Session 1

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COMMITMENT TO LONG-TERM AGRICULTURAL RESEARCH : A MESSAGE FOR SCIENCE, SPONSORS AND INDUSTRY

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ABSTRACT

Commitment to long-term research is essential to provide answers to many of the questions and problems currently besetting European and world agriculture. Without it, proper assessment of the sustainability of agricultural systems, effects of climate change, determination of pest and disease status, and detection of the environmental effects of farming and industry, are impossible. Commitment is also required for the development of improved agrochemicals and new pest control concepts and strategies. Unfortunately, governments and funding agencies do not fully appreciate the need for such commitment. There are now almost insurmountable constraints to pursuing long-term studies. These arise from the on-going reorganization of public-funded science, poor career structures and mechanisms of reward for scientists, and financial pressures on farming and the agricultural sector. The agricultural and environmental consequences of failing to invest in foresight could be expensive and, perhaps, irretrievable. The industry urgently needs to exert a strong influence on national and international research policies.

INTRODUCTION

Nationally and internationally we are experiencing a period of intense reappraisal and change in the need for and approaches to agricultural research. My credentials for being invited to speak on this topic are presumably two-fold. The first is that, in 1961, Frederick Bawden appointed me as a practising scientist to Rothamsted, a name and institution synonymous worldwide with long-term agricultural experiments, and which last year celebrated its 150th anniversary. The second is that latterly, as one of his successors, I have been involved in the planning and management of public-funded research during a decade of shifting goals and declining resources.

Many of the more important questions, aims and issues currently facing farmers and the supporting agricultural industry (such as acceptable pest control, environmental safeguards, sustainable production, wealth creation, climate change and fickle public attitudes), require commitment and long-term approaches to provide meaningful information and answers. By contrast, many pressures including Government policies, the re-organisation of public-funded science, financial squeezes on farming and agrochemical companies, public disenchantment with agriculture,

uncertain career prospects and remuneration for scientists, all tend to favour expediency and quick "fixes" based on short-term research.

I shall explain why a significant commitment of resources to long-term scientific studies should be made, underlining the dangers for science, the environment and the agricultural industry if they are not. Crop production is an integrated activity, so I have chosen illustrations and examples from the wider agrochemical scene, including fertilizers. Because of my own background and in acknowledgement of the scientist in whose name these lectures were inaugurated, I shall draw generously, though by no means exclusively, on Rothamsted's research sponsored by the former Agricultural & Food Research Council, the Ministry of Agriculture, Fisheries and Food (MAFF) and the agricultural industry.

The nature of commitment

My interpretation of "commitment" in this context is the provision of a stable research capability that allows lessons to be learned from the past and encourages creative planning for the future. Many individual projects do not need to be long-term; some aims can and should be achieved by a progression of short steps, but essential for creative agricultural research is the assurance that resources will be available to permit those consecutive steps to be taken properly. Agricultural science and scientists, particularly in the public sector, would benefit from such reassurance.

The resources required are familiar - a critical mass of quality staff, equipment and infrastructure assembled in a multidisciplinary setting with access to land of known history for field experiments. Furthermore, a successful research organisation needs to have an ethos and policy supportive of long-term work than can override the inevitable mobility of individuals. Organisations with these desirable features lie, by and large, within a few universities, in the laboratories and field stations of major multinational agrochemical companies, in laboratories of MAFF and particularly in the large Institutes of the Research Councils.

What is "long-term"? Scientifically, it depends on the nature of the investigation, whether it is laboratory or field based, conducted in controlled or seasonal conditions, and on the rate of a multitude of interactions between host crop, invading organism, treatments, and the physical and biological environment. Economically, it depends on the funding and resourcing policies of sponsors and the interval they are prepared to wait for a useable outcome. Consider two sets of data and perhaps decide for yourselves!

A century of dry herbage yields from one plot of the Rothamsted Park Grass Continuous Hay Experiment which receives inorganic fertilisers annually and is unlimed, shows no substantial changes from 1892 to 1992 (Figure 1). Yet if the experiment had lasted the fifteen years from 1944 to 1958, a long period for present sponsorship, a clear upward trend would have been recorded. Likewise, a typical 3-year grant from 1974 to 1976 or from 1988 to 1990 to study the early abundance of peach potato aphid *(Myzus persicae)* in relation to previous winter temperatures would have been misleading; a 25-year study was necessary to establish reliable information

(Figure 2) (Woiwod & Harrington, 1994). Suffice to say, "long-term" has no single, simple definition. In terms of agricultural sustainability it must be measured in generations; in terms of monitoring environmental effects and change it relates to decades or even centuries. For practical experimental studies I suggest 10 - 25 years; anything beyond this is a bonus which only a few scientists in privileged situations are likely to enjoy. Clearly, funding to sustain studies over such periods can derive only from Governments and possibly Trusts and benevolent Foundations.

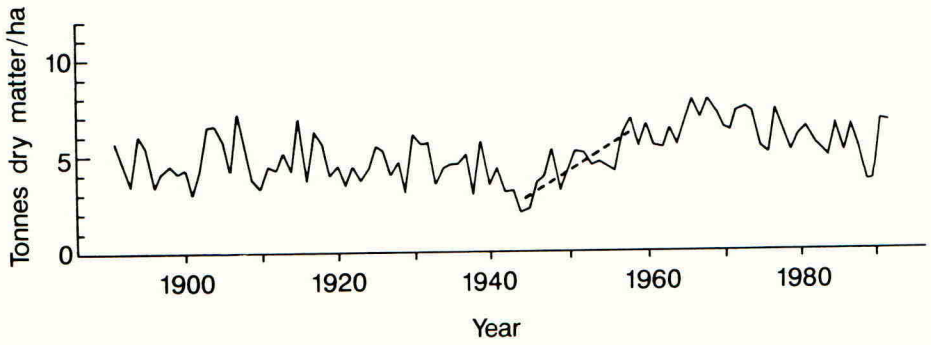

Figure 1. Yield of dry herbage on plot 16D of the Rothamsted Continuous Hay Experiment 1892 - 1992: unlimed, inorganic fertilizers annually (48 kg N as nitrate, 3kg P, 225 kg K and 10 kg Mg per ha). - - - - - best fit 1944 - 1958. (Redrawn after Jenkinson et al, 1994.)

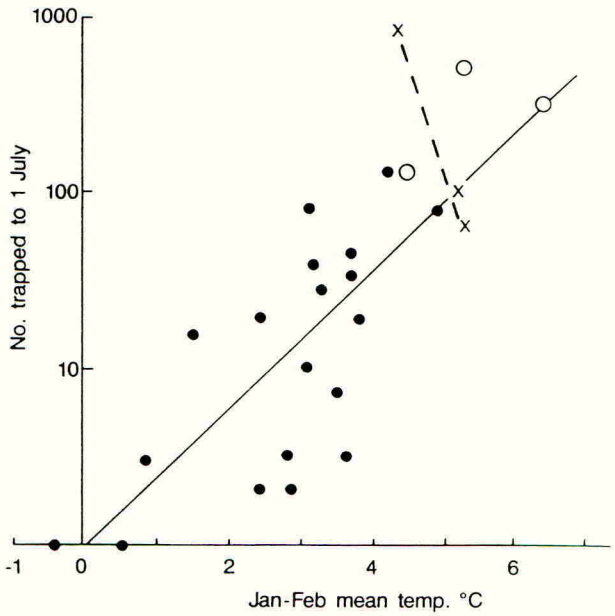

Figure 2. No. of *Myzus persicae* trapped up to 1 July vs. January - February mean screen temperature, 1968 - 1993. x -- x 1974/75/76; ○ 1988,89/90; ● other years. (Unpublished data from R. Harrington, Rothamsted.)

THE SCIENTIFIC IMPERATIVE

I have selected six broad areas of agricultural science covering a range of interests relevant to the industry to illustrate the case for commitment.

Assessment of sustainability of agricultural systems

I hesitate to introduce any element of the sustainability debate because it embraces almost all concepts benign, and the term "Sustainable Agriculture" is at risk of becoming a cliché. Nevertheless, the question as to how long any farming system can maintain land fertility and flexibility, keep environmental effects within acceptable guidelines and replenish essential resources, is pertinent, especially where improved management systems are evolving in the developing world, including eastern Europe (Greenland, 1994). It is often claimed, for example, that yields cannot be sustained in monocultures based on inorganic fertilizers. One hundred and fifty years of study on Rothamsted's Broadbalk refute this. A comparison of yields of winter wheat from 1852 to 1992 on two plots, one receiving balanced inorganic fertilizer every year, the other unmanured, and each with herbicides, fungicides and insecticides as necessary since the late 1960s, reveals that yields on both are now higher than 100 years ago. They are agronomically sustainable, although yields on the unfertilised plot remain at less than one third those on the fertilized plot so would not be economically viable (Jenkinson, 1993). No-one could have predicted after the first 10, 20 or 50 years of study that the same manurial inputs plus new cultivars and crop protection would have almost tripled yields. Not all systems are sustainable for as long, but the point is that it takes time to appreciate this. In another experiment at Rothamsted, yields of potatoes grown in consecutive years from 1876 did not decline for 15 years; thereafter, neither organic nor inorganic fertilizers could sustain yields. With hindsight, this was probably due to potato cyst nematode, which was not recognised in the 1890s, incidentally illustrating the need for a multidisciplinary approach to crop experimentation (Johnston & Powlson, 1994).

Over the past 30 years, concern has been expressed about the effects of pesticide applications on soil fertility. A field trial was started in 1974 at Rothamsted, to assess whether repeated applications of pesticides have long-term deleterious effects. Spring barley has been grown continuously on plots receiving the same pesticides each year at slightly greater than normal rates, with all combinations of up to five pesticides applied to 32 plots. Commercial formulations of aldicarb, benomyl, and chlorfenvinphos have been incorporated into the seedbed since 1974 and, since 1982, glyphosate and triadimefon applied to the soil in autumn. The plots otherwise receive standard cultural practices and fertilizers.

Yield of barley is used to detect any long-term effects on soil fertility. In some years, there were no significant differences between plot yields. In other years, plots receiving certain treatments, particularly aldicarb, yielded slightly better than others, due to aphid control, but after 20 years there is no indication that plots receiving repeated applications of several pesticides yield less than those receiving fewer or no pesticides (Bromilow & Catt, 1992). A further 5-year study without treatments has

begun to examine residual effects on fertility and soil microflora, free from the immediate effects of pesticide application.

There is no substitute for foresight leading to long-term studies to provide positive or negative information on such aspects of sustainability, for either farmers, the agrochemical industry, or as an objective assessment to inform Government and the public of the true impact of agrochemicals on modern farming systems and their productivity. Long-term fertilizer studies elsewhere in Europe, e.g. at Bad Lauchstadt in eastern Germany (91 years), Skierniewice in Poland (60 years) and Prag-Ruzyne in the Czech Republic (38 years) indicate a widespread appreciation of the need.

Assessing effects of climate change

The implications for crop protection of changing climate is one of the more obvious topics for long-term research, and one that can reasonably be studied in anticipation rather than retrospectively. By the time its effects are glaringly obvious it may be too late to investigate some of the ensuing crop problems. Within Europe this is recognised as a research priority with currently some 140 EC-funded projects on global change, though only a minority of these are on agricultural aspects.

Temperature affects the development rates of aphids and their natural enemies differently, thereby affecting the impact of natural control and, hence, the need for pesticides. The generation time of the pea aphid *(Acyrthosiphum pisum)* is 22 days at 10°C but only 5 days at 26°C, whereas the aphid pathogenic fungus *Erynia neoaphidis* kills pea aphids in 10 days at 10°C and 5 days at 25°C. Thus, at 10°C the fungus can pass through two generations while the aphids complete only one, but at 25°C their respective development rates are about the same. The warmer the climate, the less effectively would the fungus control this pest. Overlying such detailed climate-related interactions are the apparently innate periodic cycling of many pest populations, from locusts and cicadas, to midges, which are undetectable without long-term records.

Black dot disease of potatoes, caused by the seed- and soil-borne *Colletotrichum coccodes*, occurs world-wide. In the UK, tuber infestation and blemish is increased by irrigation; in drier parts of the United States of America, the disease colonises the vascular systems of plants weakened by drought, causing wilting and premature death of tubers. If European summers become wetter, levels of tuber infection would probably increase; conversely, if they become drier, black dot may affect plant growth and decrease tuber yields instead.

The heat requirements of cyst nematode pests on arable crops are approximately 500 day degrees above a base temperature of 5°C. Crops with long growth seasons (oilseed rape, sugar beet) already provide enough time for two generations, but a 2°C warming would provide all cyst nematodes over most of Europe with the conditions for two generations. *Globodera pallida* on potato would be particularly problematical with the absence, so far, of good resistant cultivars.

The effects of changing weather on the growth of crop plants are paralleled by effects on weeds. Persistent changes in winter temperature would affect the relative importance of some common species, for example, the winter hardiness of cleavers is greater than that of field pansy. Germination of weed seeds is controlled by weather during their development. In wild oats, high temperature and water stress produce seeds with low dormancy, whereas cool moist conditions produce highly dormant seeds (Leigh, 1990). Much work is needed to evaluate the effect of changing climate on these processes and phenologies, requiring investment in controlled facilities for carbon dioxide, moisture and temperature. To set field studies in a time scale, on the experimental plot on Rothamsted Park Grass Experiment (referred to earlier), yields have not changed measurably over 100 years despite a 21% increase in the CO_2 content of the atmosphere over the period and substantial changes in the quantities of chemically combined nitrogen brought down in rain or by dry deposition (Jenkinson, et al., 1994).

Determination of Pest status and forecasting

Knowledge of pest abundance in relation to environmental and cropping factors is essential to formulate models for forecasting pest and disease occurrence to ensure timely pesticide application or to prevent unnecessary spraying. This accords with European Common Agricultural Policy (CAP) which, for economic and environmental reasons, is encouraging lower pesticide inputs . One of the best and largest insect datasets in the world, compiled for this and other purposes, is that of the Rothamsted Insect Survey (RIS); this comprises over 20 million individual records of more than one thousand aphid and moth species. Continuous records of airborne aphids, currently monitored by fifteen "12-metre suction" traps in the UK, have been kept daily since 1968, and the network now extends to most EU countries, including 14 traps in France, 4 in the Netherlands and 8 in Sweden. In the UK, successful forecasting systems have been developed for aphid presence in hops, cereals, sugar beet, beans and potatoes. Answers can be provided by this system to three important questions, namely: will pests reach damage thresholds?; when will thresholds be reached or important migration occur?; are there qualitative attributes such as pesticide resistance or virus transmission potential that will make aphids difficult to control or affect their pest status? (Woiwod & Harrington 1994).

