIA FULVA*

R. HARLING, R. P. OLIVER, L. KENYON, B. G. LEWIS, J. G. TURNER AND A. CODDINGTON

Norwich Molecular Plant Pathology Group, School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ

ABSTRACT

We describe progress in a research programme aimed at cloning and analyzing genes for pathogenicity in *Fulvia fulva*, causal organism of tomato leaf mould disease. Results are presented on the production of fungal protoplasts as the first step in transformation, and on the induction and isolation of fungal mutants altered in pathogenicity. High protoplast yields were obtained using commercial enzymes and protoplasts were capable of regeneration on solid media. A pathogenicity test has been devised enabling the screening of UV-induced mutants for altered pathogenicity and a number of mutants have been isolated.

INTRODUCTION

Attempts to understand the basic mechanisms by which plant pathogenic fungi cause disease have traditionally relied upon the techniques of comparative physiology and biochemistry. From these techniques we know much about the role of pathogen enzymes, toxins and elicitors, for example, but these are factors which are readily observable and quantified, and moreover occur some time after contact between the pathogen and its host. The crucial events, determining whether a challenge will be successful or not, probably happen very shortly after first contact (Daly 1984) and are necessarily difficult to study. These determinative events, and others involved in pathogenicity that remain to be discovered, can now be investigated by the techniques which have become available in molecular genetics, in particular by the use of gene cloning.

Our approach to the study of pathogenicity is to isolate fungal mutants altered in this capacity. It should be possible to identify which genes have been affected, by complementation of the mutants with wild type DNA fragments which restore pathogenicity, then to isolate and clone these genes on the DNA fragments to study their products. Thus we should be able to identify genes important in pathogenicity. Techniques for genetic transformation in filamentous fungi are rapidly progressing (Mishra 1985) and together with a considerable knowledge of the etiology of leaf mould disease (e.g. de Wit 1981) a sound basis exists for studying the molecular biology of pathogenicity in *Fulvia fulva*.

We report here on progress in two preliminary stages of our research programme. Genetic transformation in filamentous fungi normally requires the production of protoplasts. Although the preparation of fungal protoplasts using lytic enzymes is now a well-established technique, there are many variables such as type of enzyme and osmotic stabilizer and no universal system exists. We report briefly here on conditions which we have developed for the release and regeneration of protoplasts from *F. fulva*.

In addition, mutant isolation requires a suitable test for pathogenicity which can be used to screen the large numbers of isolates necessary to give a good chance of finding mutants with altered pathogenicity. We describe a test which we have developed and the isolation of the first mutants.

MATERIALS AND METHODS

Protoplasts

Isolation

F. fulva race 2,4 (source: M. Gerlagh, I.P.O., Wageningen, the Netherlands) was grown on potato dextrose liquid medium in shake culture and mycelium harvested at intervals by filtration. The mycelium was incubated at 28°C with a number of commercially available lytic enzymes and osmotic stabilizers to determine their effectiveness in releasing protoplasts.

Regeneration

Following release, protoplasts were collected and washed by filtration and centrifugation, then spread on potato dextrose agar (PDA) containing appropriate osmotic stabilizers. Colonies derived from protoplasts were counted at suitable dilutions to assess regeneration frequency.

Induction and isolation of pathogenicity mutants

Mutagenesis

Conidial suspensions prepared from a young culture of *F. fulva* race 4 (source: M. Gerlagh, I.P.O.) were exposed to UV light (254 nm) for 4-8 min to give about 1% survival. Irradiated conidia were plated on to minimal medium (Czapek Dox (Oxoid Ltd.) plus 0,01 µg ml^{-1} thiamine, solidified with 2% agar) to exclude auxotrophs. Colonies were picked off on to PDA slopes after 10 days, then left to grow for a further 14 days when they were ready to be used for plant inoculation.

Pathogenicity test

Tomato seeds of various isolines derived from cv. Moneymaker and showing differential resistance to *F. fulva* races were kindly supplied by I.W. Boukema, I.V.T., Wageningen, the Netherlands. Isoline 1148, susceptible to *F. fulva* race 4, was used for initial screening of the mutants. Seeds were surface sterilized in NaOCl, rinsed and placed into universal bottles containing 7 ml of nutrient medium (Bollard 1966) solidified with 0,75% agar. The bottles were loosely capped and placed in a growth room at 22°C with a 16 h photoperiod. After 2 wk, seedlings were 2-3 cm high with fully expanded cotyledons and were ready for inoculation.

