Session 2

Appropriate technology and training: the way forward

REQUIREMENTS FOR THE SAFE AND EFFECTIVE MANAGEMENT OF PESTICIDES IN LESS-DEVELOPED COUNTRIES

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ABSTRACT

The use of pesticides is subjected to increasing public scrutiny, reflected in tighter national and international legislation. In the developed world, pesticide use is tightly controlled and practices are generally good. The situation in less-developed countries is often, however, quite different and although some individual countries have made substantial progress in the development and enforcement of pesticide-use legislation, with supporting monitoring facilities, many are badly in need of substantial assistance for the provision of appropriate technology and expertise to allow them to implement the necessary programmes.

INTRODUCTION

Pesticide use in less-developed countries is anecdotally synonymous with unsafe practice, misuse and abuse. Registration systems are considered weak, extension services under-manned and ill-informed, and facilities for the monitoring and policing of national and international legislation are few or non-existent.

Although there is much truth in this, recognition must also be given to those national authorities that are both active and effective, often functioning with minimal resources, and who are showing just what can be done. Much comes down to initiative, determination and the will to safeguard the public and the environment from pesticide misuse.

There is no doubt that there is a rapidly growing awareness of the risks associated with the misuse of pesticides. However in most less-developed countries, where pesticides are available and affordable, their continued use is essential for efficient crop production and protection. Integrated pest management programmes are being developed which emphasise improved cultural practices, and the use of biological control systems and natural pesticides to reduce dependence on chemical pesticides. However, conventional chemical pesticides will remain with us, for a wide range of uses, for the foreseeable future and we need to ensure that management practices are substantially improved to accommodate their safe and efficient use.

TECHNIQUES AND TECHNOLOGIES

Pesticide management and application

The safe and effective management of pesticides requires a high degree of training coupled with a thorough understanding of all the issues involved in their choice, procurement, storage and use. There are many good and professional managers involved with the use of these materials who ensure that all reasonable precautions are taken to protect the user, the consumer and the environment. Unfortunately there is also a large proportion of poor managers who, for a variety of reasons, choose to ignore the basics of good pesticide management putting the environment and, more particularly, their fellow workers at risk.

The problem is not always limited to the smallholder, who for reasons of ignorance or lack of funds may purchase cheap, substandard products, who cannot afford protective clothing, or because he/she cannot read the label instructions, prepares treatment solutions by guesswork. Some large farms and estates also exhibit a very casual approach towards pesticide use and operator training, although generally their standards are much higher.

The choice of pesticide for particular pest or disease problems is often the point at which things begin to go wrong. Growers producing crops for the export market will generally operate to defined spray regimes and use pesticides from an agreed list of approved products. Others may receive less guidance and rely on advice from fellow growers, representatives of agrochemical companies or their own judgement. Pesticide choice can also be limited by cost or availability; trade across national boundaries in cheap, banned products can become a significant factor in border areas under such circumstances.

A further range of scenarios can unfold when pesticide packaging is considered. Although standards are improving and the use of pictograms is helpful, problems still occur through poor labelling, labelling in the wrong language and illiteracy on the part of the user. Poor labelling practices often arise when pesticides are formulated or repackaged within country; registration authorities need to be more alert to this. All these factors can contribute to the misuse of the product. In many cases little harm results, but in others the consequences can be serious and deaths have resulted.

The stores in which agrochemicals are frequently kept before, after and between use are a further potential source of accident and error. Many of us have seen, I am sure, products kept in the home, sometimes in plain containers and bottles which could be, and have been, mistaken for drink or for cooking materials. The chances of accidental poisoning in these circumstances are high. By contrast there are also farms which have separate, locked stores with secure shelving, dispensing facilities, spillage control, protective clothing, stock records and water for personal decontamination. In between these two extremes lies a wide range of indifferent practices which can result in mistakes and misuse. The commonest problem is one of poor stock control where new materials are put at the front of the shelf and used first, whilst the older material, usually opened and part used, remains on the shelf, slowly deteriorating before it is eventually used. Mixing of products on a single shelf is also a common sight, allowing every opportunity for the wrong product to be taken and used.

The choice and use of protective equipment, especially in tropical conditions, is a particular problem. Reasons for not using such equipment vary between cost, indifference and the discomfort of wearing the equipment under a hot sun. Lighter, cooler alternatives exist but the use of protective clothing, particularly by smallholders, is poor and is probably the single largest factor in many of the alarmingly high number of reported cases of pesticide poisoning.

Application equipment and practices vary considerably in many developing countries and the whole spectrum from good to bad can often be seen in a short space of time. The commonest fault observed is the use of inappropriate or poorly maintained equipment. Care taken in product selection, solution/suspension preparation and in the application itself can be wasted if the delivery rates or the drop spectrum are incorrect. How many of us have seen punctured coins or washers used to replace nozzle discs on sprayers? How much or how little sprayer calibration is undertaken?

Lack of education and training in the discipline of safe and effective pesticide application is a major issue. In recognition of this, the International Group of National Associations of Manufacturers of Agrochemical Products (GIFAP) has developed its Safe Use Project (Ledru *et al.*, this volume). GIFAP will admit, however, that this merely scratches the surface of the problem and that major initiatives to address the subject of pesticide management and use must be undertaken if improvements are to be made. Such training needs to be aimed at two distinct levels:

- For the supervisor/manager primarily responsible for the handling, storage and supervision of agrochemical use.

- For the operators involved in the preparation and application of agrochemicals.

There are areas of overlap between these but also some quite distinct differences. The supervisor needs to be able to manage effectively all stages in the operation: procurement, stock record maintenance, storage, issue and measurement of pesticides and the supervision of spray activities. He/she will also have to be fully conversant with all of the activities of the operator who, although focusing on a narrower range of activities, will need to be proficient in the safe and effective preparation of treatment solutions, correct application procedures, equipment cleaning and maintenance and personal safety. These issues will be dealt with in greater depth later in the conference proceedings associated with this Symposium.

Monitoring and analysis

Residues

Effective monitoring of pesticide use and the detection of cases of misuse (including over- and underdosing, lack of observance of the pre-harvest interval and the use of illegal pesticides) and of environmental contamination, requires access to supporting analytical facilities. Similarly, national research into improved application techniques or modified application regimes to combat particular pest problems or complexes requires analytical support.