One of the most comprehensive forecasts for regional abundance in the UK has been developed for the black bean aphid (*Aphis fabae*), a species for which there are extensive historical data on crop and overwintering abundance from field sampling, and on aerial abundance from the RIS database. Crop infestation on spring-sown field beans (*Vicia faba*),can be forecast most successfully from the size and timing of the spring migration measured by RIS suction traps.

When aphids act as plant virus vectors it is important to be able to predict the timing of migration into crops as well as subsequent population size. The beginning of migration of *Myzus persicae* is closely related to incidence of sugar beet yellows virus. The forecast date based on temperatures in January and February is used routinely to advise beet growers of the likely benefits of applying insecticidal granules

at planting. On the day the first aphid is caught in a trap an updated forecast of virus incidence is issued and RIS trap catches form an essential part of the season-long spray warning scheme issued by Broom's Barn Experimental Station to the sugar beet industry. The current UK scheme is thus based on 28 years of trap records, 40 years of crop sampling and 10 years detailed study of plant virus interactions. The same type of traps are run in other beet-growing countries including the Netherlands, Denmark and Spain for different purposes, but the beet industry in these countries seems not to communicate with the trap operators to take advantage of the opportunity available (Dewar, 1994).

It is now possible to measure some of the other biological attributes of individual aphids from trap samples, such as the degree of resistance to insecticides or the identification of the viruses each is carrying, by collecting samples in a storage solution that preserves the enzymes for later immunoassay (Tatchell et al., 1988). Suction trapping also forms the basis of the infectivity-index system devised by Rothamsted and ADAS for assessing the risk to autumn-sown cereal crops from barley yellow dwarf virus (Plumb et al., 1986).

The will, commitment and resources to set up, staff and maintain these forecasting systems for so long has required immense determination. All have required long runs of data because only a single point for any relationship is obtained each year (e.g. between winter temperature and spring pest abundance), and also because wide ranges of causal variables are needed to establish the form of the relationships.

Detection of environmental effects

Many interactions between agrochemicals and the wider environment can be detected only by monitoring, an activity sometimes unwillingly accepted by laboratory scientists as scientifically valid. However, science depends on the collection of data, and monitoring is a legitimate form of this providing it has a well-defined purpose, avoids a totally open-ended resource commitment, and is backed up by experimental confirmation of the causes of observed trends. As it is rarely possible to monitor retrospectively and predict an outcome, commitment is inherently important.

As an example, the combined commitment of the British Trust for Ornithology, the Nature Conservancy Council, museums and a host of field observers, all coordinated by Derek Ratcliffe, unravelled the probable explanation of the decline and recovery of peregrine falcon populations during the middle of this century.

Over the 25 year period from 1930 to 1955 the occupation of breeding territories was fairly constant, but from 1955 - 1962 it declined to only 56% of the 1930 - 1939 average (Figure 3). By detailed scientific detection involving monitoring of bird numbers, distribution, observations on breeding success, adult behaviour, and analysis of organs and egg-shell contents, a strong case was established against insecticides, particularly the cyclodienes, as being the cause. Subsequent restrictions imposed for several reasons on the use of organochlorines, and the dieldrin group in particular, in

the UK (1962-76) and eventually the EC (1979) allowed bird populations to recover and indeed exceed their 1930's level. Similar fluctations have occurred elsewhere in Europe and North America where falcons have had opportunity for close contact with persistent organochlorines. I do not wish to argue the relative harmfulness of particular compounds in this group of insecticides which, for a time, brought great benefits to agriculture and public health, but merely to stress that nearly 50 years was required to count pre-use populations and elucidate post-use consequences which were not foreseeable (Ratcliffe, 1993).

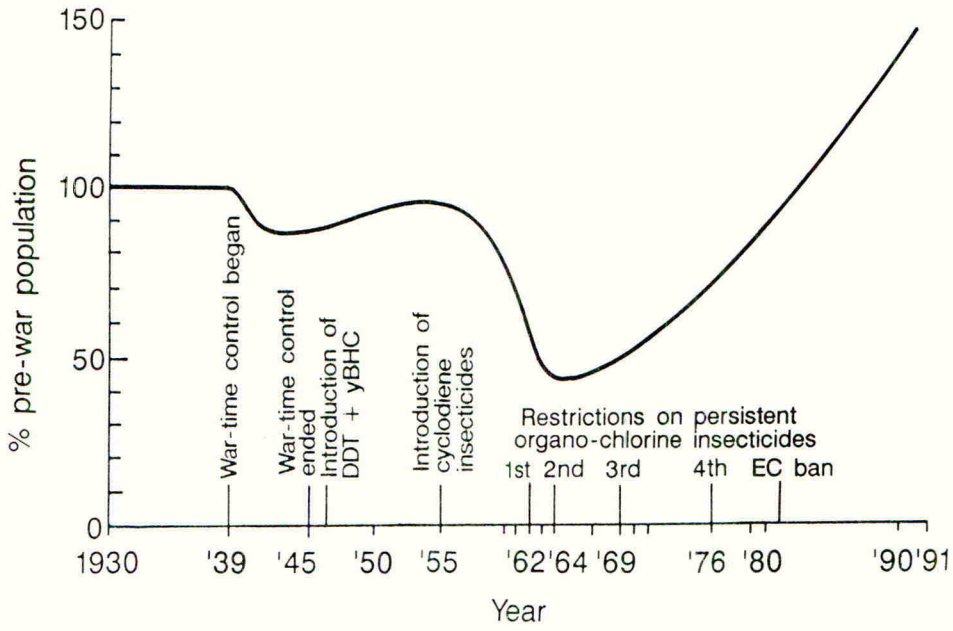

Figure 3. Decline and recovery of peregrine falcon populations in Britain. (Redrawn after Ratcliffe, 1993.)

Comparable persistence and commitment, funded almost entirely by the private sector, was required to explain and partially rectify the decline of partridges in arable areas which began in the 1940s and 1950s. The most important impact was the

indirect effect of herbicides on insects in the 1950s and 1960s which roughly halved the food supply for partridge chicks. From 1965 to 1975, the decline in undersowing further reduced chick food, especially sawfly larvae, as well as predators and parasites of cereal aphids which succumbed to post-harvest cultivations. Thereafter, foliar fungicides destroyed the food of beneficial fungivorous insects with a consequent increased requirement for aphicides which killed even more bird food. Thus, over 30 years of effort was required to elucidate the causes of the decline in the population of partridges and other arable birds, before MAFF recommended a ban on the summer use of insecticides around cereal field margins. Similar population changes occurred in the U.S. where insecticides were used heavily against Russian wheat aphid (*Diuraphis noxia*) and in Eastern Europe against cereal ground beetle (*Zabrus tenebrioides*) (Potts, 1992).

Soil contamination with heavy metals and organic pollutants is another topic requiring long term monitoring and experimentation to identify, and possibly rectify, effects often brought about in innocent ignorance of apparently useful agricultural practices. The widespread use of sewage sludge as a fertiliser is one such example. The speciation of metals in waste materials added to soil may be different from that in which they are taken up by plant roots; long periods may be necessary for added metals to equilibrate with other soil constituents. Furthermore, concentrations of heavy metals below those affecting crop growth have adverse effects on the soil microbial population. Observations made at Woburn on clover 20 years after the last application of contaminated sewage sludge, which had been applied for 20 years to increase soil organic matter, revealed that strains of *Rhizobium* able to form effective nodules on clover roots were still absent (McGrath et al., 1988). With similar results obtained recently from the Federal Research Station at Braunschweig, Germany, after ten years of sewage sludge applications, limits can now be set for the permitted accumulation of metals in soil. If the principle of "polluter pays" is strictly enforced, it is clearly in the best interests of industry to ensure that effective long-term monitoring is established.

Equally damaging is the slow build up in soil of total pollutants from atmospheric deposition such as lead, (15% increase over 140 years), cadmium (mainly since the 1940s) and various organics such as the dioxins which have been increasing since the beginning of this century (Powlson & Johnston, 1994). Crop samples show that lead in herbage has declined over the last 30 years, reflecting its decreasing use in petrol. This, and much more information, has been obtained from the Rothamsted Archive, which uniquely contains 200,000 soil and crop samples taken over 140 years and available for continuing analysis as techniques are refined and improved. It thus offers one of the relatively few opportunities for retrospective monitoring. Lawes & Gilbert could not have predicted the immense value of these preserved samples, though they did have the foresight to appreciate that future generations might benefit from the hindsight they endowed. Incidentally, funds to house the Archive and open it up to further international study are urgently needed; public-funding is an unlikely solution.

Current "set-aside" practices carry a different range of environmental implications, viz their effect on soil carbon, nitrogen and acidity, and the dynamic balance of pests, diseases, weeds and beneficial organisms. Arguably the ultimate demonstration of set-aside is Broadbalk Wilderness at Rothamsted, which clearly shows the consequences on soil and flora of leaving part of a wheat field uncultivated for 114 years. Now woodland with mature trees, it continues to evolve to climax vegetation as the understory gradually dies out, with still increasing soil organic matter and nitrogen and decreasing pH. Is this what the Government and EU intend to achieve? Are they even aware of the long-term consequences of set-aside? Nowadays, who would make a committment to even 14 years of study, let alone 114 years?

The Development of New Compounds

One of the most successful and lucrative classes of insecticide so far developed, the synthetic pyrethroids, illustrate emphatically the rewards of long-term commitment by basic and applied scientists working on a multi-disciplinary group. The story, familiar to many, is worth outlining again, not least because it shows how the investment of a few million pounds of largely public funds over 50 years led to a class of insecticides extremely potent against insects, but relatively safe to man and the environment, and now with annual sales approaching £1000 million.

From 1924, Frederick Tattersfield, an organic chemist, and colleagues worked at Rothamsted to see whether pyrethrum could be grown in the U.K. and how the flowers compared with those from Dalmatia and Japan. Seed from these early studies contributed to the establishment of the natural pyrethrum industry in Kenya. In 1936 Charles Potter, an entomologist, was appointed and soon introduced techniques for raising insects year round, to speed up investigations. In 1947, he judged that fundamental studies on the constituents of pyrethrum might eventually lead, if required, to alternatives to organochlorine, organophosphate and carbamate insecticides. In 1948, Michael Elliott joined Rothamsted and gradually built up an impressive team of organic chemists and entomologists.

The rest is history and fully documented (Elliott, 1993). Over the next 25 years, with support from the National Research Development Corporation since 1962 and with the benefit of interactions with the Medical Research Council and the University of California at Berkeley a succession of compounds, resmethrin, bioresmethrin, permethrin, cypermethrin and deltamethrin was developed. Many industrial firms have contributed to the commercial production and development of these compounds and have ultimately greatly benefitted from this research. What is less widely appreciated is that "independent" reviews of the work in the 1950s and 1960s twice suggested that it was unpromising; it was to the credit, foresight and tenacity of Potter and Elliott that this advice was rejected. Regrettably, such external strictures delivered nowadays in the pervasive climate of "market forces" and rapid "wealth creation" would be much more difficult to resist. Indeed, depending on the source of public funding, which is often no more than an historical quirk, the option to ignore so-called "impartial" reviews may not exist.

Another important point to draw from the pyrethroid story of general relevance to insecticide, fungicide and herbicide development, is the necessity to maintain on-going basic biochemical and chemical studies to identify novel targets and thence provide the compounds, preferably biodegradable, to reach and disrupt them.

Development of new pest control concepts and strategies

Since the end of the last war the agrochemical industry has devised, by elegant synthetic chemistry, an immense range of successful pesticides. Essentially, the approach has been to find compounds that interfere with the pest's metabolic biochemistry when applied directly to the harmful organism or its host crop. While acknowledging superb advances in formulation, dosages, application techniques, and limitation of side effects, the basic tenet of this 50-year evolution has been to bring the pesticidal agent into contact with the harmful organism, and to "zap" it. Furthermore, if a potential control agent was not relevant to several major crops and pests, it was unprofitable to develop. As public perceptions, however irrationally formed, force new concepts and approaches on the industry, we realise what a high proportion of private- and public-funded effort has gone into this conceptionally simplistic strategy. How much more effort and time is now needed to devise, develop and integrate the new complex chemical, biological, behavioural and managerial approaches, often for single crops and pests, that current pressures from European risk-assessment requirements demand? A few examples illustrate the point.

New strategies for control of fungal diseases will depend on judicious exploitation of the natural or genetically manipulated defence mechanisms of plants and efficient use of fungicides. Excessive use of fungicides fosters resistance in the pathogen and may impose stress on the host. Decision-making strategies, therefore, will need accurate disease forecasting schemes which in turn require a deep understanding of the interactions between pathogen, host and environment. Such schemes should be able to forecast the development of an epidemic and to assess the potential damage to the crop. At present, much of the information required for disease forecasting models for many crop/pathogen systems is lacking, especially for relatively new crops such as oilseed rape, linseed and lupins.

Effective biological control frequently relies on obtaining the correct pest/natural-enemy ratio at an early stage in the development of an outbreak. Also, factors which slow the development of pest or disease outbreaks, such as host plant susceptibility, usually enhance the impact of natural agents. The survival and spread of organisms applied as biological control agents is poorly understood. Quantitative information on these relationships, which are crucial to the successful manipulation of natural enemies in the field, is generally lacking, even for those insects which have been most intensively studied. Research on the biological control of fungi, nematodes and weeds is still largely empirical.

Some of the most exciting prospects for new crop protection strategies lie in the study of chemical messengers that regulate insect or nematode plant interactions by non-toxic mechanisms. Aphid sex pheromones and antifeedants active against beetles (e.g. ajugarin I from *Ajuga remota* or polygodial from the tree *Warburgia ugandensis*) are promising candidates. Although semio-chemicals are not yet commercially competitive with conventional pesticides they are compatable with beneficial organisms and could be developed, given time and effort, to improve their effectiveness. In simulated field studies, antifeedants are proving to be particularly effective in protecting growing tips of plants, thus forcing pests to aggregate on the lower leaves where they can be killed by slow-acting but highly selective insecticides. However, this is only a prelude to using fungal pathogens of insects, now under development, which are particularly effective in the more humid lower plant canopy away from the damaging effects of sunlight.

There is much enthusiasm for genetically engineered resistance in crops to simplify pest control, but there are many steps requiring careful long-term assessment between the production of a transgenic insecticidal crop, such as B.t.-containing cotton, and its widespread usefulness. The insecticidal properties in such a crop would probably rapidly lose effectiveness unless the 'treated' crop was planted next to fields of 'untreated' crops where the target pest remained unmolested. This is because within a large population of insects a few individuals will not be susceptible to the toxin, and so will have a survival advantage. The onset of resistance could be particularly rapid for crops producing their own toxic agent because it will be exerting an effect at population levels well below those that would merit control by conventional pesticides, thereby encouraging even faster development of resistance. Financial incentives, or even legislation, to persuade farmers to adopt crop refugia may be necessary - but they would not be cheap, or logistically and politically easy to enforce (Mallet & Porter, 1992).