Spores were washed off mutagenized colonies with 0,5 ml water plus 0,01% Tween 80, and 0,25 ml pipetted over the upper surfaces of the cotyledons. Each isolate was tested on one seedling at this stage. The bottles were loosely capped and incubated as before for 14 days when presence or absence of infection, i.e., mycelium growing over the surfaces of the cotyledons, was scored. Isolates causing normal symptoms were identified and discarded and those seedlings not showing infection were left for a further 14 days. The potentially mutant isolates were re-tested by inoculation on to six seedlings of isoline 1148 and six seedlings of 83314, an isoline susceptible to all *F. fulva* races.

The reliability of the pathogenicity test was assessed by inoculating six different F. fulva races on to six tomato isolines possessing differential resistance to these races. All combinations of host and pathogen genotype were tested, i.e. compatible and incompatible combinations, with sixfold replication. Control seedlings received sterile water plus 0,01% Tween 80.

RESULTS

Isolation and regeneration of protoplasts

Protoplasts were released from F. fulva mycelium by the enzyme Novozym 234, either alone or in combination with other lytic enzymes (Table 1). The other enzymes which we tested were not able to produce protoplasts without the inclusion of Novozym 234 although other enzymes contributed some lytic activity: later experiments showed that numbers released were higher if Novozym 234 was used in conjunction with another enzyme (data not shown).

enzyme(s)	protoplasts released
Novozym 234	***
Cellulase CP	-
Snail enzyme	-
Novozym 234 + Cellulase CP	***
Novozym 234 + Snail enzyme	***
Cellulase CP + Snail enzyme	-
Rhozyme + Driselase + Cellulase CP	-

TABLE 1

Protoplast release using different lytic enzymes. Mycelium age 48h; stabilizer 0,6M $MgSO_4$ + 0,05M Na maleate buffer pH 5.8.
*** protoplasts released, at least 5 x 10^4 ml^{-1} enzyme solution.
- no protoplasts.

Having successfully found an enzyme which would release protoplasts, factors affecting yield and regeneration were then studied. Inorganic salts containing Mg^{2+} were most effective as osmotic stabilizers (Fig 1). $MgSO_4$ gave large, highly vacuolate protoplasts which floated, making subsequent collection and washing easy. Culture age was important in obtaining high protoplast yields: cultures of 24 or 48 h, which were prior to the logarithmic growth phase, gave the highest yields which generally exceeded 10^7 protoplasts ml^{-1} enzyme solution. As a standard protoplast release recipe we adopted Novozyme 234 plus Cellulase CP, each at 7,5 mg ml^{-1}, in 0,8M $MgSO_4$ buffered with 0,05M Na maleate, pH 5,8.

Protoplasts were capable of regeneration on solid media incorporating an osmotic stabilizer. Regeneration frequencies of about 13% were obtained on sucrose-stabilized PDA. Observations on regenerating protoplasts showed that protoplasts either produced a germ tube directly, or did so after a period of growth by budding: in either case, normal colonies resulted on agar plates. Nuclear staining using a fluorescent dye showed that many protoplasts were enucleate.

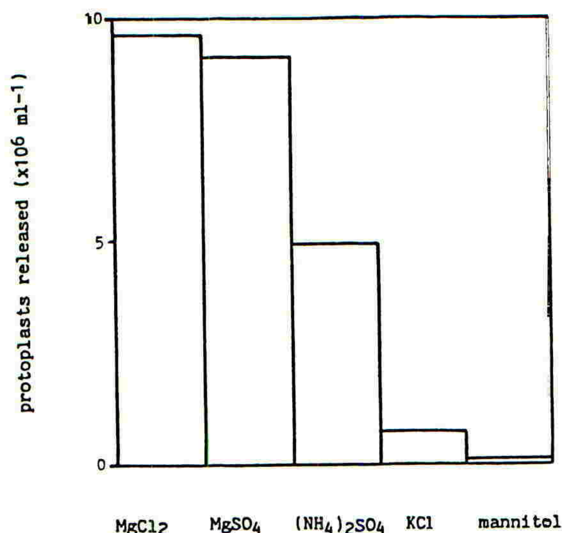

FIG. 1

Effect of different osmotic stabilizers on protoplast release. All stabilizers were at 0,8M and included 0,05M Na maleate buffer, pH 5,8. The enzymes used were Novozym 234 plus Cellulase CP, each at 7,5 mg ml^{-1}. Mycelium age 48 h.