Some developing countries have introduced good quality analytical laboratories, sometimes with donor support, others have no facilities at all. The reasons for this are varied, and include the lack of suitably qualified, trained staff, but there is no doubt that those countries with a substantial or developing horticultural export trade have had the incentive to develop full and appropriate laboratory services; in the current climate of competition between producing nations and in the face of ever-stricter legislation on Maximum Residue Levels (MRLs), the detection of excessive or illegal residues in foodstuffs, by an importing country, could jeopardise future trade links.

The problem faced by many laboratories, and the largest single factor restricting their establishment and maintenance, is one of cost. Quite apart from the set-up costs, which can be prohibitive, there are the increased costs of maintenance where the laboratory is remote from service facilities in the region, and the cost of general consumable materials, particularly solvents and other chemical reagents. For many countries these need to be imported and there are, additionally, problems of supply and logistics which need to be addressed. In some cases these problems are insoluble; in others the basic choice of equipment may be a factor and a more considered choice based on reliability, local service arrangements, minimum of replaceable parts, reduced material consumption etc. may improve the viability of the laboratory and reduce instrument downtime.

Technologies and procedures used by the different laboratories depend essentially on the laboratory's function -- whether support of research studies on one or two known compounds or multi-residue screening of export crops prior to shipment. These laboratories can be poles apart in terms of the complexity and breadth of the techniques used and the equipment required. Analytical procedures commonly used in Europe or the United States may not be appropriate or affordable to some of these laboratories in terms of material requirements.

There is a desperate need for a re-evaluation of analytical practices to meet the competing demands of the analyst seeking ever-smaller limits of analytical detection and using the most sophisticated of methods to achieve them, and the needs of overseas laboratories where the nature of the analysis, and the equipment/material requirements, puts it almost beyond their capabilities. Whilst recognising the need to analyse for extremely low residues and that sometimes there is no alternative, there is a tendency for analysts to use the most sophisticated procedure rather than a more mundane one that could achieve the same result. Similarly, there is a tendency to create new methods of analysis whereas the same result may be achievable by expanding the use of an existing procedure.

There is also scope for the development and use of simplified screening procedures. The use of such procedures is something of a bone of contention with some analysts. Many insist on the principle that *if you are going to do the job, you've got to do it properly* and that laboratories must use a standard, fully validated procedure without any *corner cutting*. Whilst the principle of only using validated procedures is absolutely correct, there is scope for simplified procedures for screening as long as they too are properly validated and their limitations are known. Samples containing residues detected with such a procedure and at a level near to the reporting level or to the MRL can be re-examined as necessary, using a full reference procedure. In this way, the analytical integrity can be maintained whilst at the same time the analytical throughput can be increased and the costs to the laboratory decreased.

There are, regrettably, few such methods published and accepted by the scientific community. Many others exist, I am sure, unpublished, but used in routine national or other surveillance programmes. We need to make these methods more widely available or provide resources for their development if we are to promote the development of residue analysis in developing country laboratories.

Thin-layer chromatography (TLC) is a relatively low-cost technique which may well be appropriate for some routine screening activities and deserves increased attention. Although used extensively in India and parts of Europe, interest in TLC seems to have waned somewhat although some excellent new coatings have been developed. A wide range of chromogenic reagents have been evaluated and excellent results achieved for most pesticides. This work is well documented in the scientific literature, providing a sound basis for uptake and development by interested laboratories.

The use of test kits based on cholinesterase or enzyme-linked immunosorbent assay (ELISA) may also have a role to play although their relevance to multi-residue screening is limited. For particular analyses, especially those using the more specific ELISA procedures, there may be distinct advantages and their use should be considered, although it is important that the limitations of the procedures are known and understood (Cox, 1993).

Formulated products

Similarly, there are relatively few developing country laboratories involved in the quality control of formulated pesticides. The relevant analytical procedures for both chemical and physical properties are well defined, and of necessity, standardised. Equipment needs vary and can utilise a range of analytical techniques; for those countries using a wide range of formulated products, the requirements can be high and, accordingly, the establishment of a quality control laboratory can be difficult to fund. The importance, however, of effective quality control cannot be stressed enough.

Old and deteriorated materials and substandard or wrongly labelled products must be detected and eliminated through effective quality assurance programmes supported by active legislation. If used, such products bring the agrochemical industry into disrepute, cause financial losses to growers, help the development of pest resistance and can lead to rejection of exported horticultural produce. Unscrupulous dealers will continue to sell such products until they know that quality testing is being carried out and that they run the risk of being detected and prosecuted. An excellent example of the benefits of introducing such a programme comes from Costa Rica where, in 1988, a Quality Control Laboratory within the Ministry of Agriculture and Livestock was established with the help of the German Agency for Technical Cooperation (GTZ). Following the introduction of a rigorous quality monitoring programme, the percentage of samples of formulated product meeting national standards in terms of active ingredient content rose from 43% in 1989 to 88% in 1992 (Mesen and von Dueszelen, 1993). When physical properties are included the comparable figures are 32% in 1989 and 80% in 1992. Similar experiences have been reported elsewhere.

The agrochemical industry is a large one and competition for sales is fierce; some small companies, often regrettably in less-developed countries, capitalise on this by producing cut-price products at the expense of product quality. These practices must be detected and stopped through the introduction of effective national quality assurance systems.

TRAINING

Thorough and effective training of all personnel involved in the management and use of pesticides is essential. This should start with the most senior member of the organisation and proceed down towards those responsible for day-to-day operations. The resulting training requirements must be met through international cooperation and the sharing of experience and expertise. Training should only be delivered by competent authorities and not be seen as just another business opportunity by those with minimal expertise but with a good public relations image. Unfortunately this is all too common an occurrence in the developing world where profits are made at the expense of those who can least afford it.

Training should be viewed as an asset and not as a distraction; it is time well spent. Of particular importance are the requirements for training in pesticide analytical techniques, where training inputs are much less than they should be. The training period depends upon the abilities of the individual and his/her projected duties. For an inexperienced chemist expected to be capable of analysing a wide range of pesticides, a minimum of three -- and preferably six -- months' training is recommended. This training must be under the supervision of an experienced, senior analyst and be part of a practically oriented course supplemented by discussion/seminars as new topics are introduced. Pesticide analysis is a subject requiring much practice and cannot be taught from behind a desk. The facilities should, where possible, reflect the equipment to be used by the student on return home. Where this is not possible, he/she must be familiar with all the operations such that there are no difficulties in switching between different makes or models of equipment.