Clearly, many of these apparently elegant laboratory solutions to pest and disease control will require long-term work to effect successful transfer to every-day farm practice. There are unlikely to be any "quick-fix", "all-time" solutions. Fungi and insects are simply too genetically plastic.

CONSTRAINTS ON COMMITMENT

Having convinced you, I trust, of the need for long-term approaches to crop production and protection, I now consider the increasing constraints on pursuing them, in the hope that collective efforts can reverse the trend to short-termism.

Reorganisation of public-funded science

Over the last 18 months, public funding of science in the UK has been under scrutiny perhaps as never before, and I have no doubt, that in so far as such fashionable upheavals are "contagious", many other countries are or will be involved in similar exercises. The basic question being asked is whether the state and the

country's manufacturing sector are getting the best value for money from public investment in science as organized over the last two decades and, if not, what structures should replace the established norm. UK Government philosophy has decreed that research efficiency could be improved and with benefit, refocussed, to create national wealth. Responsibility for funding for most biological research, excluding medicine but including agriculture, has been delegated from April 1994 to a new Research Council, the Biotechnology and Biological Sciences Research Council (BBSRC) . In so far as biotechnology is the application of biological systems and processes to the production and/or manufacture of new products, this is logical. The agriculture and food industries have long been prime examples of traditional biotechnology. Plants provide food, fibres, pharmaceuticals and construction materials. Insects and birds have made an ongoing contribution to pest control. Biological processes, through the action of micro-organisms, have been exploited in bread, cheese, beer and wine making, silage fermentation and the enhancement of plant nutrition. In this sense, biotechnology has been a creator of wealth and a sustainer of the community. The BBSRC intends to continue to encourage traditional biotechnologies, though regrettably prefers not to identify the principle component, agriculture, by name. In addition, the Research Council will particularly foster new biotechnologies derived from the biological revolution of the past two decades. DNA or protein molecules can, for example, be used as probes to detect beneficial soil micro-organisms or harmful vectors of disease in livestock, crops and food. New monoclonal antibodies can be used for diagnostics in farm animals and crop plants. There are exciting opportunities for the use of cells and whole organisms to create new products and to provide new strategies for introducing resistance to bacterial and viral diseases into plants, for combatting insect pests, and for increasing salt, drought and heat tolerance into crops and improving their quality.

All this is laudable and, given a balanced approach, desirable. But, with a few exceptions, the thrust and the consequent downside of industry-based wealth creation is short-term. Often pressure from boardroom and financial institutions mitigates against long-term planning and development in industry; how much more so for the type of research I have discussed?

A further unfortunate and spurious element that has crept into this situation is the perceived relative quality of university and institute research. This is a sterile debate; each is complementary to the other, but, by and large, the managed atmosphere of staff and resources in an institute better provides the continuity required to investigate long-term problems. How often has one seen valuable equipment bought for a talented researcher on a short-term grant at a university, standing idle after the incumbent has moved on to his next 3-year stint?

A further recent complication in public-funding of agricultural research in the UK has been the transfer, since 1991, of MAFF R & D funds to fund-holding policy groups which operate in consultation with the Chief Scientist's Group, rather than the latter having final authority for commissioning research. Given that MAFF has primarily economic and social objectives and does not regard itself as responsible for the nation's scientific base (even though it supports some £125M worth of research

annually), policy changes generated by Government and ultimately public perceptions and pressure, cause rapid changes of support for research projects operating on customer/contractor principles. The withdrawal or transfer of funds to accommodate frequent short-term changes in policy, assumes a broad, stable and well founded research base to which new commissions can be assigned. But, if there is no reasonable guarantee of support for the agriculturally orientated research base, it will be rapidly eroded by receiving predominantly short-term projects, thereby squandering knowledge and expertise irreplaceable within a generation. There is also a basic incompatability between the ever-increasing requirement for competitive tendering for funds and the desire to eliminate overlap in research capability between public-funded Institutes and Departments.

Career Structures for Scientists

The rapid growth of short-term appointments in UK universities and institutes has also seriously eroded the planning of long-term studies and commmitment to them. Concern relates to a possible reduction in the quality of research output because of the constant flux in staffing, the poor value for money of 3-year appointees, who often need a period of training before they become effective and then spend much time seeking their next appointment, and the danger for individual scientists that a succession of short-term contracts may diminish their employment prospects by making them overspecialized.

In UK AFRC Institutes from 1981 to 1993 total science group staff halved while the short-term science staff rose from nil to one-third of science employees. Furthermore, the introduction of large "co-ordinated programmes" to increase competition for research council funds between institutes and universities has created pools of high-quality young scientists who are hired and released on to the labour market at about the same time. There has been no parallel increase in permanent posts elsewhere in the public service to which young scientists can move after several short-term contract, post-doctoral posts. This has been especially damaging to crop protection in which much of the skill of advisory workers is built up only by many years of experience on the job.

The proliferation of short period contracts has also resulted in much time being spent unproductively by project leaders in preparing proposals and application for funds in order to secure the next contract, a situation widespread throughout the EU in the quest for community support. (It may not be common knowledge, but the grant proposals received by the EC for its Human Capital Mobility programme in 1992 weighed 6 tonnes!) Despite the EU's emphatic interest in the future of agriculture, its approach to research funding is no more geared to long-term studies than most national or private-sector funding schemes. Combined with the switching of policy-driven goals by Government Departments, in the UK particularly by MAFF, and the ever-changing CAP, it is difficult for young scientists to develop their own research interests in a consistent way. Disillusionment and loss of intellectual independence

set in, and for students, the perceived instability and insecurity dissuade them from choosing a research career in agricultural sciences. We must put mechanisms in place to allow our brightest scientists to flourish at a young age. On average, 34-year-olds produce the most innovative science - just about the age at which our present system ceases to sustain so many!

If the capacity of the science base to offer stable careers to the best researchers is to be improved, there must be an increase in the availability of long-term, independent studies, many of which should be of the types I have outlined. This will involve a reduction of resources devoted to other purposes, including three-year projects. One approach that maintains some flexibility would be to offer many more three- or five-year fellowships at mid-career level with a mutual decision by sponsor and researcher at five, six or eight years (say age 33/34) to award 'tenure' or not. Alternatively, an approach favoured in many larger American universities would be to create a system of "rolling tenure" whereby research staff have up to three years or more guaranteed employment beyond the time when their short-term contract funds cease.

Whatever system is devised, it is clear that no funding organisation can achieve both flexibility in funding to meet new priorities and stable career structures with the same funds; a balance must be struck.

Performance related pay

One of the strongest demotivations for research scientists is performance-related pay, which for all its superficial attractions is largely a "con". Having been obliged to operate such a scheme for 5 years, I believe that annual pay based on performance indicators encourage short-term fixes at the expense of long-term organisational development. This is because the system inevitably focuses minds on the short-term aspects of any job; it is divisive and damages the trust essential to the free flow of information without which efficient team effort cannot occur. One could argue that the apparent need for performance-based pay reflects inadequate performance not by individuals but by their management, which in the public sector means Government Departments and Research Councils.

My perception of the great majority of research scientists is that they like to work well and in a "high-performing" manner. They are not happy when underperforming. Where an organisation is well managed, quality and performance will thrive and performance-related pay will not be needed.

In a multidisciplinary research environment team effort, mutual stimulation and the sharing of ideas are paramount to progress. The atmosphere of mutual trust necessary for this disappears if individuals are competing for a limited, and usually scanty, monetary incentive. Furthermore, in practice it leads to intellectual dishonesty; if individuals are to be judged annually on performance they are reluctant to discuss any possible areas that need improvement! It is simply not a mechanism suitable for encouraging long-term commitment, and it is significant that

many multinational companies are moving away from the approach just as the public sector embraces it!

Financial pressures on the farming and agrochemical industries

Disappointingly, even farmers themselves, a group from which one would have hoped for collective encouragement for a long-term research view, are not currently enthusiastic. This attitude can be traced to the requirement of financiers generally to obtain a rapid return on investments, and particularly on uncertainty about the industry's future throughout Europe. At present, in the UK, area compensation payments on specific arable crops range from 15 to 70% of income from them. Within the next 1 - 2 years some reform of the enormously costly CAP seems inevitable, probably involving a decrease or even cessation of income support payments. As a result, perhaps half of the 23000 UK arable farmers growing more than 40 ha of cereals may leave the industry over the next decade. With this bleak future, little wonder that most are lukewarm about long-term research; many are more interested in contracting out land or attracting contract farming rather than establishing and extending their personal stewardship. Even under these arrangements longer-term research on maintenance of soil fertility is essential if farmers are to enter contracts which sustain the fertility of their soils, one of their most important capital assets.

The knock-on effects of this uncertainty have been felt by the agrochemical industry, especially the fertilizer sector, which, in the UK and mainland Europe has been damaged by cheap imports (£60 - £70/tonne for N) over several years from the Baltic States and CIS compared with £90 - £100/tonne from Western European sources. Understandably farmers bought imports at these prices, but as Eastern European producers gear their energy costs to economic levels, our farmers may be left with little price advantage. Furthermore, the financial problems for the fertilizer industry have resulted in less research and advisory support for farmers.

Surprisingly, the British Agrochemicals Association has not detected the expected fall in pesticide use in the UK as 15% of arable land has been set-aside, but in France there has been a 20% fall in sales over two years and a 15% reduction in Germany in 1993. This will inevitably lead to a loss of private-sector jobs, research capacity, and a will to commission research in the public sector. Already Shell have withdrawn from agrochemical research and the pesticide industry, and Hoechst and Schering have merged their agrochemical operations under pressure from the high cost of individual research programmes, with an overall loss to research funding. Such reduced commitment must have implications for farming and mean, inevitably, that the environmentally benign pesticides that public pressures more and more require farmers to use, are less likely to be forthcoming. The quest for lower prices by an industry uncertain of its future, with consequent mergers and rationalisations, all mitigate against long-termism.

Being neither an economist, a financier nor industrialist, I need to ask a question: why can the major Japanese agrochemical companies pursue patient basic research

with goals some 20 years ahead, and be satisfied with a 2 to 3% return on invested capital, whilst their European and American counterparts are pulling back on research, even when in recent years they received an average of 8% return on investment? I can only conclude that long-term commitment is being squeezed out by boardroom and shareholder demands for quick returns, or by comparisons with the pharmaceutical industry where returns in a similar risk business have been over 20% in the same period.

CONCLUSIONS

You have heard a case for commitment to long-term research in agricultural science needed to safeguard and maintain productive farming and food supplies for the burgeoning world population. You have heard of the increasing constraints on either beginning or sustaining this type of work. The message is clear. The agricultural and environmental consequences of failing to invest in foresight could be expensive and perhaps irretrievable.

However, I am an optimist. Because of the changing requirements and organisation of science in the UK and elsewhere, the broad agricultural community is in a much stronger position than in the recent past to influence Government and Research Council policies. For the first time in the UK the thrust of public-funded science is acknowledged to be directed towards wealth creation through take up by industry. You have been given a voice. Indeed, industry has been widely consulted and involved in setting up the new structures and their *modus operandi*. Now is the time and opportunity for serious appraisal and definition by industry and Government of future research strategies. If you accept a significant role for long-term research speak up collectively to ensure its establishment and survival.

ACKNOWLEDGEMENTS

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Session 2

New Compounds, Formulations and Uses - Insecticides

Chairman	Dr R O Clements
Session Organiser	Mr C Furk
Papers	2-1 to 2-8

DIOFENOLAN - A NEW INSECT GROWTH REGULATOR FOR THE CONTROL OF SCALE INSECTS AND IMPORTANT LEPIDOPTEROUS PESTS IN DECIDUOUS FRUIT AND CITRUS

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ABSTRACT

Diofenolan is a new insect growth regulator for the control of scale insects and lepidopterous species in pome and stone fruit, citrus, grapes and olives. It disrupts the insect-specific transformation from the crawler to the sessile scale insect or, in lepidopterans, from the egg to the larva or from the larva to the pupa. The product shows excellent activity at low rates against all important scale insects, e.g. *Aonidiella aurantii* and *Quadraspidiotus perniciosus* (Diaspididae), *Saissetia oleae* (Coccidae), *Planococcus citri* (Pseudococcidae), *Icerya purchasi* (Margarodidae), as well as lepidopterous pests, e.g. *Cydia pomonella*, *Cydia molesta*. The favourable mammalian toxicity coupled with its good selectivity in respect to beneficials make diofenolan an excellent IPM tool. Field experiments are discussed.

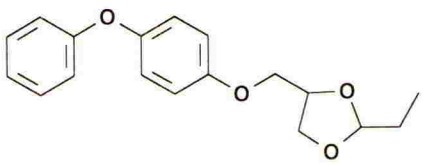
INTRODUCTION

The most important group of pests in fruit (pome fruit, stone fruit and citrus) are mites, lepidopterans and scales. Conventional, broad spectrum insecticides are currently the most important tools used for the control of these pests. Amongst the pests, scales have an abundance of insect predators which have a better chance in perennial fruit trees to play their role as natural antagonists. Broad spectrum insecticides often disrupt this relationship and can lead to the outbreak of secondary pests. In addition, a number of established products encounter resistance. Therefore, more specific products with novel modes of action are gaining in importance, especially within concepts of IPM and Insect Resistance Management (IRM). Specific products like diofenolan with a different type of action preserve most of the natural antagonists and enable the grower to slow down or circumvent the development of resistance.

CHEMICAL AND PHYSICAL PROPERTIES

Code number : CGA 59205

Structural formula :



Common name	: Diofenolan
Chemical name	: *cis, trans-(±)*-2-Ethyl-4-(4-phenoxyphenoxymethyl-) 1,3-dioxolane (mixture of the four configurational isomers)
Molecular formula	: $C_{18}H_{20}O_4$
Molecular weight	: 300.3
Physical state at 20°C	: Clear, pale yellow viscous liquid
Vapour pressure at 25°C	: 1.1×10^{-4} Pa
Partition coefficient n-octanol / water (Log P)	: 4.4 (*cis* isomers), 4.3 (*trans* isomers)
Water solubility	: 4.9 mg/l
Formulation	: 500 g/l Emulsifiable concentrate (EC 500)

TOXICOLOGY AND ENVIRONMENTAL PROPERTIES

Diofenolan is of low acute toxicity in the rat (WHO class III), (Table 1). It is not irritant to skin and eyes and is not a dermal sensitizer. GA 59205 is not mutagenic or teratogenic.