Induction and isolation of pathogenicity mutants

At the initial screening, 97% of mutagenized colonies gave typical disease symptoms on the tomato seedlings after 14 days. From the remaining 3%, when seedlings inoculated with these were left for a further 14 days, symptoms eventually appeared on most and the respective isolates were confirmed as being weakly pathogenic on re-testing. A few isolates gave typical symptoms after 14 days on re-testing: these isolates therefore gave false negative results at the initial screening. We have isolated a number of mutants which are slower to give symptoms and/or cause less severity of disease. In addition we have obtained one isolate which was unable to cause disease on either isoline 1148 or 83314, i.e., has lost pathogenicity. All these mutant isolates showed colony morphologies and growth rates similar to the wild type on PDA.

In the cross inoculation experiments in which various compatible and incompatible combinations of host and pathogen were tested, all seedlings inoculated with compatible *F. fulva* races became infected. The seedlings from incompatible inoculations remained healthy or showed visible hypersensitivity (necrotic patches on the cotyledons) but no disease.

DISCUSSION

We have developed conditions for the release and regeneration of protoplasts from *F. fulva* giving yields suitable for transformation. High yields were obtained using Novozym 234 with $MgSO_4$ as an osmotic

stabilizer. The regeneration frequency of the protoplasts may be improved by further trials but this is unlikely to be increased greatly as many protoplasts were enucleate and therefore would not remain viable. These techniques for the release and regeneration of protoplasts are currently being applied to the development of a transformation system in F. fulva.

A number of fungal mutants showing weak pathogenicity, and one showing loss of pathogenicity, have been induced by UV irradiation and isolated by testing on young tomato seedlings. Auxotrophs were excluded by plating directly on minimal medium: this would exclude mutants whose altered pathogenicity was due to a growth requirement which may not be supplied by the host.

The pathogenicity test fulfils certain requirements as a screen for mutants, especially that it be economical on time, apparatus and space, and provide optimal conditions for infection. Cross inoculations with compatible and incompatible host/pathogen combinations gave results as predicted, proving that seedlings at the cotyledonary stage, grown under the above conditions, provide a reliable test for pathogenicity.

The induction and isolation of mutants is continuing until a collection has been built up. The mutants will be characterized and used in complementation studies.

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BACILLUS THURINGIENSIS: TAILORING THE STRAIN TO FIT THE PEST COMPLEX ON THE CROP

P. JARRETT, H.D. BURGES

Glasshouse Crops Research Institute, Littlehampton, W.Sussex, BN17 6LP, UK

ABSTRACT

Most Bacillus thuringiensis (B.t.) products for agricultural use contain a single strain (HD-1) active against a wide range of lepidopterous pests. Sometimes, low susceptibility of one or more species to HD-1 leaves control of Lepidoptera incomplete. Host ranges of wild B.t. strains differ, some strains being active against pests that HD-1 does not control, e.g. Spodoptera littoralis. Others have improved activity against present key, major, target species, e.g. Heliothis virescens. The toxic protein crystals of many strains contain more than one toxin, each coded by single genes, often carried on different plasmids. Transcipients from plasmid exchange by a conjugation-like process have been tailored for use on crops offering a large insecticides market and simultaneously for markets too small to warrant development of separate specialised strains. Improved toxin combinations might be incorporated into plants by genetic engineering to control larvae that burrow into plants.

INTRODUCTION

Most Bacillus thuringiensis (B.t.) products for agricultural use at present contain a single strain. This is HD-1, effective against a wide range of lepidopterous pests. Sometimes one or more important species, among a complex of pest species on a crop, cannot be controlled by the HD-1 strain. Often this is because species that survive have only limited susceptibility to HD-1. For example, greenhouse pepper crops in the UK were attacked by a mixed infestation of larvae of the tomato moth, Lacanobia oleracea and the cabbage moth, Mamestra brassicae. The tomato moth was eradicated by a commercial product (containing strain HD1), applied with a thermal fogger. In contrast, most cabbage moth larvae survived, even at increased dosages. Another reason for control failure is the feeding habits of larvae of some species; e.g. after hatching, young larvae of cotton bollworms (Heliothis spp.) browse on plant surfaces then bore into the cotton squares. They may not eat a lethal dose of B.t. (which can take effect only after ingestion) from the treated plant surfaces, before reaching B.t.-free internal plant tissue, on which they will feed until they pupate.

In the present work, to overcome some of the above problems, recombinants produced by genetic manipulation of strains superior to HD-1 were selected for improved potency and host range. In the future, genes controlling the production of these toxins might be inserted into plants, thereby overcoming the limitations that result from the feeding habits of larvae.