Training requirements should also include inputs on basic equipment installation and servicing, preferably from the manufacturer of the equipment in question, and preventative maintenance and diagnostic testing. Such training is important for the maximisation of instrument usage and to minimise downtime through simple faults that could be easily remedied, or prevented, by basic training. Any additional training costs incurred will, in the long term, benefit the laboratory concerned.

The further requirement for laboratories to be accredited or to work to Good Laboratory Practice (GLP) adds extra pressure, particularly for those laboratories providing surveillance data, monitoring export crops or providing analytical back-up to research for international product registration. The discipline required for this is strict and depends on a thorough understanding of all the factors associated with an analytical laboratory. It will be very difficult for new laboratories to conform to GLP in the early stages of their lives unless they are staffed or managed by senior analysts already familiar with the requirements. Accreditation to international standards or full GLP will not be an option for many such developing country laboratories in the foreseeable future, and for most there is a need to introduce a half-way house. In this, the standards would still be high; the principles of GLP would be operated with the laboratories also participating in collaborative ring exercises. The introduction and recognition of such a standard is important, however, if the laboratories are to be recognised as a source of reputable data and their development encouraged.

The future career development of good quality, trained staff must also not be neglected. There are many examples of where the lack of reward or motivation for such staff has led to their loss to other employment, with the efficiency of their departments suffering in consequence. Good staff, trained in the relevant technical disciplines, are difficult to replace and there is, inevitably, a considerable time lag in training them to a comparable standard.

CONCLUSIONS

In summary, and without wishing to minimise the substantial progress made by some individual countries, there are many challenges for the nations of the developing world to address before safe and effective pesticide management can be achieved. Training and equipment requirements must be met, effective accommodation and test areas must be provided and the implications of national and international legislation must be understood. Modern technology has a role to play in supporting these processes, but care needs to be taken in its selection such that the most appropriate is chosen for the situation rather than what may be the latest development in the field. Procedural developments, often demand-led from the developed nations, must bear in mind that there are many other potential users and that their needs must be similarly addressed.

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BIOLOGICAL CONTROL OF INSECT PESTS AND WEEDS IN THAILAND: A CHALLENGE AND A THEORY OF MULTIPLE EFFORTS

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ABSTRACT

Crop protection practices in the developing world are generally presumed to be primitive, inappropriate and inadequately scientific, according to the standards of the developed world. Such cultural intimidation is also applied to the biological control practices being developed or already accomplished in the developing world. In contrast to such negative assumptions, the accomplishments in biological control of insect pests and weeds in Thailand, one of the less-developed countries, are presented. Some successes are listed with reference to theories of biological control, where applicable. The necessity for multiple efforts is stressed to encourage fellow countries in the developing world to confront crop protection problems, relying on biological control either as a single control measure, or as a major component in integrated pest management systems.

INTRODUCTION

Economically the world has been sharply divided into the rich and the poor, the North and the south, the East and the West, the First and the Third World, the developed and the developing, the more affluent and the less fortunate, and so on. Such economic divisions are obviously made from the materialistic viewpoint, and fail to consider other parameters such as culture, way of life, climatic and biological factors.

Likewise, crop protection, or the struggle against insect pests, diseases, weeds and other pest problems, has also been similarly divided such that the crop protection methods in developed countries are regarded as superior to those currently practised in the less-developed countries, labelled as the "Third World". This assumption has been repeated continually in the crop protection literature, and has also been deeply rooted both in the minds of westerners who have little knowledge of crop protection, and in the minds of qualified crop protection experts in various national and international development and assistance agencies. But are there significant differences in the crop protection practices employing certain synthetic chemicals: their effects on users; their target and non-target organisms; and their effects on the environment in the developed and developing worlds? The same question can be addressed to other crop protection practices, including those using non-chemical methods such as biological control.

This paper presents a challenge to the conventional view of crop protection in the developing world and emphasizes the need for multiple efforts, by drawing supporting evidence from various attempts at the biological control of insect pests and weeds in Thailand. For this purpose it is appropriate to consider various definitions of biological control, and to refer to some theories of biological control and relate them to current practices.

DEFINITIONS OF BIOLOGICAL CONTROL

Classical biological control is defined as "the action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average than would occur in their absence" (DeBach, 1964). It can be *disciplinary*, defined as "the study and utilization of parasites, predators and pathogens for the regulation of host population densities" (DeBach, 1964), further simplified as "the utilization of natural enemies to reduce the damage caused by noxious organisms to the tolerable level" (DeBach & Ronsen, 1991). With the advent of rDNA technology, genetic engineering and biotechnology, biological control has been defined and modified as "the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests), and to favour desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms" (NAS, 1988); this description is referred to here as *augmentative* biological control. With the worldwide attempts to search for alternatives to pesticides, biological control has been described as "any pest control measure not employing synthetic chemical pesticides". The latter definition embraces other biology-based control measures such as host resistance, botanical pesticides, use of insect growth regulators, etc., all of which have been coined as *parabiological control* by Sailer (1981).

From these definitions, van den Bosch *et al.* (1982) preferred a concept of biological control that embraces other control measures, and regarded the definition of biological control in DeBach (1964) as a classical and traditional one. The definition of biological control by the National Academy of Sciences (NAS, 1988) has also received wide objections from numerous conventional biological control workers worldwide.

It has been strictly in the context of these definitions that attempts to develop biological control in Thailand have followed the route from DeBach's (1964) definition through that of DeBach & Rosen (1991), and to a very large extent through that of Sailer (1981), and are venturing at an increasing pace into that of NAS (1988). The trend of research and development in biological control in Thailand has thus far progressed in the same manner as in developed countries.