Table 1. Acute mammalian toxicity of technical diofenolan

Acute oral LD_{50} (rat)	: > 5000 mg / kg
Acute dermal LD_{50} (24 h) (rat)	: > 2000 mg / kg
Eye irritation (rabbit)	: none
Skin irritation (rabbit)	: none

Table 2. Effects of diofenolan on environment

Bobwhite quail, Mallard duck, oral LD_{50}	: > 2000 mg/kg
Bee (adult) LC_{50}	: > 96 µg/bee
Fish LC_{50} (96h)	: 1.1 - 1.7 mg/l
Daphnia LC_{50} (48h)	: 0.5 mg/l
Algae LC_{50} (72h)	: 0.072 mg/l
Earthworm LC_{50} (14d)	: 204 mg/kg soil

Technical diofenolan has a high toxicity to the brood of the honeybee.

The immobility in soils (RMF 0.15) and the fast dissipation rate (T1/2 approx. 3 days) indicate that diofenolan will not move into ground water and will not accumulate in the food chain.

Toxicity to beneficial arthropods

Diofenolan proved to be highly selective against a broad range of beneficial insects and predatory mites. It did not show adverse effects on the parasitoid *Aphytis melinus* (Hymenoptera), *Cales noacki*, an important parasitoid of the citrus whitefly, species of the family Syrphidae, the flower bugs *Anthocorus nemorum* and *Orius majusculus*, or on the predatory mites *Amblyseius fallacis* and *Typhlodromus pyri* in laboratory and field trials. An inhibition effect was observed on immature stages of *Coccinella septempunctata* in the laboratory. For more information about the selectivity of diofenolan, refer to Sechser *et al.* (1994).

Type of action

Diofenolan inhibits the development of the first and the second instar larvae of scales. Treated San José scale first instar larvae which initiated the first moult were not able to shed their old exuviae. Treated second instar larvae did not moult to adult stages. Diofenolan has also an effect on young adult females. An increased number of San José scale females died after a treatment with diofenolan, a large proportion of the progeny of the treated females was stillborn (Hippe *et al.* 1994). In lepidopterans diofenolan disrupts the development from the egg to the larva or from the larva to the pupa.

BIOLOGICAL ACTIVITY UNDER FIELD CONDITIONS

Pome fruit

Quadraspidiotus perniciosus: the San José scale is an important pest in deciduous fruit nearly all over the world. In some countries it is listed as a quarantine pest. Diofenolan at significantly lower dosage rates than standard insecticides exhibited excellent activity against *Q. perniciosus* and other important armoured scales (Diaspididae), e.g. *Q. ostreaeformis*, *Lepidosaphes ulmi*. For the control of the San Jose scale best results were achieved with diofenolan at rates of 10 gAI/100 l as dormant spray on overwintering first instar larvae at the phenological stage B-C before bud burst (Table 3). Figure 1 demonstrates an example of the efficacy against the summer generation at the beginning of crawler migration.

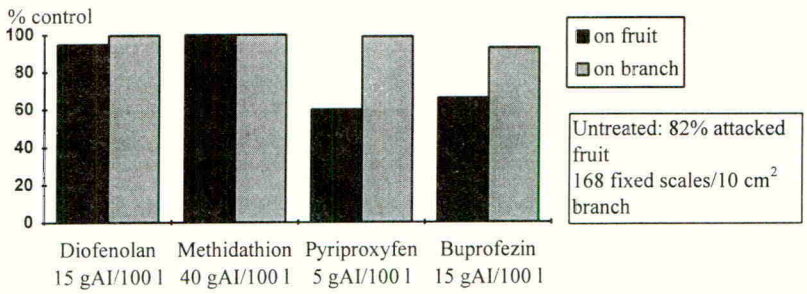
Table 3. Control of *Quadraspidiotus perniciosus* on apple after dormant spray (Geneva,Switzerland, 1993)

Treatment	Formulation	Dose [1] (gAI/100 l)	% control
Diofenolan	50 % EC	15	100
Diofenolan	50 % EC	10	100
Methidathion	40 % WP	40	99
Mineral oil	99 % EC	3500	99
Untreated [2]			(153)

[1] Application date: 19.3.1993 using 2000 litres / ha

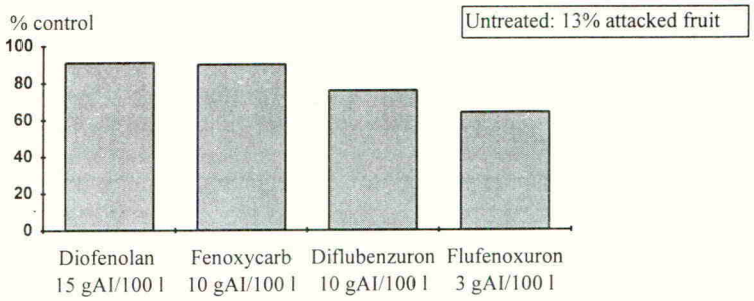
[2] Number of infested fruit per 300 at harvest

Figure 1: Control of summer generation of *Quadraspidiotus perniciosus* on apple (Italy, 1993)



Cydia pomonella: Although the activity of diofenolan is mainly against scales, it also performs very well against various important lepidopterous pests. Figure 2 shows the good activity of the product against *C. pomonella.* The first application was done at the beginning of the oviposition, followed by 6 applications until the end of the moth flight (12.5.1992 - 17.8.1992). The activity of diofenolan at 15 gAI/100 l was equal or superior to that of commercial standard insecticides.

Figure 2: Control of *Cydia pomonella* on apple (Aigues Vives, France, 1992)



Stone fruit

The white peach scale (*Pseudaulacaspis pentagona*) and the oriental fruit moth (*Cydia molesta*) are important pests on peaches. Diofenolan at 15 gAI/100 l provided excellent activity against these pests in trials conducted in Italy (Figures 3 and 4). The product offers, with only one spray, an excellent opportunity for the control of both pests which often occur simultaneously.

Figure 3: Control of *Pseudaulacaspis pentagona* on peach (Italy, 1992)

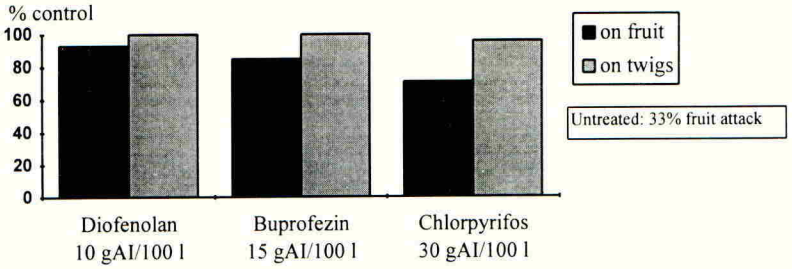
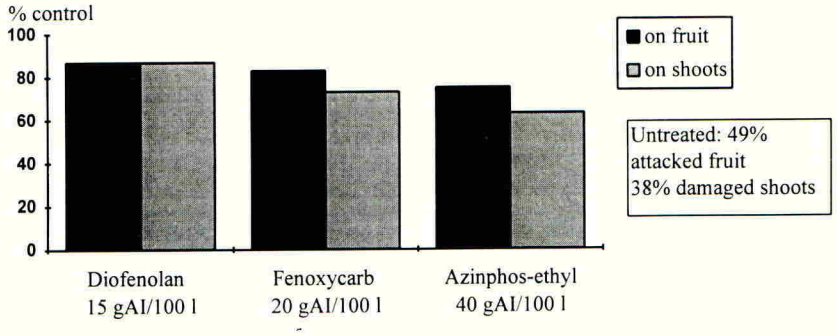


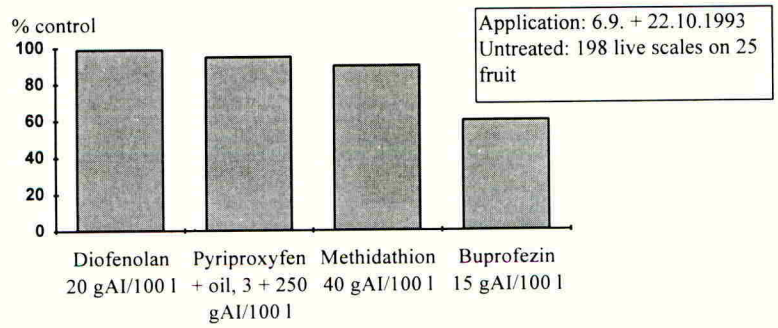
Figure 4: Control of *Cydia molesta* on peach (Italy, 1992)



Citrus

The California red scale (*Aonidiella aurantii*) is a pest of major economic importance in citrus and the development of resistance to conventional insecticides makes its control very difficult. Two sprays of diofenolan at 20 gAI/100 l applied at the time of the first and the second crawler movements gave excellent control of *A. aurantii* under high population pressure in South Africa (Figure 5).

Figure 5: Control of *Aonidiella aurantii* on citrus after two applications (South Africa, 1993)



Often a number of different scale species occur simultaneously in a citrus orchard and have to be controlled. Excellent control of the whole scale complex (Diaspididae) was demonstrated with diofenolan at 20 gAI/100 l in Spain (Table 4). The application in this trial was timed for *A. aurantii*.

Table 4. Performance of diofenolan 50 % EC against different armoured scales (Diaspididae) on citrus (Spain, 1992)

Treatment	Formulation	Dose [1] (gAI /100 l)	% control		
			Lepidosaphes beckii	*Aonidiella aurantii*	*Parlatoria pergandei*
Diofenolan	50 % EC	20	93	90	82
Methidathion	40 % EC	60	92	88	78
Pyriproxyfen	25 % EC	5	94	81	84
Buprofezin	25 % WP	15	93	73	68
Untreated [2]			(28)	(24)	(11)

[1] Application date: 11/6/92 using 3200 litres/ha

[2] Number of live scales on 20 fruit

Good results have also been obtained against different soft scales (Coccidae). Diofenolan at rates from 5 to 10 gAI/ 100 l provided good control of *Ceroplastes floridensis* (Table 5).

Table 5. Control of *Ceroplastes floridensis* (Coccidae) on citrus (Egypt, 1993)

Treatment	Formulation	Dose [1] (gAI/100 l)	% control
Diofenolan	50 % EC	10	99
Diofenolan	50 % EC	5	97
Methidathion	40 % EC	40	97
Buprofezin	25 % WP	15	89
Untreated [2]			(420)

[1] Application the time of crawler migration

[2] Number of live scales on 50 twigs

Mealybugs have increased in pest status over the past few years on citrus, probably due to the reduced use of OP compounds. In South Africa, diofenolan with two sprays at 20 gAI/100 l (4500 l/ha) performed very well (equal to chlorpyrifos) against *Planococcus citri* (Table 6).

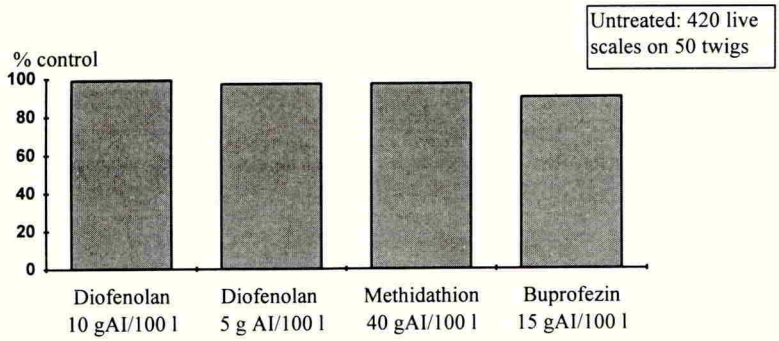
Table 6. Control of *Planococcus citri* on citrus (South Africa, 1993)

Treatment	Formulation	Dose (gAI/100 l)	Dates of application	% control (1.3.94)
Diofenolan	50 % EC	20	06.9. + 8.10. 93	92
Diofenolan	50 % EC	20	22.9. + 25.10.93	93
Chlorpyrifos	48 % EC	48	06.9. + 8.10.93	93
Untreated[1)]				(75)

[1)] Percent attacked fruit

In addition to its activity against a broad range of scale insects, diofenolan at rates from 10 to 30 gAI/100 l has proven to be effective against important lepidopterous pests on citrus, e.g. *Cryptophlebia leucotreta*, *Phyllocnistis citrella*. Figure 6 demonstrates an example of the efficacy against the citrus leaf miner *P. citrella* (Gracilariidae).

Figure 6: Control of *Phyllocnistis citrella* on citrus (California, USA, 1993)



Other crops

Diofenolan provided also excellent control of scales on olives (*Saissetia oleae*), mango (*Aulacaspis tubercularis*) and ornamentals (*Asterolecanium pustulans*) and of the tea leaf roller (*Caloptilia theivora)* on tea.

CONCLUSION

Diofenolan represents a promising new insecticide with outstanding strength in the control of the important scales and lepidopterous pests in fruit crops. The original mode of action and its good selectivity together with a good toxicological and ecotoxicological profile make it especially useful for IPM / IRM.

ACKNOWLEDGEMENTS

The authors thank all of their colleagues in Basel and many countries who have contributed to research and development of diofenolan.

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WATER DISPERSIBLE GRANULE: A NOVEL FORMULATION FOR NEMATODE-BASED BIOINSECTICIDES

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ABSTRACT

Stable formulations for current nematode products have been achieved by immobilizing and/or partially desiccating infective stage nematodes. These formulations are suitable in high and medium value crops such as mushrooms, ornamentals, citrus, turfgrass and berries. However, more advanced formulations are needed for the nematodes to become commercially competitive to chemical insecticides especially in low-value agricultural markets. In this regard, a breakthrough was achieved with the development of a water dispersible granular formulation which allows the nematodes to enter into anhydrobiotic (desiccation) state preserving nematode survival and pathogenicity for at least 6 months at 4-25°C. The formulation is scaleable, and easy to apply without the time consuming preparation steps. It is available in 0.7 litre and 1.2 litre products containing 350 g ($100X10^6$ nematodes) and 680 g ($250 X 10^6$ nematodes) of granules (5-10 mm in diameter), respectively.

FORMULATION: GENERAL ASSESSMENT

Entomopathogenic nematodes in the genera *Steinernema* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae) are attractive biological control agents that are commercially available for the control of a wide-range of soil-inhabiting insects (Georgis, 1992, 1994). Significant progress achieved with steinernematid nematodes in the last five years in liquid culture production (15,000 - 80,000 litre fermenters), application technology, field efficacy and formulation technology have resulted in the introduction of a number of nematode-based products in various markets (Georgis, 1992).