METHODS

Production of mutants, bacterial growth, sporulation and purification of crystal delta-endotoxin has been described by Jarrett (1985). Crystals

were dissolved in 0.05M carbonate buffer, pH 10.0, containing 10 mM dithiothreitol and further digested by addition of 1 mg/ml trypsin (Sigma) and incubated at 37°C for 1 hour. Sodium dodecyl sulphate (SDS) polyacrylamide (PA) gel electrophoresis and plasmid analysis on agarose gels are described by Jarrett (1983, 1985). Plasmid transfer was performed using the conjugation-like method of Gonzalez _et al_. (1982).

Bacteria were cultured and harvested for insect bioassay by the method of Dulmage (1970). Activities of strains in _Galleria mellonella_ were assessed using the bioassay technique with artificial food described by Burges (1976). Activities in _Heliothis armigera_, _H. virescens_, _Mamestra brassicae_, and _Spodoptera littoralis_ were assayed by addition of a series of concentrations of bacteria to an artificial agar-based diet, described by Payne (1981). For _Pieris brassicae_, the diet of David & Gardiner (1965) was used. All larvae for bioassay were 6 days old. Mortality was recorded after 6 days.

RESULTS

The crystals of many _B.t._ strains contain more than one larvicidal polypeptide. This was shown when crystals were solubilised with alkaline reducing buffers, digested with trypsin and electrophoresed on SDS-PA gels (Table 1). Each toxic polypeptide was active in different lepidopterous

TABLE 1

Size and potency of crystal polypeptides in three wild-type strains of _B. thuringiensis_ var. _aizawi_ and two mutants with small crystals

Treatment	Wild types			Small-crystal mutants	
	a	b	c	d	e
Solubilisation (kDa)	138 130	138	138	138	138
Proteolysis (kDa)	65 58	58	65	58	65
Potency: _P. brassicae_	+	+	–	+	–
Potency: _G. mellonella_	+	–	+	–	+

species (Table 1) and retained full activity after dissolution and digestion. Using the conjugation-like plasmid transfer process discovered by Gonzalez _et al_. (1982), genes coding for the delta-endotoxins in some strains were found to be carried on certain large plasmids (Fig. 1). Different _B.t._ strains were found to have widely different potencies in different host species (Table 2). Using the conjugation-like process (Fig. 2), transcipients were obtained at very high frequencies, e.g. 64%, 43% and 22%. Thus, potencies of the progeny following conjugations could be readily screened directly by insect bioassays or by plasmid profiles.

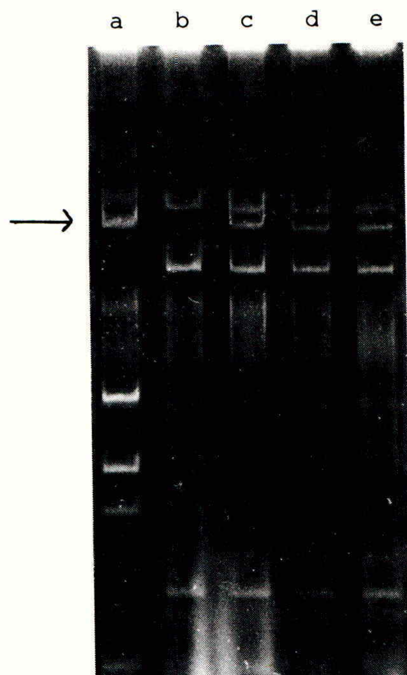

Fig. 1. Plasmid profiles on agarose gels: a, crystal-producing donor strain; b, acrystalliferous recipient; c, d and e, transcipients able to produce crystals after transfer of a plasmid (⟶) from donor into recipient.

TABLE 2

The relative activities* of four distinct B. thuringiensis strains against four noctuid pests

Insect pest	B. thuringiensis strain			
	a (HD1)	b	c	d
Heliothis virescens	42	2	100	52
H. armigera	90	6	100	49
Spodoptera littoralis	7.5	100	<1	24
Mamestra brassicae	15	100	2	32

* Activities expressed as percentages of the highest potency (nominally 100) observed with each insect species