BIOLOGICAL CONTROL OF INSECT PESTS AND WEEDS IN THAILAND

Systematic research and development for the implementation of biological control in Thailand was not initiated until 1975, when the National Biological Control Research Centre (NBCRC) was established with support from the National Research Council of Thailand, and implemented by Kasetsart University in collaboration with 17 other national institutions comprising various universities, government agencies and enterprises. In addition to its headquarters located at the Bangkhen (Bangkok) campus of Kasetsart University, NBCRC has established regional centres: Central at Kasetsart University, Kamphaengsaen Campus (Nakhon Pathom); Northern at Maejo University in Chiangmai; Northeastern at Khon Khaen University (Khon Khaen); and Southern at Prince of Songkla University, Hat Yai, Songkla. With the headquarters, these NBCRC regional centres serve as focal points for research and development activities and implementation of biological control projects specific to regional insect pest and weed problems.

Prior to the establishment of NBCRC, attempts at biological control carried out in Thailand were negligible. The first accounts on the parasitic Hymenoptera in Thailand included reports by Ladell (1930, 1931, 1933a,b) in one of which a eulophid egg parasite, *Tetrastichus schoenobii,* was found attacking rice stem borer. It was not until 1963 that the very first attempt at classical biological control was made when a scolid wasp, *Scolia ruficornis*, was introduced from the then Caroline Islands for the control of the coconut rhinoceros beetle, *Oryctes rhinoceros*. This was soon followed in 1965 by the introduction of *Brachymeria* sp., *Cotesia plutellae*, *Tetrastichus sokolowskii*, and *Diadromus (Thyraella) collaris* from India for the control of the diamondback moth, *Plutella xylostella*. An event that helped place Thailand on the world map of biological control took place in 1974, when a braconid larval parasite, *Cotesia erionotae,* was introduced from Thailand to Hawaii resulting in complete control of the banana skipper, *Erionota thrax,* in the Hawaiian Islands. *C. erionotae* was later introduced to Papua New Guinea, resulting in highly satisfactory control of the banana skipper.

Substantial achievements in biological control of insect pests and weeds in Thailand since 1975 have been reviewed by Napompeth (1982) and updated by Napompeth (1989, 1990a,b, 1992a,b). Details of some of these accomplishments are given in NBCRC publications as extension leaflets, technical bulletins, special and miscellaneous publications and research reports. Such accomplishments in biological control encompass both augmentative and classical biological control of insect pests and weeds, and the use of biological control as a major component of integrated pest management systems.

Within a span of about three decades, a less-developed country such as Thailand has been able to build up specialized infrastructure and other supportive mechanisms, enabling the country to initiate, use and share its natural enemy resources, and to implement, collaborate and sustain biological control activities in a self-sufficient manner to such an extent that NBCRC has been considered as the key "natural enemy" of chemical control advocates and chemical companies within Thailand.

SOME THEORIES OF BIOLOGICAL CONTROL

The discussion of biological control concepts and questions by Doutt & DeBach (1964) describes a number of theories of biological control: the *sequence theory* of Howard and Fiske (1911), criticized by Thompson (1923); the *island theory* related to Imms' ecological islands (Imms, 1931); and the *three-generation* or *three-year theory* of Clausen (1951). Other theories relating to biological control are the *theory of new and old association* of Hokkanen & Pimentel (1984), and the *refuge theory* of Hawkins *et al.* (1993). All these theories were proposed in connection with classical biological control, where introductions of natural enemies are the main activities. Waage (1990) also attempted to justify the application of ecological theory in two approaches to the selection of biological control agents for introduction: *reductionist* and *holistic*. The theories propounded in biological control may or may not be applicable in a practical situation, because in many cases sufficient evidence is lacking.

How important are the better-known theories in biological control: the sequence theory, the island theory and the three-generation or three-year theory, for example, to less-developed countries in their attempts to initiate and develop biological control programmes? The less-developed countries can draw upon the experiences of the developed countries to improve their own programmes, by attempting the successes achieved through repeated effort, and avoiding the mistakes or failures previously experienced elsewhere.

The sequence theory

The sequence theory of Howard & Fiske (1911) states that with the gypsy moth or the brown-tail moth, parasitic (biological) control must come about through a variety of parasites, working together harmoniously, rather than through one specific parasite. The theory was challenged and criticized by Thompson (1923) who stated that, while a sequence of parasites may sometimes be desirable, nevertheless a single effective parasite on one stage of the host may well bring about control. Biological control of the banana skipper, *E. thrax*, in Thailand, Hawaii, Guam and Papua New Guinea could provide supporting evidence to Howard & Fiske (1911) as well as Thompson (1923).

In Thailand, *E. thrax* was parasitized by a number of parasitic insects. The more dominant and effective ones were the egg parasite, *Ooencyrtus erionotae*, the larval parasite, *Cotesia erionotae*, and a number of pupal parasites of which the chalcid *Brachymeria excarinata* dominated. The banana skipper is thus a good target for augmentative biological control in its native range, using a sequence of agents already available. Both *O. erionotae* and *C. erionotae* were introduced from Thailand to Hawaii and Guam but it turned out that *C. erionotae* was the more effective agent, contributing to the complete control of *E. thrax* in Hawaii but only substantial control in Guam. *C. erionotae* was later chosen as an agent and introduced from Guam to Papua New Guinea, where more or less complete control was achieved. Thus the sequence theory was at work in Thailand, while a single parasite on one stage of the host was also effective in Hawaii, Guam and Papua New Guinea.

An issue of importance in classical biological control is single *versus* multiple introductions. Parasites for diamondback moth control with single introductions to the country were *C. plutellae* (firmly established); *Macromalon orientale* (only recovered after a lapse of almost three decades); *Diadegma insulare* from Canada in 1964, *D. semiclausum* from Taiwan in 1989; and *T. sokolowskii* from India in 1976 and Pakistan in 1981-82. An egg parasite, *Trichogrammatoidea bactrea fumata*, found attacking the diamondback moth in Thailand, was also introduced to Taiwan in 1988.

A single-introduction approach was also adopted when the mottled water hyacinth weevil, *Neochetina eichhorniae*, was introduced from Florida to Thailand in 1977. It was not until 1990, when *N. eichhorniae* had become widely distributed in Thailand and had moved to neighbouring countries including Malaysia, that the second species of the chevroned water hyacinth weevil, *N. bruchi,* was introduced from Florida via Australia. Both the weevils are complementary in biological control of water hyacinth in Thailand.