To maintain nematode virulence, current technology utilizes gel polymers or clay to immobilize or partially desiccate the nematodes (Georgis, 1992). These formulations reduce nematode metabolism and improve their tolerance to temperature extremes. However, issues related to extracting, mixing and applying these formulations have limited their commercial potential. Additionally the amount of products needed to cover large areas have been in many cases impractical. For example 4.0 litre alginate gel formulation products that contain $250 X 10^6$ nematodes treat up to 930 m^2 and 0.2 litre clay formulation products cover up to 50 m^2. Nematode stability at high temperatures is also a limiting factor with these formulations. Therefore a more concentrated, reliable and stable formulation such as a water

dispersible granule is needed to strengthen the position of nematode-based products in various market segments (Tables 1 and 2).

TABLE 1. Product size and coverage of major nematode-based formulations

Formulation	Product Size (litre)	Nematode Density	Maximum Coverage (m^2)
Alginate gel[a]	4.0	250 X 10^6	930
	1.0	10 X 10^6	50
Clay[b]	0.2	50 X 10^6	50
Water dispersible granule	1.2	250 X 10^6	930
	0.7	100 X 10^6	232

[a]: Nematodes spread on mesh screen impregnated with calcium alginate
[b]: Nematodes spread on clay material

NEMATODE QUALITY

The successful market acceptance of the nematode-based formulations depends heavily on their stability during shipping and storage as well as their ease of use and consistent performance under field conditions. Thus it is important to maintain nematode quality throughout all stages of product development. The first step in standardization is aimed at producing reliable and consistent nematodes. Inoculum batches from *in vivo* cultures are produced from stocks of nematode strains that are stored by cryopreservation (Popiel and Vasquez, 1991) to minimize variation in nematode pathogenicity among *in vitro* production lots. Subsequent steps are focused on maintaining the viability and pathogenicity of the nematodes immediately after the nematodes are harvested from the fermenter until the product is applied by the end-user. To assure this process, LT_{50} (the time needed to kill 50% of test insects) performance standards and lipid contents of infective juvenile nematodes have been determined and are used to measure product stability.

Another aspect of product assurance is timing of the production according to the market needs. The primary time of production is accomplished from January to March for products needed from May to August. However, for August to December markets, nematodes are produced from March to June. Certainly, such considerations are dependent

on the nematode species, type, storage requirements, market forecast and the distribution channels.

Stability at various temperatures and ease of use are the major obstacles in expanding the commercial potential of nematode-based products. Shelf-life of the infective juvenile nematodes is a function of stored energy and rate of its utilization. Lipid is a major energy reserve for infective juveniles (Popiel and Vasquez, 1991) and initial lipid level appears to have a direct impact on shelf-life. The rate of utilization of stored energy depends on many factors such as temperature, environmental stress and nematode activity and behavior. These factors should be taken into consideration when conducting formulation research. Nematode immobilization on alginate gel has been the most widely used formulation to preserve stored energy. This formulation allows ease of handling, shipping and application of large quantities for the high value markets. However, more stable and ease of use formulations are needed for the nematodes to become commercially competitive with chemical insecticides in most of these markets and in low-value traditional agricultural markets such as cotton and corn.

TABLE 2. Description of water dispersible granule formulation.

Water Dispersible Granule	Description
Components	Infective juvenile nematodes, dispersent, binder, diluent, wetting agent
Sizes	5-10 mm in diameter; each granule contains an average of 40,000 infective juvenile nematodes
Commercial products	100 X 10^6 and 250 X 10^6 infective nematodes formulated in 350 and 680g of granule material, respectively

WATER DISPERSIBLE GRANULE FORMULATION

Recently, a breakthrough in nematode formulation was achieved with the development of water dispersible granular (WDG) formulation (Table 2) that allows the nematodes to enter into an anhydrobiotic state preserving nematode survival and pathogenicity for up to 6 months at 4-25_C and up to 8 weeks at 30_C (Table 3). The formulation is scaleable and is easy to apply without any time consuming preparation steps (Table 3). The formulation is suited for a wide variety of consumer, agricultural and horticultural applications (Table 4). For example, 1.2 litres of product containing 250 X 10^6 nematodes (680g) treats up to 930 m^2 compared to 4.0 litres for the same amount of nematode formulated on alginate gel (Table 1).

TABLE 3. Comparison analysis between water dispersible granule (WDG) and alginate gel-based formulations

Character	WDG	Alginate Gel
Stability	Up to 6 months at 4-25°C Up to 8 weeks at 30°C Up to 6 days at 36°C	Up to 5 months at 4-25°C Up to 2 weeks at 30°C Few hours at 36°C
Ease of use	Dissolve quickly in water Ready to use. Easy to measure	20-30 minute preparation steps Once diluted the entire product must be used
Product size: coverage ratio	Comparable to chemical products and adequate for use in various markets	Only adequate in certain markets.
Disposal	Minimal product disposal	An issue in certain markets
Usage range	Various nematode species are compatible with WDG	Suitable for few nematode species
Field efficacy	Comparable to alginate gel	Comparable to WDG
Cost	Cheaper than alginate gel	Higher than WDG

Since nematode species differ in behaviour, size and tolerance to temperature and moisture conditions, it is important to develop an optimum material and production scale up process for each nematode species. Careful consideration should be placed on temperature and duration of exposure of WDG to temperature to optimize the desiccation process for each nematode species (Fig. 1).

Once WDG is mixed in the tanks, a spray volume of 750-1890 litre/ha is usually required for most nematode species to reach the target insect. Nematodes can be applied to the target zone with nearly all commercially available ground and aerial spray equipment. These include ground equipment such as small pressurized sprayers, mist blowers, electrostatic sprayers, as well as the traditional sprayers used in aerial application via helicopters. In addition, nematodes are commonly applied using drip and sprinkler irrigation systems. Pressures of up to 1068 KPa have no detrimental effect on nematodes. The nematodes can pass easily through sprayer screens with openings as small as 100 microns in diameter.

TABLE 4. Products based on water dispersible granule (WDG) formulation

Trade Name[a]	Market Segment	Company
Exhibit	Turf and Ornamentals	Ciba Geigy, USA
BioSafe	Home and Garden	biosys, USA
Vector-T&L	Turf and Lawns	Lesco, USA
Bio Flea Halt	Pet/Vet	Farnam, USA
Interrupt	Pet/Vet	Farnam, USA
Vector MC	Turf	Lesco, USA

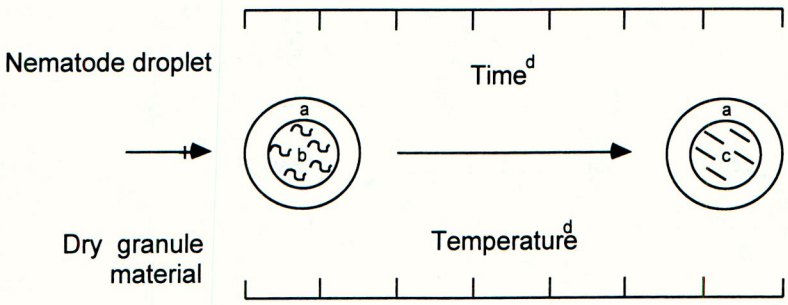
[a]: All products are based on *S. carpocapsae* except Vector MC which is based on *S. riobravis*.

Nematodes prepared from WDG should be applied to moist soil. Post-application irrigation and continuous moderate soil moisture are essential for nematode movement, persistence, and pathogenicity, and for nematodes to achieve insect control at a level comparable to standard insecticides. Although nematodes are recommended to be applied during early morning or evening to avoid the effects of ultraviolet radiation and temperature extremes, in many situations nematodes can be applied at any time of the day as long as post-application irrigation is employed within 30 minutes.

Numerous field data generated over the last two years showed that nematodes in WDG can protect crops from insect damage, thus, they are not slow biological control agents. Insect such as white grubs (Scarabaeidae) and root weevils (Curculionidae) are controlled successfully within 2-4 weeks post-application, whereas, lepidopteran are generally controlled within 3-7 days.

The compatibility of WDG with various chemical pesticides is a major concern when considering their inclusion in integrated pest management systems. WDG can be mixed safely with commercial preparations of *Bacillus thuringiensis*, pyrethroids, and other pesticides and fertilizers. Some pesticides can adversely affect nematodes, however they still can be used in sequence if nematodes are applied before the pesticide or vice versa, thus allowing time for the pesticide to become absorbed or degraded to a level non-toxic to the nematode. To date, several successful attempts have been made to increase nematode efficacy further when employed in conjunction with chemical and microbial agents.

FIG. 1. Schematic diagram process showing nematode desiccation in water dispersible granule (WDG) formulation.



a: Granular material
b: Active nematodes
c: Desiccated nematodes
d: Each nematode species has an optimum temperature and exposure time for desiccation

CONCLUSION

The quality of commercial nematode products is critical if entomopathogenic nematodes are to realize their full potential as biological insecticides. The stability and ease of use of water dispersible granular formulation and the excellent quality of nematodes grown in liquid culture are significant steps towards this goal. All commercial formulations including water dispersible granules have been developed to maintain product stability during storage and transportation, and they are applied as a spray in water against the target pest. Granular, capsules and bait pellets that can be applied by aircraft and standard granular applicators that protect and (or) release nematodes in the soil are also desirable and worth further investigation.

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YI-5301, A NOVEL OXAZOLINE ACARICIDE

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ABSTRACT

2,4-Diphenyloxazoline derivatives are a new type of basic chemical structure that have been investigated and shown to provide good control of mites and aphids. YI-5301, one of the derivatives, has been selected for showing high activity against mites. The compound has excellent contact activity on eggs, larvae and nymph of mites under 1 mg AI/l with no effect on adults. The role of YI-5301 appears to be due to an inhibition of moulting process similar to hexythiazox. YI-5301 is equally active in controlling hexythiazox-resistant and susceptible strains of mites. In field trials, low mite population is consistent over a month after application of YI-5301. YI-5301 is unique since currently marketed moulting inhibitors (e.g., flufenoxuron) provide no control of aphids. YI-5301 exhibits low mammal toxicity and no injury to commercial crops.

INTRODUCTION

In research into oxazoline derivatives, a new class of acaricidal compounds was discovered by Yashima Chemical Industry Co., Ltd. YI-5301 was selected as the most active compound among many oxazoline derivatives. YI-5301 will be developed worldwide as a promising acaricide for citrus, apple, grape, vegetables, flowers and tea. This paper describes the properties and performance of the compound under laboratory and field conditions in Japan.

CHEMICAL AND PHYSICAL PROPERTIES

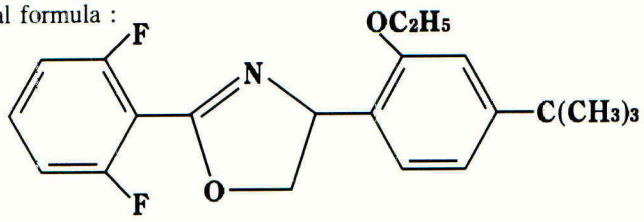
Code name : YI-5301

Chemical name :
4-(4-*tert*-butyl-2-ethoxyphenyl)-2-(2,6-difluorophenyl)-4,5-dihydrooxazole

Molecular formula : $C_{21}H_{23}F_2NO_2$

Molecular weight : 359.4

Structural formula :



Appearance : white crystalline powder

Melting point : 101 – 102 ℃

Vapour pressure : 2.18×10^{-6} Pa at 25 ℃

Solubility (g/l solvent at 20 ℃) :

water	75.4×10^{-6}	acetone	450
methanol	100	ethanol	100
cyclohexanone	550	tetrahydrofuran	850
acetonitrile	100	ethyl acetate	400
xylene	400	n–hexane	15
n–heptane	15		

Stability : no decomposition for 30 days at 50 ℃

Log pow : 5.59 at 25 ℃

Formulation : 100 g/l Suspension Concentrate

TOXICOLOGY AND ENVIRONMENTAL PROPERTIES

Acute oral LD_{50} :	rat	male	>5000 mg/kg
		female	>5000 mg/kg
	mouse	male	>5000 mg/kg
		female	>5000 mg/kg
Acute dermal LD_{50} :	rat	male	>2000 mg/kg
		female	>2000 mg/kg
Ames test :			Negative
Irritation :	skin	rabbit	Non–irritant
	eye	rabbit	Non–irritant

Environmental toxicity :

fish	Japanese carp	LC_{50} (96h.)	0.89 mg AI/l
soil	half–life (laboratory) in loam		9 days at 25 ℃

BIOLOGICAL PERFORMANCE

Laboratory Tests

YI-5301 was tested to determine biological activities in various species of mites and aphids compared with conventional acaricides and insecticides under laboratory conditions. The formulated product of a 10% suspension concentrate was diluted with water, and set for dipping in all laboratory tests.

Acaricidal activity

The activity of YI-5301 on eggs of four major mite spp was evaluated and demonstrated to be over 100 times higher than hexythiazox against *Tetranychus* spp. and *Panonychus* spp. (Table 1).

TABLE 1. Ovicidal activity of YI-5301 and hexythiazox against *Tetranychus* spp. and *Panonychus* spp., using leaf-disc dipping.

Compound	LC_{50} (mg AI/l)			
	T.u.	T.k.	P.c.	P.u.
YI-5301	0.003	0.005	0.001	0.002
Hexythiazox	0.8	0.9	0.4	0.6

T.u. : *Tetranychus urticae* P.c. : *Panonychus citri*
T.k. : *Tetranychus kanzawai* P.u. : *Panonychus ulmi*

The ovicidal activity of YI-5301 on three egg stages of *T. kanzawai* was equally active (Table 2).

TABLE 2. Ovicidal activity of YI-5301 against various stages of eggs of hexythiazox-resistant *Tetranychus kanzawai*, using leaf-disc dipping.

Compound	LC_{50} (mg AI/l)		
	1 DAO	3 DAO	5 DAO*
YI-5301	0.05	0.05	0.05

* DAO : Days After Oviposition

YI-5301 was consistently active in controlling eggs of *T. kanzawai* in temperatures from 15 ℃ to 30 ℃ (Table 3).

TABLE 3. Ovicidal activity of YI-5301 against hexythiazox-resistant *Tetranychus kanzawai* under various temperature conditions, using leaf-disc dipping.

Compound	LC_{50} (mg AI/l)		
	15 ℃	25 ℃	30 ℃
YI-5301	0.06	0.05	0.05

YI-5301 has excellent activity on eggs, larvae, protonymphs, and deutonymphs of susceptible mites, but has no activity against adult mites. YI-5301 has sufficient effects on hexythiazox-resistant strains of *T. kanzawai* and *P. citri* at 50 mg AI/l (Table 4).

From observation under a microscope, the mode of action appears to be an inhibition of the moulting process similar to hexythiazox.