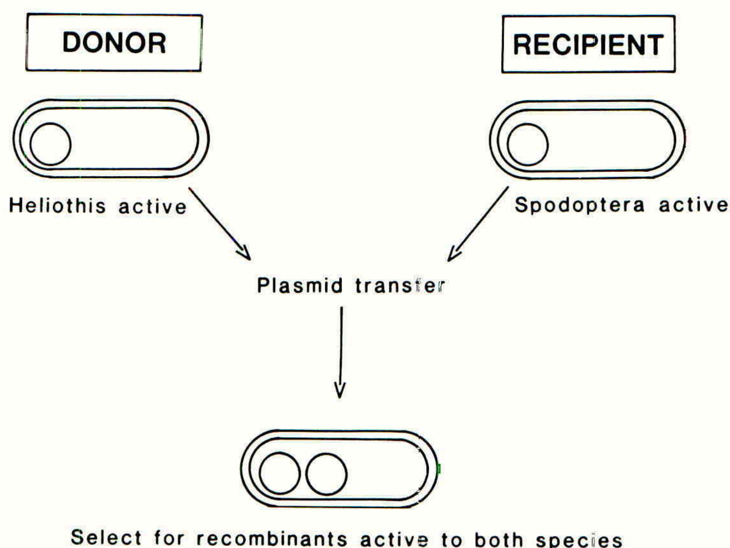

Fig. 2. Strategy of plasmid transfer in B. thuringiensis by the conjugation-like process.

Some transcipients contained a combination of toxins more potent than the combination in the commercial strain, HD-1, against two major pest species, Heliothis armigera and Spodoptera littoralis (Table 3). The particular toxins involved were chosen because they also had high potencies against other, selected host species (Table 4).

TABLE 3

Potency of parent and manipulated isolates of B. thuringiensis against S. littoralis and H. armigera

Insect pest	LC_{50} (ug bacteria/g food)		
	Donor	Recipient	Transcipient
S. littoralis	>10,000	298	220
H. armigera	48	598	39

TABLE 4

Potency of a transcipient strain of B. thuringiensis compared with the HD-1 strain

Insect species	LC_{50} (ug bacteria/g food)	
	HD-1	Transcipient
Galleria mellonella	2,600	18.4
Mamestra brassicae	1,510	162
Pieris brassicae	0.64	0.72
Heliothis virescens	8.6	4.8
Heliothis armigera	42	44
Spodoptera littoralis	5,780	330

DISCUSSION AND CONCLUSIONS

Krieg & Langenbruch (1981) made a list, admittedly incomplete, of susceptibilities of Lepidoptera species to B.t. They classified 146 species as highly susceptible (+++) and 93 as rather less so (++) to the most active strains tested in the artificial conditions of laboratory and small field trials. A major B.t. manufacturer in the USA has registered B.t. for use in favourable circumstances in various countries against 93 species, 34 being in the USA. In a recent leaflet the same firm listed only 14 species in the USA as prime targets. The reductions in these numbers are to some extent the combined result of inadequate potency and limited host range of the almost universally used HD-1 strain. This strain was adopted around 1972 as the best strain available then, and has persisted ever since. B.t. is used infrequently on cotton, probably its largest potential world market, because HD-1 is not potent enough and does not kill newly-hatched larvae of some species quickly enough to prevent them penetrating cotton bolls - away from the protective deposit of B.t. Synthetic pyrethroids are less expensive over a whole season. However, resistance to these chemical insecticides is appearing in bollworms such as Heliothis virescens in the USA.

The transcipient strain illustrated in Table 3 is tailored primarily for the potentially largest market, cotton. It incorporates a delta-endotoxin active against Heliothis armigera (Europe and Africa), with improved activity against H. virescens (USA) and it is also tailored for another quite large market in Europe and Africa, the various field crops attacked by S. littoralis. Its use should enable dosages to be lowered.

The other side of industry, i.e. that of the insecticide user, often faces the problem of finding pesticides developed for crops that provide only small markets, and also registered with regulatory agents for those crops. The income from these markets is judged not to justify the expense involved. With B.t., because the expense of obtaining data for registration is relatively low, this has been partly overcome by genetic manipulation using strains that are also capable of killing "small market pests". Thus the new strain, developed primarily for cotton, is also

designed to be a specialist product for three much smaller markets where it is effective against lepidopterous pests which are not very susceptible to strain HD-1: (1) European brassicas, on which it is effective against the cabbage moth *Mamestra brassicae*; (2) many European greenhouse crops, on which it is effective against *M. brassicae* and *S. littoralis* and (3) worldwide apiculture, in which it is effective against the wax moth, *Galleria mellonella*.

By inserting toxin genes into plants, it might be possible to overcome a major limitation to the effectiveness of *B.t.*, namely larvae burrowing into plants away from surface deposits of *B.t.* Already, expression of the toxin is claimed in genetically engineered tobacco plants (Yanchinski, 1985). Recognition of the host ranges of individual toxic polypeptides will be essential in designing plants protected against whole complexes of lepidopterous pest species.

AKNOWLEDGEMENTS

Heliothis spp. and *Spodoptera* spp. have been reared under licences from the Ministry of Agriculture Fisheries and Food.

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