A multiple-introduction approach was adopted in biological control of the giant sensitive plant, *Mimosa pigra*. The introduced agents were the seed bruchids, *Acanthoscelides puniceus* and *A. quadridentatus*; the top-shoot-feeding chrysomelid, *Chlamisus mimosae*; the young flower-bud-feeding apion, *Coeloecephalapion aculeatum*; and the stem-boring sesiid, *Carmenta mimosa*, from South America via Australia. Of these introduced agents the seed bruchids are doing a very good job while others have not shown much promise. The same bruchids released in Northern Territory, Australia, did not perform as well as their counterparts in Thailand. Here the frequency of field releases could make a difference: since their introduction into Thailand in 1983, multiple field releases have been made consistently until the present.

The island theory

The island theory was based on the striking successes of biological control in the islands of Hawaii and Fiji, and in Imms' ecological islands (Imms, 1931) such as California. The island theory attempts to confirm and limit classical biological control methods to the insular areas, and suggests that biological control projects in non-insular areas are likely to meet with failure. The theory has received objections from biological control workers on the grounds that the successes in biological control in Hawaii, Fiji or California are not necessarily due to their insular location.

Thailand, although located on the Southeast Asian continent, could be considered an ecological island from a geographic and topographic point of view. Thus, according to the island theory, the country should be suitable for classical biological control. However, some of the classical biological control projects carried out in the country have met with failure. Examples are the failure to become established of several introduced parasitic Hymenoptera for the control of the diamondback moth, in spite of repeated introductions and field releases; and the failure of an arctiid moth, *Pareuchaetes pseudoinsulata*, introduced from Guam for the control of the exotic Siam weed, *Chromolaena odorata*, to become established although field releases covering diversified habitats were made.

In reality, most biological control projects, augmentative or classical, are carried out in locations and habitats with ecological limits and boundaries, the so-called *ecological islands*. It should be emphasized that it is mainly determination, combined with effort and given resources, that will generate success in biological control attempts no matter whether the country is geographically insular or not.

The three-generation or three-year theory

The three-generation or three-year theory of Clausen (1951) states that an effective parasite or predator might be expected to show evidence of control at the point of release within a period of three host generations or three years. The three-year theory is obviously applicable to target pests for biological control in temperate regions where most insects are univoltine. Under tropical conditions three generations could take much less than a year, and thus the three-generation theory would be more applicable. This theory is here restricted to the introduction, liberation and establishment of natural enemies in classical biological control, and has received both support and criticism.

Several coccinellids introduced to Thailand could not be recovered at all after field releases. Two coccinellids introduced and released for the control of the *Leucaena* psyllid, *Heteropsylla cubana*, behave differently: while *Curinus coeruleus* became established in less than a year but after several prey generations, *Olla v-nigrum* was found established once in one location in abundance but disappeared altogether afterwards. An encyrtid nymphal parasite, *Psyllaephagus yaseeni*, introduced for the same purpose, was firmly established in less than a year and certainly after several host generations. There is no clear explanation in a situation like this why the two coccinellids performed differently within the same habitat, while in Hawaii, where they had also been introduced a long time previously from Mexico, both *C. coeruleus* and *O. v-nigrum* do occupy the same habitat but with different population densities. Efforts were also made to introduce *O. v-nigrum* from Tonga to Thailand, but its fate was the same as before.

In classical biological control of weeds, the three-generation or three-year theory has to be modified. While the three-generation theory could apply to annual weeds, it will definitely not be applicable to perennial weeds; in the latter case the three-year theory would be more acceptable. Two weevils introduced for the control of water hyacinth in Thailand, *N. eichhorniae* and *N. bruchi,* showed obvious evidence of their establishment in less than a year. The same evidence was also obtained with the releases of two seed bruchids, *A. puniceus* and *A. quadridentatus*, for the control of the giant sensitive plant, *M. pigra*. However, the top-shoot-feeding chrysomelid, *C. mimosae*, was discovered in less than a year at the point of release only once and has not been found since.

Some parasitic Hymenoptera introduced for the control of the diamondback moth in the mid-1960s could not be traced. Among these parasites, only *C. plutellae* and *M. orientale* were discovered again in the early 1990s. This is caused not by the parasites themselves, but rather by failure to monitor them using the concept of the three-generation or three-year theory. No matter how superficial the theory, its application in classical biological control would be of help in terms of timely evaluation of the project. In any case, the project should not be assumed discontinued after three host generations or three years in compliance with the theory. In almost all biological control projects, additional and cumulative efforts would be worthwhile as long as such an effort is cost-effective and justifiable.

Other theories of biological control

Other theories of biological control worth mentioning are the theory of old and new association of Hokkanen & Pimentel (1984), and the refuge theory of Hawkins *et al.* (1993). So far there are no cases of biological control in Thailand which relate to the theory of old and new association. However, in the evaluation of the success or failure of various biological control projects, the refuge theory is highly applicable. Using a model proposed by Hawkins *et al.* (1993), it is possible to quantify the extent that parasites will depress host populations below the densities that hosts could achieve in the absence of parasites. The refuge theory predicts that hosts which occupy small refuges (that is, a low proportion of their population is in the refuges) will be highly exploitable by parasites, and as a result the host population will be severely reduced; conversely, for hosts that occupy sufficiently large refuges, parasites will be unable to exploit the host population sufficiently to depress its density appreciably. The theory concludes that the success of biological controls is inversely related to the proportion of insects protected from parasitism.

However, the refuge theory does not take into consideration the basic nature of biological control agents as the density-dependent mortality factor. In a refuge, large or small, density-dependent action is always in operation and self-destined in accordance with the population size, large or small. However, in the absence of substantial quantitative evidence to analyse, there is a need to ascertain whether the refuge theory could be used to evaluate the success of biological control projects.

ACHIEVING SUCCESS BY MULTIPLE EFFORTS

The development of biological control in Thailand has passed through a primitive phase lacking direction, to a phase in which systematic approaches were adopted and substantial achievements have been accomplished, to the extent that it can serve as a model for other countries in the developing world to follow. In all cases, in reference both to single successful projects and to overall biological control research and development activities, these achievements were the outcome of coordinated and multiple efforts. The various case studies in biological control of insect pests and weeds in Thailand, described above in relation to definitions and theories or biological control of insect pests and weeds, demonstrate the need for multiple efforts. It is the author's experience that *the more effort one devotes to the development of matters related to and supportive of biological control in terms of cost-efficiency and resources, the more likely are the successful achievement and desirable accomplishment in biological control regardless of any anticipated limitation and constraint. Multiple efforts should include multiple introductions and multiple parasites where appropriate.* The national profile on biological control of insect pests and weeds in Thailand serves to validate this statement.