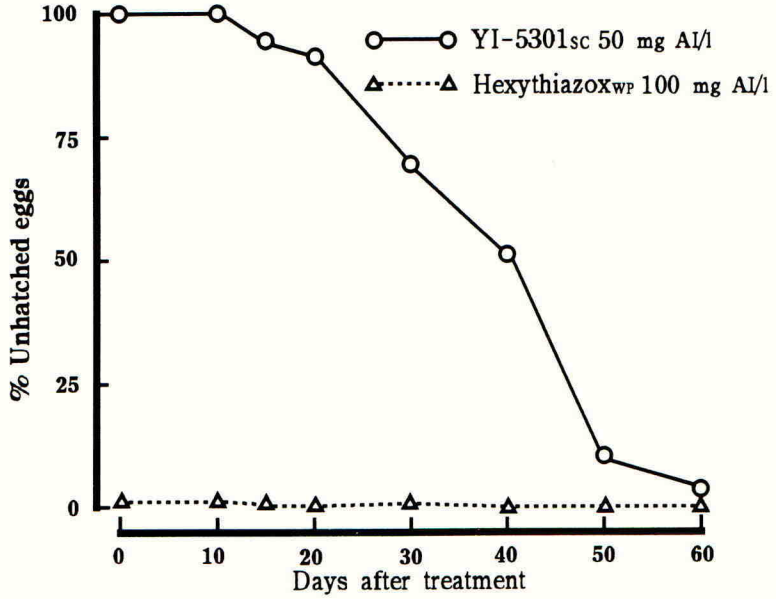
TABLE 4. Activities of YI-5301 and hexythiazox against different stages of susceptible and resistant mites, using leaf-disc dipping.

Species	Compound	LC_{50} (mg AI/l)				
		Eggs	Larvae	Protonymphs	Deutonymphs	Adults
T.k.[S]	YI-5301	0.005	0.0001	0.0001	0.0001	>1000
	Hexythiazox	0.9	0.4	0.5	0.5	>1000
T.k.[R]	YI-5301	0.05	0.0005	0.0005	0.0005	>1000
	Hexythiazox	>1000	50			>1000
P.c.[S]	YI-5301	0.001	0.0006	0.0006	0.0007	>1000
	Hexythiazox	0.4	0.4	0.3	0.5	>1000
P.c.[R]	YI-5301	0.5	0.08	0.08	0.08	>1000
	Hexythiazox	>1000	>1000			>1000

T.k. : *Tetranychus kanzawai* P.c. : *Panonychus citri*
[S] : Susceptible strain [R] : Resistant strain

YI-5301 has long residual activity at 50 mg AI/l against eggs of hexythiazox-resistant *P. citri* (Fig. 1).

FIGURE 1. Residual activity of YI-5301 and hexythiazox against eggs of hexythiazox-resistant *Panonychus citri*, using pot tests.



Aphidicidal activity

YI-5301 shows good effect on juvenile stages of aphids with no effect on adults. In observation on the aphids treated with YI-5301, the moulting process was incomplete. YI-5301 seems to inhibit the moulting process of aphids in the same way as it does to mites. This effect is unique since currently marketed moulting inhibitors provide no control of aphids. YI-5301 is also active against insecticide-resistant strains of aphids as well as susceptible strains (Table 5).

TABLE 5. Insecticidal activity against *Myzus persicae* and *Aphis gossypii*, using leaf dipping.

Species [stage](resistance)	LC50 (mg AI/l)				
	YI-5301	Permethrin	Acephate	Carbaryl	Flufenoxuron
M.p.[N](S)	1.2	11.4	21.6	78.9	>200
M.p.[N](R)	2.16	45.1	257.2	346	>200
M.p.[A](S)	>100				
A.g.[N](S)	0.5	0.582	11.0	4.53	>200
A.g.[N](R)	0.805	2115	216	26.2	>200

M.p. : *Myzus persicae*
[N] : Nymph stage
(S) : Susceptible strain
A.g. : *Aphis gossypii*
[A] : Adult stage
(R) : Resistant strain

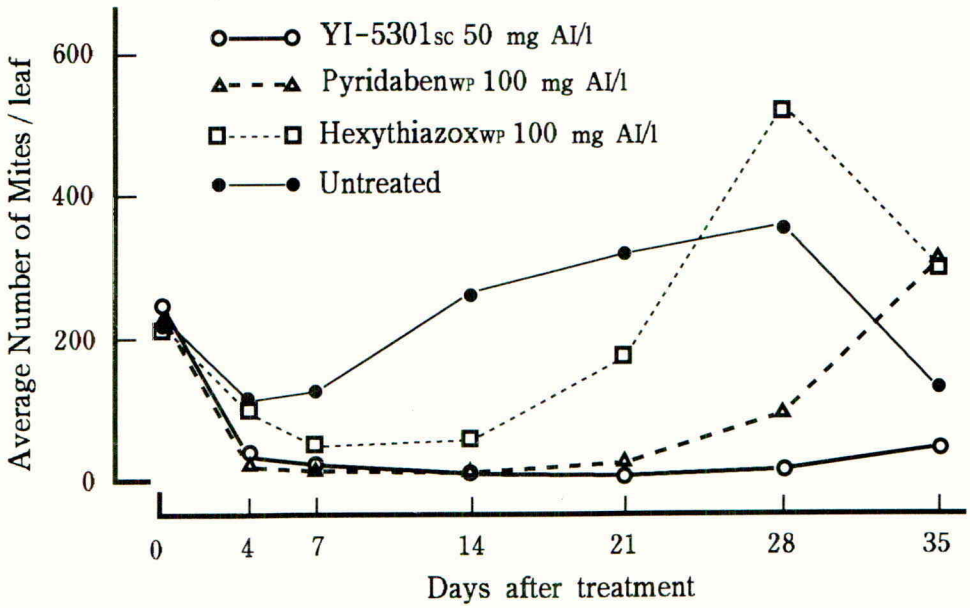
FIELD PERFORMANCE

The biological effects of YI-5301 against mites and aphids in economically important apple, citrus and cucumber were evaluated under field conditions in 1991 and 1992.

Apple

In 1991, YI-5301 (50 mg AI/l) was compared with pyridaben (100 mg AI/l) and hexythiazox (100 mg AI/l) for the control of *T. urticae* on apple. In this trial, YI-5301 gave excellent control of *T. urticae* over a period of a month (Fig. 2).

FIGURE 2. Control of *Tetranychus urticae* on apple in Nagano Prefecture, Japan, after treatment on 19 July, 1991.



Mandarin

In 1992, YI-5301 (50 mg AI/l) was compared with pyridaben (66.7 mg AI/l) and hexythiazox (33.3 mg AI/l) for the control of *P. citri* on Satsuma mandarin. YI-5301 showed as good control of *P. citri* as pyridaben (Table 6).

TABLE 6. Control of *Panonychus citri* on Satsuma mandarin in Shizuoka Prefecture, Japan, after treatment on 11 September, 1992.

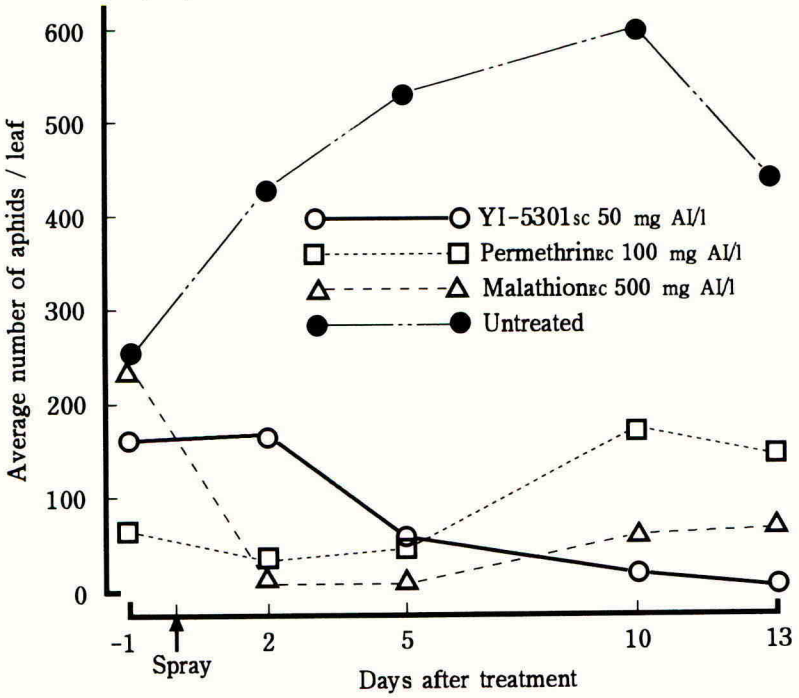
Compound	Dose mg AI/l	Average number of females / 100 leaves				
		Pre-spray (7/Sep.)	3 DAT (14/Sep.)	10 DAT (21/Sep.)	19 DAT (30/Sep.)	33 DAT (14/Oct.)
YI-5301SC	50	85	38	3	2	2
Pyridaben	66.7	98	0	0	0	0
Hexythiazox	33.3	66	35	34	44	173
Untreated		72	98	172	506	1380

Source : Shizuoka Prefectural Citrus Experiment Station

Cucumber

In 1992, YI-5301 (50 mg AI/l) was compared with permethrin (100 mg AI/l) and malathion (500 mg AI/l) for the control of *A. gossypii* on cucumber. The initial activity of YI-5301 was not as rapid as that of the fast-acting insecticide malathion, but the residual activity of YI-5301 continued with a low level of aphid population throughout the trial (Fig. 3).

FIGURE 3. Control of *Aphis gossypii* on cucumber in Nagano Prefecture, Japan, after treatment on 27 June, 1992.



CONCLUSION

YI-5301 is useful for the control of the mites and aphids on fruits, vegetables, flowers and tea. The compound is highly active against juvenile stages of mites and aphids with no effect on adults. It shows good efficacy against mites and aphids resistant to conventional acaricides and insecticides as well as susceptible strains.

YI-5301 has stable ovicidal activity against eggs of mites at various egg ages and temperature conditions.

YI-5301 appears to inhibit the moulting process of mites and aphids. The activity is unique since conventional moulting inhibitors have no effect to aphids.

In field trials, YI-5301 showed a promising residual effect against mites and aphids.

ACKNOWLEDGMENTS

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EVALUATION OF NATURALIS-L FOR CONTROL OF COTTON INSECTS

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ABSTRACT

Naturalis-L, which contains an insect-specific fungus, *Beauveria bassiana*, ATCC 74040, is a new proprietary biological control product that provides excellent control of many of the major cotton insects, as well as those on vegetables and ornamentals. Naturalis-L at 750 ml/ha, applied by conventional and aerial application equipment, effectively controls the sweet potato whitefly, *Bemisia tabaci;* boll weevil, *Anthonomus grandis;* cotton fleahopper, *Pseudatomoscelis seriatus;* bollworm, *Helicoverpa zea;* budworm, *Heliothis virescens;* and others. Field studies have demonstrated excellent field efficacy throughout the world, including the United States, Mexico, Nicaragua, Paraguay, Egypt, Turkey, Turkmenistan, and Australia. Naturalis-L does not deleteriously affect bees or natural predators and parasites, such as *Geocoris spp., Encarsia spp., Eretmocerus spp.*, and *Chrysoperla spp.* Its chemical and physical properties address current environmental issues and reflect a very favorable profile to mammals & terrestrial and aquatic organisms. Naturalis-L also is being effectively used in insect resistance management programs.

INTRODUCTION

The continued development of insecticide resistance to current commercial products and the inherent toxicity of existing synthetic pesticides to nontarget organisms and their persistence in the environment are strong dictates for identification of alternative control measures by the agricultural industry. The development of a biological alternative with similar control activity has been sought for many years with few successes, e.g. *Phytoseiulus persimilis* and *Bacillus thuringiensis.* We are here today to report on a biological alternative that can be successfully used for insect control and that also addresses these important concerns today in agriculture throughout the world. We have developed laboratory and field data in the United States and other countries on a variety of crops and in many different environments. The crop, cotton, affected by numerous insect pests, is the one which we will address today and explain our strategies for the use of Naturalis-L. Registration is impending with the Environmental Protection Agency (EPA) in the United States and should be issued by time of this presentation.

Naturalis-L is a commercial formulation that contains an insect specific fungus, *Beauveria bassiana* (ATCC 74040 = ARSEF 3097 = FCI 7744) that was isolated from a coleopteran boll weevil in Texas. The activity of this fungus is manifested as a contact material in which the conidia of the fungus attaches to the insect cuticle. The fungus uses the

insect for nutrition and growth, which results in the death of the insect. This biological activity is expressed over a period of time, and expression of death is variable depending uponthe species of insect attacked. Insect behavior, i.e., feeding and sexual activities, may be affected immediately after fungal attachment and entrance within the target insect.

TOXICOLOGICAL AND ENVIRONMENTAL SAFETY

An analysis of the toxicological and environmental data for Naturalis-L may be summarized as having no immediate or potential negative effects against tested organisms.

Background levels of *B. bassiana* present in cotton fields averaged about 7.0 X 10 colony forming units (C.F.U.). After 10 applications at 7 day intervals of Naturalis-L at rates of 750 ml/ha (2.37 X 10^7 conidia per ml), there was no significant change in this background level. The evaluations were repeated and performed over a three year period. These results indicate that Naturalis-L does not accumulate in the environment even after repeated applications.

Numerous studies with cotton in different environments have investigated the effect of Naturalis-L on key predators and parasites of pest species. In all studies, Naturalis-L was found not to have a detrimental effect. Examples of beneficials studied are: *Chrysoperla spp., Geocoris spp., Encarsia spp., Eretmocerus spp., Scymus spp., Micromus spp., Nabis spp.* and various arachnids.

Results of a 30 day dietary and contact study with the honeybee, *Apis mellifera*, indicate that Naturalis-L does not significantly affect this important pollinator. These laboratory studies were confirmed in field observations in cotton and vegetables in which no effects were observed on honeybees within the fields or their hives located at the field edges.

Fresh water toxicological studies indicate Naturalis-L does not affect fish embryos, larvae, or adults and also has no effect on water fleas.

Dermal, oral, and inhalation toxicity studies with Sprague Dawley rats indicate that the fungus should be considered non-toxic and non-pathogenic. Additional toxicological studies with other organisms yield the same conclusions: Naturalis-L is non-toxic and non-pathogenic.