CONCLUSIONS

In less-developed countries, the failure to initiate biological control projects is primarily due to the myth that biological control is expensive and difficult to achieve, and requires enormous investment and manpower, while the chances of success are remote and highly unpredictable. The governments of less-developed countries (or even developed countries) are very hesitant to provide adequate support for biological control projects. In this situation, a gap can be identified, which opens up the opportunity for outside expertise to enter, at a cost out of proportion to local and national standards, and still with no guarantee of success. Less-developed countries desiring to carry out biological control projects are thus toured by "safari" experts trying to convince the least knowledgeable high-ranking government officials and policy makers to accept their services at cost. The proposed commitment of multiple efforts needs to be applied to enable biological control projects to be economically realizable, and to enable competence in biological control to become deeply rooted and proliferated in the developing world.

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BIOTECHNOLOGY AND THE PROSPECTS FOR IMPROVING CROP RESISTANCE

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ABSTRACT

The worldwide output from agriculture is unable to meet the global demands for food today. Not only is the current application of agriculture unsustainable, but the demands upon it are continuing to increase at a significant rate. The requirement for improved crop resistance and the impact that biotechnology can have are discussed with specific reference to the use of plant-derived pest control genes, their specificity of action and their durability. The major beneficiaries of this technology should be the less-developed countries but the application of biotechnology in such regions is not straightforward. A number of these problems are considered.

INTRODUCTION

Biotechnology encompasses a wide range of techniques, of varying degrees of sophistication, which may be of value in increasing the resistance of crops to pests, pathogens, weeds and biotic stresses. This paper is confined to discussion of those involving the introduction of *foreign* genes into crop plants -- the genetic engineering of resistance -- and will concentrate in particular on resistance to pests. However, the potential for biotechnology to enhance other forms of resistance and other agronomic characters will be of similar significance to the agriculture of the future.

THE NEED FOR IMPROVED CROP RESISTANCE

Worldwide agricultural output is unable to meet even the most basic needs of a sizeable fraction of humanity, with around one billion people suffering from hunger or malnutrition today. In 18 countries of sub-Saharan Africa it has been estimated that 45% of the population lives in absolute poverty; in 12 of these countries the figure is above 65% (Myers *et al.*, 1985). This problem is exacerbated by the rate at which growth of the human population is outstripping improvements in agricultural productivity. In some less-developed countries this is not a matter of mere statistics, but of life and death itself. But even the high-unit-output agricultural systems of the developed world are coming under increasing pressure due to their heavy dependence on high levels of industrial inputs. The view is now widely held that such systems are unsustainable because of their huge cost in terms of non-renewable resources; their inefficiency in terms of the proportion of these resources which actually miss their intended target; the environmentally unacceptable consequences of the preceding criticisms, such as contamination of food chains and water sources and physical degradation of the environment; and growing consumer dissatisfaction with the publicly perceived consequences of high-input agricultural practices.

We are faced, therefore, with the dilemma of accomplishing a massive increase in food production, by an estimated 75% by the year 2000 (Blaxter, 1986), but doing so in a sustainable, environmentally friendly way. A substantial contribution to this end could be made by preserving more of what is grown for its intended end use. It is estimated that approximately 40% of agricultural production is lost to pests, pathogens and weeds; this despite the current approximately US$27 billion spent annually on crop protection. There is clearly scope for improvement here, and increasing the inherent resistance of crop plants is an obvious target -- indeed, one which has been a major goal for many conventional plant breeding programmes for years and one of the earliest identified objectives of plant genetic engineering.

Crop losses due to insect pests are crudely estimated at around 14% of potential global yield (range for specific crop per area = 0-100%), despite the expenditure of some approximately US$8 billion per year on synthetic chemical insecticides. Crops with inherent insect resistance have the potential to offer a huge saving in production costs, substituting for some of the expenditure on insecticides, and to enable huge savings in yield.

Similarly, nematodes are pests of major economic importance worldwide, but are of particular importance in the tropical and sub-tropical regions where they may be a major constraint on agriculture. For example, losses due to parasitic nematodes on bananas and plantains have been estimated as varying from 5-263% (over several crop cycles) and the progressive decline of ratoon crops may necessitate replanting within 5 years (Gowen and Queneherve, 1990). There are at present few effective, acceptable options for nematode control. Development of more nematode-resistant crops would allow the substitution of the highly toxic chemical nematicides currently in use and permit cultivation where farmers could not previously afford or justify nematode control.

Securing anything like an adequate world food supply in the future will depend on the development of sustainable agricultural systems which will not be dependent on high inputs of non-renewable resources. The technical advantages of using cultivars with high inherent resistance, relative to the use of exogenously applied chemical protectants, have been outlined before (*eg*. Boulter, 1993a). Many modern, high-yielding cultivars are considerably less resistant than their wild relatives. Enhancing inherent resistance by genetic engineering might offer new solutions to some of these problems of crop protection.

WHAT CAN BIOTECHNOLOGY DO?

Genetic engineering offers a number of unique benefits over conventional plant breeding for resistance. It widens the potential gene pool from which *resistance* genes may be taken, as genes from any source, not just interbreeding plants, may be selected for transfer. It allows a number of different desirable genes, from different sources, to be introduced in a single event into a crop species, and reduces the time required to introgress introduced desirable characters into an elite genetic background.

The practical application of plant genetic engineering involves two equally important constituents, cellular and molecular biology. The list of crop species which are amenable to genetic engineering has grown steadily and now includes some of the major grain monocots, such as rice, maize and wheat, and some of the legumes, such as soyabean and pea, which were previously recalcitrant. Transforming many of the most important crop species remains, however, a far from trivial exercise and is frequently genotype-dependent, with elite lines usually proving harder to transform than model varieties. Many of these transformable crops are of major importance in the developing world, but there remain many *orphan* crops for which transformation systems have not been successfully developed. The development of transformation systems for these orphan crops should be a priority funding target area for the international aid agencies. Transformation technology continues to be developed at a rapid pace and recent advances have been made using established technology, *eg*. the use of *Agrobacterium tumefaciens* to transform rice at high efficiency (Hiei *et al.*, 1994) as well as the development of new technologies, *eg*. silicon carbide fibre-mediated DNA delivery (Kaeppler *et al.*, 1990; Wilson *et al.*, 1994). These two developments demonstrate that transformation of such *orphan* crops should be possible in the future, and that methodologies should become available that do not depend on expensive equipment or consumables.

The list of genes which might usefully be transferred into transgenic crops has not grown at a similar pace, although there are now a number of genes which confer enhanced resistance to various yield-limiting agents. By far the greatest research effort in developing pest-resistant transgenic crops has gone into expression of *Bacillus thuringiensis* (*Bt)* toxins in plants.

Transgenic crops expressing *Bt* toxins

The crystal toxin genes produced by the insect pathogenic bacterium *Bacillus thuringiensis* have been in limited field use as biological control agents for more than 25 years. Expression of modified genes encoding the *Bt* toxin provided the first examples of genetically engineered insect resistance in plants (Barton *et al.*, 1987; Fischhoff *et al.*, 1987; Vaeck *et al.*, 1987). *Bt* toxins are very effective in controlling neonate larvae of susceptible lepidopteran insect species, but the range of pest species which are susceptible to any particular *Bt* toxin is extremely limited. In order to obtain adequate levels of expression in plants of this bacterial gene it has had to be substantially modified -- truncated, altered in codon usage and fused to highly efficient promoters -- to the extent that the current generation of *Bt* toxin genes are essentially 'rebuilt' (Perlak *et al.*, 1991). *Bt* toxin has now been expressed in such major crops as cotton (Perlak *et al.*, 1990), maize (Koziel *et al.*, 1993) and rice (Fujimoto *et al.*, 1993), with the transgenics showing good resistance to the major lepidopteran pests in both laboratory and field trials. Commercialisation of the first of these genetically modified, insect resistant crops is expected within the present decade.

Transgenic crops expressing plant-derived pest control proteins

An alternative source of pest control genes are plants themselves: exploiting the plants' solutions to the plants' problems in a *copy nature* strategy (Boulter, 1993b). Within the Axis Genetics Ltd/Durham University Insect Resistance Programme, a number of quite different types of plant protein have been identified which have pest control properties, including enzymes, enzyme inhibitors and lectins. Work is in progress with over a dozen different plant-derived genes, representing more than six different classes of pest control protein (reviewed by Gatehouse *et al.*, 1992; Hilder *et al.*, 1992).

The paradigm of this approach is cowpea trypsin inhibitor (CpTI), expression of which in the leaves of transgenic plants enhances their resistance to various lepidopteran pests

(Hilder *et al.*, 1987). Such limited field trial data as is available suggests that a similar level of partial control is achieved in the field (Hoffmann *et al.*, 1992). There are unpublished reports of successful field trials of CpTI-expressing transgenics in China.

CpTI serves to illustrate two important general characteristics of these plant-derived pest control proteins. (1) Their absolute toxicity is relatively low, especially compared with synthetic pesticides or *Bt* toxins. They rarely produce 100% kill of insects in any realistic trial, tending rather to increase mortality to a limited extent but significantly to retard insect development and growth. (2) They tend to have an effect against a broad spectrum of pests (Table 1). Thus CpTI has been demonstrated to have some significant effect not only against a number of pest species of lepidopteran and coleopteran insects, but also against some orthopterans (Good *et al.*, 1994) and plant-parasitic nematodes (Atkinson, 1993). Similarly, the mannose-specific lectin from snowdrops (GNA) is effective not only against lepidopterans and coleopterans, but expression of GNA in transgenic plants offers the potential to control the sap-sucking homopteran insects (Hilder *et al.*, 1994), a very important group of pests.

TABLE 1. Spectrum of activity of pest control proteins.

	Protease inhibitors		Lectins		Chitinase	*Bt* toxins	
	Serine	Thiol	Mannose	NAcGlu		cryI	cryIII
Lepidopteran	+	-	+	+	+	+	-
Coleopteran	+	+	+	+		-	+
Homopteran	-		+	+	-	-	-
Nematodes	+	+	+				
Fungi			+	+	+	-	-
Mammals	±	-	-	+	-	-	-

SPECIFICITY OF ACTION

Different considerations apply to the spectrum of activity of transgenic plants expressing pest control proteins than to synthetic, exogenously applied pesticides. The aim with transgenic plants should be to provide a broad spectrum of protection, whereas there is a trend to favour highly selective, narrow-spectrum pesticides. Because the transgene products are essentially confined within the host plant, they are intrinsically specific to those pests which are heinous enough to invade and eat the crop. It should be remembered that most crops are not subject to attack by a single pest species but by an entire complex of different pests. For example, the cowpea crop in Africa is vulnerable to attack by a range of insects throughout the growing season until post-harvest storage. Major pests belong to the orders homoptera, lepidoptera, coleoptera and thysanoptera, with any single species capable of reducing yield by 20-100% (IITA, 1984). Similarly cotton, although grown under a number of different cropping systems, is subject to losses to a surprisingly similar pest complex worldwide, principally heliothines, mirids, aphids, spider mites and thrips (Lutterell *et al.*, 1994). The advantage of, say, transgenic cotton with protection against boll worms would be seriously limited if it were still necessary to make a dozen applications of insecticide to control other members of the pest complex.

Within a transgenic plant, the broader the spectrum of activity against different pests the better. From the farmer's point of view, what ultimately matters is not how many dead individuals of a particular pest species there are, but what is the yield in the face of the challenge from the whole pest complex.

It is, of course, important that intended consumers of the transgenic crop are not included within the spectrum of activity. Many insecticidal plant-derived proteins are notoriously toxic to humans (*eg*. the lectins wheat germ agglutinin and *Phaseolus* bean agglutinin). This has to be a major concern in the selection of genes; proteins such as CpTI and GNA have been demonstrated to be benign in independent mammalian feeding trials (Puzstai *et al.*, 1990; 1992). Further work is needed to clarify their possible effects on beneficial insects in insect-pollinated crops.