COTTON: BIOLOGICAL PERFORMANCE UNDER FIELD CONDITIONS

Trial Arizona: Sweet potato whitefly, *Bemisia tabaci*

Deltapine 5415 upland cotton was planted in a sub-drip irrigated field. Fields (8 ha each) were established and divided into subplots for replications of Naturalis-L and the pyrethroid, bifenthrin. Naturalis-L was applied at the rate of 750 ml/ha and bifenthrin at the label recommended rate. An untreated check field was left untreated. Five ground applications of each product were applied in August-September. Applications were made with

a John Deere 6000 Hi-Cycle sprayer with an 18 meter boom with nozzles oriented at 45 degrees to spray upward for under-leaf coverage.

All life stages of the sweet potato whitefly were sampled weekly; eggs and immatures were counted on 4 leaf/discs from each of the 5 leaf/subplots. Adults were sampled by leaf turn, sticky card, and vacuum samples.

RESULTS

This two year study clearly demonstrated that Naturalis-L was an efficient control agent against the sweet potato whitefly. The numbers of nymphs were significantly reduced compared to the bifenthrin and untreated control fields (Table 1). Adult control was comparable to that of bifenthrin as evaluated by leaf turn and sticky card samples; however, it was significantly better than bifenthrin when compared by vacuum sampling and leaf turn (Table 2).

TABLE 1. Number of sweet potato whitefly immatures one week after last application in DPL 5415 cotton in Arizona, U.S.A.

Naturalis-L	3.3 a
Bifenthrin	15.4 b
Untreated check	18.8 b

Means with differing letters are significantly different ($P < 0.05$)

TABLE 2. Number of sweet potato whitefly adults one week after last application in DPL 5415 cotton in Arizona, U.S.A.

	Number of adults		
	Total	Mean	
	Vacuum samples	Leaf turn	sticky card
Naturalis-L	418 a	3.8 a	69.7 a
Bifenthrin	2300 b	4.0 a	106.8 b

Means with differing letters are significantly different ($P < 0.05$).

Cotton: Texas Boll weevil, *Anthonomus grandis*
Cotton fleahopper, *Pseudatomoscelis seriatus*

Tests were conducted to evaluate the use of Naturalis-L in an IPM program for control of overwintered boll weevils and early infestation of the cotton fleahopper. These tests were performed on irrigated sandy loam soil. The fields were bordered by cotton on the north and south and by brush on the east and west - excellent habitat for the two pests. This area has a history of higher than normal insect infestations.

The field evaluations were arranged as a randomized block design with three replicates. Each block was 10 ha. Deltapine 50 was the cultivar. Treatments were applied with a mechanical air blast, tractor mounted, Berthoud B-N sprayer that delivered 93 liters per hectare. Naturalis-L was applied at the rate of 750 ml/ha. Four treatments were applied at 5-7 day intervals and insecticides thereafter. In the other field tests, Naturalis-L only and insecticides were applied throughout the cotton production season. Label recommended rates of acephate, azinphos-methyl, bifenthrin, methyl parathion, and lambdacyhalothrin were applied. An untreated block of cotton served as a comparative field. For the early season applications of Naturalis-L in which four applications were made pre-bloom, nine additional insecticide treatments were used. For the full season insecticide and Naturalis-L only treatments, thirteen applications were applied.

Insect counts, percent of fruit damaged, fruit set per plant, and lint production and yield were obtained from all fields. Beneficials were also monitored.

RESULTS

All treatments had significantly increased yields over the untreated controls but there were no significant difference between treatments (Table 3). Beneficials were present in significantly higher numbers in the Naturalis-L treated fields. Damage by boll weevils and cotton fleahoppers was significantly suppressed in the Naturalis-L and insecticide treated fields.

TABLE 3. Yield of cotton in the Rio Grande Valley of Texas, U.S.A., for Naturalis-L treatments

Treatment	Kg lint per hectare
Naturalis-L full season only	1295 a
Naturalis-L/insecticide IPM	1190 a
Insecticide only	1145 a
Untreated	189 b

Means with differing letters are significantly different ($P < 0.05$)

Cotton: Paraguay, boll weevil, *Anthonomus grandis*

Cotton was planted on three separate planting dates, and Naturalis-L was applied with conventional backpack sprayers. Comparisons was made with cypermethrin for control of the boll weevil in replicated plots of 4 ha per plot. Naturalis-L was applied at the rate of 750 ml/ha and cypermethrin at the label recommended rate. Three applications were at a 5-8 day schedule due to the heavy infestation of boll weevils. Fruit damage by boll weevils was determined in the replicated plots.

RESULTS

Planting dates influence the level of infestation by the boll weevil and, for all three planting dates evaluated, the Naturalis-L was as effective in control as cypermethrin (Table 4). Insect control by a biological product such as Naturalis-L reduces the exposure of workers within the treated cotton and also reduces the development of resistance to insecticides.

TABLE 4. Results of Naturalis-L treatments of cotton for boll weevil control in Paraguay

Treatments	Planting dates	% fruit damage
Naturalis-L	Oct 7	16 a
cypermethrin	Oct 7	17 a
Naturalis-L	Oct 12	6 a
cypermethrin	Oct 12	5 a
Naturalis-L	Oct 20	2.6 a
cypermethrin	Oct 20	3 a

Means with differing letters are significantly different ($P < 0.05$)

Cotton: Texas, fleahopper, *Pseudatomoscelis seriatus*
Sweet potato whitefly, *Bemisia tabaci*

Ten-hectare fields (3) of cotton received pre-bloom applications of Naturalis-L for treatment of cotton fleahoppers. A total of five applications with a Berthoud B-N sprayer that delivered 93 liters per ha was made. Naturalis-L was applied at the rate of 750 ml/ha. Fleahopper numbers and fleahopper damage was determined prior to application and monitored thereafter for 4 weeks.

Assessment of honey-dew levels in Naturalis-L and insecticide-treated fields was used to determine the effects of the treatments on the sweet potato whitefly. Insecticides used were bifenthrin and fenpropathrin at label recommended rates. No untreated plots were available for comparison.

RESULTS

The first field observations prior to applying the Naturalis-L treatment showed that 32% of the cotton squares were damaged by cotton fleahoppers. Over the next 5 weeks, there was a significant decline in numbers and damage by cotton fleahoppers following the applications of Naturalis-L (Table 5). Also no damage by boll weevils or bollworms occurred. The sweet potato whitefly invaded the cotton fields and Naturalis-L was very effective in controlling the populations. In the insecticide-treated fields, whitefly populations were not controlled. Beneficial species increased in the Naturalis-L fields and declined in the insecticide-treated fields. At the end of the season, the insecticide field had 200% more open bolls contaminated with honey dew from the sweet potato whiteflies and the field treated with Naturalis-L had a significantly higher yield than that of the insecticide (1023 kg per hectare compared to 800 kg per hectare).

TABLE 5. Reduction in numbers and damage of cotton fleahoppers by Naturalis-L in Texas, U.S.A.

Treatment dates	fleahoppers/ meter row	% fleahopper damage
April 27	7.6 a	32.2 a
May 5	7.0 a	34.6 a
May 13	4.6 b	18.6 b
May 19	1.8 c	9.0 c

Means with differing letters are significantly different ($P < 0.05$)

Australia Boll worms, *Heliothis spp.*

In Australia, the major cotton pests are lepidopterous species. Resistance to insecticides, particularly the pyrethroids, is especially severe and control of the boll worms is extremely difficult, if not impossible. Naturalis-L is effective on the eggs and larvae. The timing of applications, directed towards the newly-deposited egg, increases the efficacy of Naturalis-L.

Evaluations were done on large commercial fields of cotton in the Gwyidir Valley, N.S.W., Australia. Applications were by both conventional ground and aerial sprayers. Three approaches were used in the testing procedure: (1) IPM approach with Naturalis-L as early season treatments followed by conventional insecticides; (2) IPM approach with

Naturalis-L as early season treatments followed by B.t. applications; and (3) conventional insecticides throughout the cotton production season. Insecticides used at label recommended rates were endosulfan, chlorfluazuron, lambdacyhalothrin, thiodicarb, methyl parathion, dimethoate, and bifenthrin. Standard research evaluations such as damage, fruit retention, and final yield were recorded. Beneficials were also monitored and recorded.

RESULTS

The results indicated that the IPM treatments of (1) Naturalis-L and insecticides and (2) Naturalis-L and B.t. provided excellent control of cotton insects and that the resulting yield was equal to the best conventional insecticide program now available on cotton in Australia. Table 6 clearly demonstrates that no significant differences between the yields of the different treatments. The biological control method integrated with an IPM approach and then total biological control treatments were equally effective as the conventional insecticide program. Additionally, numbers of beneficials built up in the biological approaches and should contribute to the following year's population for increased beneficial activity. The Naturalis-L treatments (1) and (2) and the unsprayed fields had significantly more beneficials than the insecticide program (Table 7). There was no significant difference between the Naturalis-L treatments and the unsprayed control in the numbers of beneficials.

TABLE 6. Results of Naturalis-L trials on cotton in Australia.

Treatment	Yield kg/hectare
Naturalis-L/insecticide IPM	2005 a
Naturalis-L/B.t. IPM	1950 a
Insecticides only*	2052 a

Means with differing letters are significantly different ($P < 0.05$)
* See text for insecticide program

TABLE 7. Naturalis-L Does Not Harm Beneficials: Australian Cotton

Treatment	Number per sample per meter				
	C. repanda	*D. notescens*	*Chrysopa spp.*	*N. capsiformis*	*D. bellulus*
Naturalis-L	0.225a	0.212a	0.100a	0.158a	0.092a
Naturalis-L+B.t.	0.264a	0.208a	0.092a	0.082a	0.091a
Conventional	0.071b	0.046e	0.015b	0.042b	0.030b
Unsprayed	0.213a	0.388b	0.142a	0.213a	0.142a

Means with differing letters are significantly different ($P < 0.05$)

CONCLUSIONS

Naturalis-L is a new biological insect control product that has excellent activities for control of important economic pests of cotton worldwide. Its active ingredient, *B. bassiana*, has excellent environmental and toxicological safety properties. Evaluations of Naturalis-L, alone and in IPM systems, have yielded excellent control data as well as final yield data. It is especially significant that Naturalis-L effectively controls the whitefly, which is highly resistant to commonly used insecticides, not only in the cotton production systems but also in vegetables, ornamentals, and other agricultural systems where this pest is considered one of the most damaging insects in modern history.

Naturalis-L can be applied with conventional application equipment, including backpack sprayers and aerial, conventional ground, and air blast sprayers. The product has an excellent shelf life exceeding two years.

The success encountered worldwide, its excellent safety profile, compatibility with beneficials, and use in IPM systems indicate that Naturalis-L should be a powerful new product for insect pest management not only in cotton, but in a wide range of crops. In crop protection programs, Naturalis-L should become a standard as part of the IPM systems.

ACKNOWLEDGEMENTS

The development of a new biological control insect product, and especially one such as Naturalis-L, which is the first such true biological product to be introduced in an international arena, is a team effort, and the effort of all our colleagues is gratefully acknowledged. Special thanks to Mr. Peter Glennie and Kylie May of B.S. Glennie & Son, Moree, NSW; Dr. Robert Mensah, ARC, NSW Agriculture, Australia; and Dr. Arthur Rhodes, Rhodes Consultants, Johannesburg, South Africa.

RELATIVE RESIDUAL ACTIVITIES OF AZADIRACHTIN, DIHYDROAZADIRACHTIN AND TETRAHYDROAZADIRACHTIN

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ABSTRACT

Azadirachtin is the key active ingredient present in extracts of the tropical neem tree. The hydrogenated derivatives, dihydroazadirachtin (DAZA) and tetrahydroazadirachtin (TAZA) show significantly longer residual activity than the parent molecule in the presence of sunlight. The Biological Half Life values (BHL_o) for Colorado potato beetle at 30 mg a.i./l were calculated to be 17.1 days, 14.1 days, 24.6 days and 29.0 days respectively for technical azadirachtin (TECH), pure azadirachtin (AZA), DAZA and TAZA respectively. It can be concluded from these studies that hydrogenation significantly improves the photostability of azadirachtin and this in turn can be expected to provide 1.7x to 2.0x increased residual activity in the field. Azadirachtin formulations are currently being marketed in the greenhouse and food crop segments. Second generation formulations comprising hydrogenated azadirachtin analogues should provide prolonged shelf life and enhanced residual efficacy in the field.

INTRODUCTION

Azadirachtin (AZA) is a complex tetranortriterpenoid molecule (molecular weight=720) and is one of the key active ingredients present in the seed extracts of the tropical neem tree (*Azadirachta indica* A. Juss). In India, it has been known for hundreds of years that the neem tree possesses remarkable properties against stored product pests and common bacteria as well as aiding in prevention of tooth decay (Koul *et. al.*, 1990). Ancient writings have often refered to the tree's healing attributes and in fact, it has recently been hailed as a "village pharmacy" by the National Research Council Panel on Neem (National Research Council, 1992). Azadirachtin has been determined to be one of the key active principles in neem extracts that provide the insect growth regulator activity which kills insects, and that the anti-feedant/repellant properties are due to other chemicals notably salannin. Extensive testing has shown that azadirachtin is active against many insects including whiteflies, leafminers, Colorado potato beetles, sawflies, fungus gnats and a wide range of lepidopterous insects including *Heliothis spp.*, and *Spodoptera spp.* and somewhat inconsistent control against certain aphids and thrips have also been shown. It has also been determined by extensive laboratory and field tests that rates of 25-50 g AZA per hectare on a weekly basis are required to give the level of control required

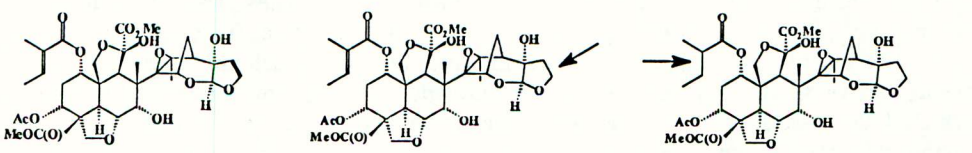
for commercial acceptability (Immaraju *et. al.*, *in press*). Prolonging the interval between applications to greater than 7 days often result in marginal control, especially at rates below 25 g AZA/h. These findings place a premium on extending the residual activity of azadirachtin. Prolonging the residual activity in the field would translate into fewer applications per season, and therefore reduce seasonal pest control costs.

The objective of this experiment was to determine the relative residual activities of (i) technical grade azadirachtin, (ii) pure azadirachtin as well as the (iii) dihydro and (iv) tetrahydro derivatives under full exposure to sunlight and to quantify the residual activties in terms of Biological Half Life values (BHL_5).