Arguments for broad-spectrum activity may also apply to where and when in the plant the protein is expressed. There appears to be little reason to restrict the activity of broad-spectrum pest control proteins within the plant by the use of tissue-specific, developmentally regulated or inducible promoters. It has been suggested that such regulated expression would minimise any yield penalty associated with transgene expression, but all the available evidence from laboratory trials (Hilder and Gatehouse, 1991) and field trials (Table 2) suggests that there is no such penalty to minimise. It has also been suggested that restricting expression in the plant could contribute to management of resistance build-up in the pest, although it unclear how this is supposed to work. There are some cases where specific promoters might be advantageous, *eg*. for root-feeding nematodes which modify expression at the feeding sites and tend to inactivate general promoters.

DURABILITY OF RESISTANCE

The development of resistance to *Bt* toxins in the laboratory and the field has set alarm bells ringing in many quarters (McGaughey and Whalon, 1992; Tabashnik, 1994). This is particularly acute due to the position of *Bt* as the leading bio-insecticide, and the toxin's high kill rate leading to high selective pressure for resistant insects. Both of these should prove less severe problems with the types of plant-derived proteins described above. Various resistance management strategies have been proposed, of which the use of more than a single resistance factor, and the provision of refugia to ensure survival of susceptible genotypes, appear to be of most value. The ability of pests to break down host-plant resistance is always a grave risk where single factor resistance is involved. The durability of transgenic crops is likely to be much higher if they are deployed with multigene, multimechanistic resistance within them (Boulter, 1993b). One demonstration of *pyramiding* different resistance mechanisms in transgenic plants has been described (Boulter *et al.*, 1990). Much of the argument concerning resistance management is based on mathematical models and laboratory studies, rather than on experience in the field. Determination of the optimum strategies for deploying transgenics in specific cropping systems must be a key area for future research.

TABLE 2. Field performance of transgenic plants expressing resistance genes in the absence of challenge.

Crop	Gene	Expression	Yield	Reference
Tomato	TMV-CP	0.02%	=CON	Nelson *et al.*, 1988
Tobacco	*bar*	0.01%	=CON	De Greef *et al.*, 1989
		0.1%	=CON	
Potato	*bar*	0.01%	=CON	De Greef *et al.*, 1989
		0.1%	=CON	
Potato	PVX-CP	0.005%	=CON	Kaniewski *et al.*, 1990
	+PVY-CP	0.01%	=CON	
Flax	*als*	N/A	=CON	McHughen & Holm, 1991
Oilseed rape	*bar*	N/A	=CON	Crawley *et al.*, 1993

=CON, no significant difference from untransformed controls.

INTEGRATED PEST MANAGEMENT

It is frequently stated that transgenic crops with enhanced resistance will be used within integrated pest management (IPM) programmes, the acronym sometimes appearing as a talisman to ward off any remaining criticism of the technology. Would resistant plants of the type discussed above actually be of benefit to IPM? Many IPM practices, such as the use of short-season varieties and conservation of predators, are aimed at preventing the build-up of pest populations to catastrophic levels, rather than total elimination of the pest. Retardation of development, leading to a slower rate of population build-up, and the relative weakness of surviving pests should mean that even in those situations where transgene expression did not keep the pest population below the threshold for intervention, it should allow a much wider window within which intervention can be successfully employed. This might encourage greater confidence in the IPM approach on the part of farmers.

BIOTECHNOLOGY AND THE DEVELOPING WORLD

Most biotechnology research for agriculture is taking place in the developed world, with a very large part of it occurring in the commercial sector. To date, more than 74% of transgenic plant field trials have been carried out by 60 private companies worldwide (Ahl Goy *et al.*, 1994). This research effort is largely driven by the problems in high-input agricultural systems of the developed world, with an eye on the huge agrochemical markets. However, most of the developments to date are applicable to general agricultural problems. As these problems often pose a greater constraint to agricultural output in less-developed countries than in developed ones, the benefits of any solutions are likely to be of even greater significance to the former. Whereas relatively high degrees of resistance are sought in the engineering of crops for the developed world, consultation with the UK Overseas Development Administration (ODA) suggests that in a number of cases, engineering an increase in resistance of just a few percent would make a huge difference to productivity in the less-developed countries.

The location of much of the research effort in the commercial sector of developed countries has led to various spectres being raised concerning restrictions on the transfer of this technology to less-developed countries. It should be pointed out that several companies have entered into agreements whereby their proprietary technology can be used royalty-free in less-developed countries. Walgate (1990) has suggested a number of reasons why the commercial sector would be willing, in fact, to transfer its proprietary technology to the developing world. However, it is sad that he does not consider the possibility that those involved would seriously like to see this technology employed to assist less-developed countries out of common humanity. It should be remembered that there are very few agricultural systems in the less-developed countries from which companies could recover a significant fraction of the development costs for their proprietary technology.

In addition to inherent difficulties with the cellular and molecular biology involved in the production of engineered crops, there are a number of other factors that could limit the application of this technology in less-developed countries. These include, in particular: (1) The inherently high cost of this technology. Biotechnology is not cheap and the cost of applying it to locally grown varieties would probably have to be borne by the less-developed country or by aid agencies. (2) The necessity to devise and implement adequate biosafety regulatory procedures and legislation. It is essential that full consideration is given to these issues before the production of material for field testing.

APPROPRIATE TECHNOLOGY

Genetically engineered crops are intrinsically *user-friendly*. The goals of biotechnology are essentially the same as those of traditional plant breeding, *i.e.* the provision of improved crop varieties that require the minimum input of non-renewable resources to give a consistent, improved yield. As such they are appropriate to any agricultural system. The use of genetically engineered seeds would require no additional inputs or technical skills from farmers -- indeed no other change in farming practice is necessary. They are applicable to any scale of agriculture and would not favour large-scale farmers. The availability of transgenic seeds should simply afford the farmer a greater choice.

CONCLUSIONS

There is clear potential for genetically engineered crops with enhanced resistance to have a significant impact on agricultural systems in both the developed and developing worlds. The application of such technology offers the advantages of being environmentally friendly, user friendly and consumer friendly. The development of this technology has depended on an expensive and sophisticated research effort, much of which has been carried out in the commercial sector. There is little doubt that the technology is becoming ripe for transfer to less-developed countries where it might have the most beneficial impact on agricultural production.

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