METHODS AND MATERIALS

The experiment was conducted using Colorado potato beetle (*Leptinotarsa decemlineata* Say) on potato (*Solanum tuberosum*) as the host. This was a five-treatment single factorial, repeated measures experiment with five replicates for each treatment. The five treatments were: Technical grade azadirachtin (a refined neem extract containing 15.4% azadirachtin), Pure azadirachtin (92% azadirachtin), Dihydroazadirachtin (92% purity), tetrahydroazadirachtin (95% purity) and a control. For the sake of convenience, these compounds have been abbreviated as TECH, AZA, DAZA and TAZA respectively. AZA, DAZA and TAZA were all extracted from TECH and DAZA and TAZA were hydrogenated as appropriate. All compounds were weighed out and dissolved in 25ml MeOH. Solutions were made up to 1 litre with deionized water containing Triton B1956 at the rate of 10 g/litre to give a final concentration of 30 mg/l of the active ingredient. Control sprays were prepared by mixing 25 ml MeOH and 975 ml of deionized water with the same concentration of Triton B1956. Klocke and Yamasaki (1991) had previously determined that the relative toxicities of AZA, DAZA and TAZA were the same to three species of insects and therefore all compounds were sprayed at the same concentration.

Figure 1. Stuctures of azadirachtin, dihydroazadirachtin, and tetrahydroazadirachtin showing the sites of hydrogenation.



Azadirachtin Dihydroazadirachtin Tetrahydroazadirachtin

Fifty 3-4 week old potato plants (*Var*: Russet Burbank) were grown individually in 20 litre pots. All shoot terminals were flagged prior to spraying to ensure that when leaf samples were taken for subsequent bioassays no unsprayed leaves were sampled. Ten potato plants per treatment were sprayed once to the point of run-off using a CO_2 backpak sprayer ensuring thorough coverage. After the sprays had dried, the plants were randomized and placed on greenhouse benches set sufficiently apart to prevent leaves of adjacent plants from rubbing each other. In order to get the full spectrum of natural sunlight, the poly covering of the greenhouse roof was removed. Over the 35 day test period, daylength ranged from 14.5 h to 12.7 h. Plants were watered and fertilized as required. Plants were covered and protected from rain as needed.

Sampling of leaves was always done by incising healthy leaves below the flagging tape (approximately 15-20 leaves/treatment/sample date). The leaves were collected and brought to the laboratory for bioassay. Sampling was done on Day 0 (soon after the spray had dried), and on 3,7,10,17,21,28, and 35 days after treatment.

Bioassay

With minimal handling, the leaves were assayed for residual activity using a petri dish bioassay. Up to three potato leaves (4.5 to 5.5g biomass) were placed over two moistened filter papers in a 150mm x 15mm plastic petri dish. Five second instar Colorado potato beetle larvae from a susceptible laboratory colony were transferred into each petri dish and treatments were incubated in a growth chamber at 28° C ($\pm$2) under 14:10 h photoperiod. During incubation, the filter papers were moistened as needed to prevent desiccation of leaves. Mortality was assessed after five days. If a larva did not exhibit movement when prodded with a brush, it was scored as dead. Similar bioassays were set up for each sampling date and percent mortality data were measured. Mortality data in the various treatments were corrected by using Abbott's formula (Abbott, 1925)

ANOVA and Tukey's test of significance for means separation ($p > 0.05$) were performed with Systat software package (Version 5.0). Data were subject to transformation using Y' = square root (Y+0.5). Biological Half Life (BHL) for a given concentration c (in this case, c = 30 mg a.i./l), was calculated by regressing Percent Corrected Mortality (PCM_c, independent variable) with Days Post Treatment (DPT_c, dependent variable) and solving the regression equation with PCM_c = 50: or;

$$DPT_c = (PCM_c * \text{slope}) + \text{constant}$$

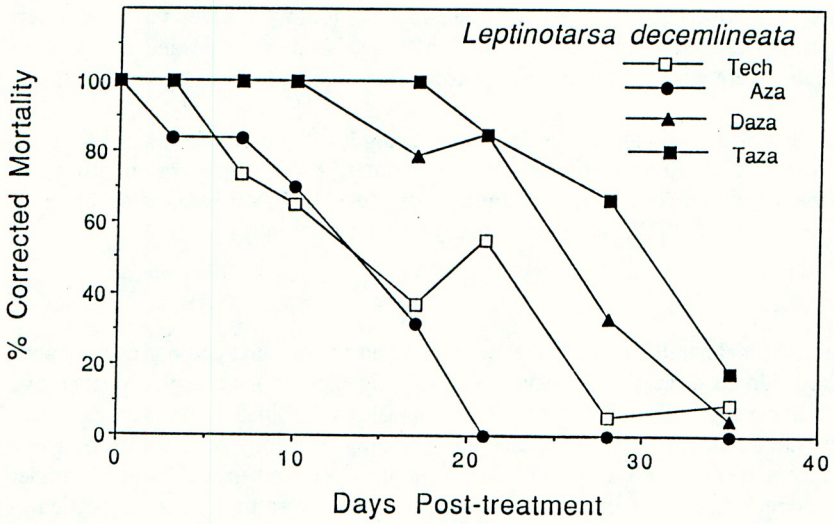
$$BHL_c = (50 * \text{slope}) + \text{constant}$$

By definition, BHL_c will provide an estimate of the residual activity of a given compound at a given concentration because it indicates the number of days required for mortality to decline to 50%.

RESULTS

The results of the experiment are presented in Figure 2 below:

Figure 2. Relative residual activities* of TECH (technical azadirachtin), AZA (pure azadirachtin), DAZA (dihydroazadirachtin) and TAZA (tetrahydroazadirachtin) against second instar Colorado potato beetle on potato.



*All compounds were sprayed at 30 mg a.i./litre.

Table 1. Regression equations, r^2 and Biological half life (BHL_c) values for TECH, AZA, DAZA and TAZA at 30 mg a.i./litre.

Compound	Regression Equation	r^2	BHL_c (Days)
TECH	Y = 32.806 - 0.31480X	0.878	17.1
AZA	Y = 27.667 - 0.27154X	0.894	14.1
DAZA	Y = 36.223 - 0.23241X	0.919	24.6
TAZA	Y = 39.903 - 0.21732X	0.952	29.0

Table 2. Biological activity summary for TECH, AZA, DAZA and TAZA and comparison of statistical differences among the various treatments.

Days after Treatment	Decreasing biological activity* = = = = = = = = = >
0	TAZA = DAZA = TECH = AZA
3	TAZA = DAZA = TECH > AZA
7	TAZA = DAZA ≥ TECH ≥ AZA
10	TAZA = DAZA ≥ TECH ≥ AZA
17	TAZA ≥ DAZA ≥ TECH ≥ AZA (TAZA > TECH) (DAZA > AZA)
21	TAZA ≥ DAZA ≥ TECH > AZA
28	TAZA ≥ DAZA > TECH ≥ AZA
35	TAZA ≥ DAZA ≥ AZA ≥ TECH

* ≥ Indicates a better numerical performance, but no statistically significant difference
> Indicates a better numerical performance and a statistically significant difference
= Indicates a numerically and statistically equal performance

DISCUSSION

Figure 2 and Table 2 show that TAZA and DAZA provided consistently better performance than AZA or TECH. The AZA and TECH performance was generally equal showing that azadirachtin is the key active ingredient in TECH and the other compounds that constitute 84.6% of TECH contribute little to the biological activity. Table 1 also shows the usefulness of calculating Biological Half Life values. The BHC_c values for DAZA and TAZA indicate 1.7x and 2.0x longer residual activity respectively, when compared to the parent molecule AZA.

We propose that BHL_c is a useful index to quantify the relative residual activities for compounds that have similar toxicity profiles at a given concentration. This index could be used effectively in an insecticide screening program to evaluate candidate compounds for optimal UV/sunlight stability in the field.

Azadirachtin has two sites of unsaturation- the 2',3' tigloyl moiety and also at the 22,23 enol-ether double bond on the furan ring (Hansen *et. al.* 1994). Under a set of defined conditions, hydrogenation is first observed at the 22,23 position giving the

dihydro derivative and further hydrogenation results in saturation of the 2'3' site giving tetrahydroazadirachtin. Klocke and Yamasaki (1991) showed that TAZA was the only compound that was resistant to UV degradation up to 400 hours. However, it is clear from this study, that hydrogenation of one site (DAZA) or both sites (TAZA) results in prolonged residual activity. It is possible therefore, that the DAZA metabolites are still biologically active.

The increased residual activity with hydrogenation is significant for commercialization. It has been speculated in the literature that the 22,23 enol-ether double bond may be susceptible to base-meditated hydrolysis (Hansen *et. al.,* 1994), and saturating this site by hydrogenation will also provide increased shelf life stability of formulated products. Ongoing studies indicate that there is merit to this theory (unpublished data). The technology to produce hydrogenated azadirachtin is available and it is anticipated that this technology will play a key role in the manufacture and formulation of a second generation of azadirachtin products in the near future.

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NC-196, A NEW SYSTEMIC JUVENILE HORMONE MIMIC; ITS PRACTICAL PERFORMANCE AS A CONTROL AGENT OF THE BROWN PLANTHOPPER, *NILAPARVATA LUGENS*

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ABSTRACT

NC-196 is a new juvenile hormone mimic(JHM) and is a potent control agent of the brown planthopper, *Nilaparvata lugens*, in rice cultivation. NC-196 shows strong metamorphosis-inhibiting and ovicidal activities against *N. lugens* as a foliar application at extremely low doses (LC_{50}'s are 0.003 mg/l and 0.74 mg/l, respectively). In addition to the contact activity, this chemical is also very active as a soil drench: showing good systemic activity in rice plants. In field trials conducted in southern Japan, granular NC-196 applications at 300 g AI/ha provided perfect suppression of population build-ups of *N. lugens* with its powerful growth-disturbing activity in concert with remarkable systemic characteristics.

INTRODUCTION

In British Crop Protection Conference 1988, Nissan Chemical Industries Ltd. made a presentation on a new JHM, NC-170 (Miyake *et al.*, 1988). Structurally, this chemical is a 2-phenyl pyridazinone derivative and is completely independent of any existing JHM's. Biologically, this chemical is very unique, particularly in its spectrum; it is active only against leafhoppers and planthoppers. Its unprecedented mode of action, outstanding selectivity and environmental safety were attractive for *N. lugens* control, but the practical efficacy was sometimes unsatisfactory. For instance, NC-170 acted rather slowly against field populations of *N. lugens*.

In the course of further researches on this chemical group of pyridazinones, we have discovered two other series of JHM's: one is the 2-haloalkyl pyridazinones (JHM's with a wide range of biological spectrum; Miyake & Ogura, 1992) and the other is the 2-benzyl pyridazinones (Fig. 1). NC-196 is a

representative of the latter group and is a very powerful JHM against hoppers and some lepidopterous insects (Table 1). Comparing its practical performance with that of NC-170, NC-196 appears to compensate for the above-mentioned shortcoming of NC-170 by adding strong ovicidal activity and systemic characteristics on the fundamental metamorphosis-inhibiting activity.

In this paper, we describe the technical properties of NC-196, its biological properties under laboratory and greenhouse conditions, and the corresponding efficacy under field conditions.

Fig. 1. Three series of pyridazinones with JH-like activity

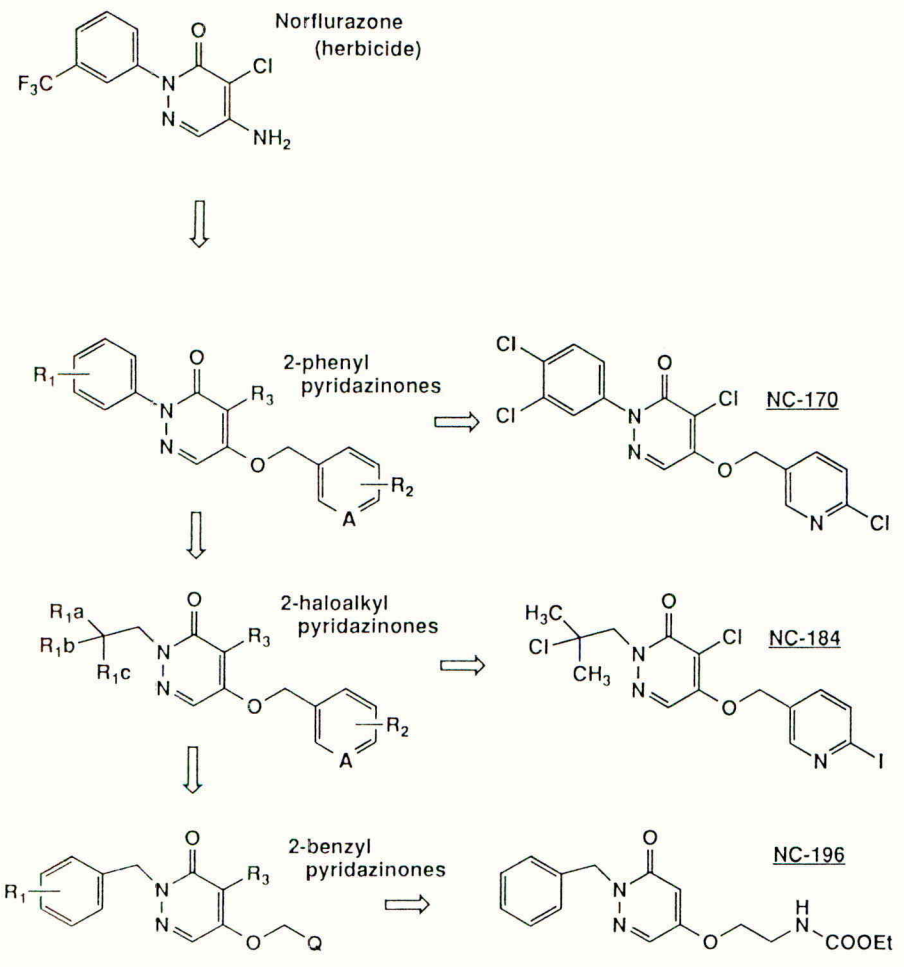


Table 1. An outline of the biological spectrum of NC-196

Order	Species	Metamorphosis-inhibiting acitivity NC-170	NC-196
Lepidoptera	*Spodoptera litura*	X	△
	Plutella xylostella	X	○
Coleoptera	*Tribolium castaneum*	X	△
	Tenebrio molitor	X	X
Hemiptera	*Nilaparvata lugens*	○	○
	Myzus persicae	X	X
Diptera	*Musca domestica*	X	X
	Culex pipiens palens	X	X
Dictyoptera	*Blatella germanica*	X	X

○: strong △: moderate X: weak or no

CHEMICAL AND PHYSICAL PROPERTIES

Code number: NC-196

Structural formula: see Fig.1

Molecular formula: $C_{16}H_{19}N_3O_4$

Chemical name: 2-benzyl-5-[2-(ethoxycarbonylamino)ethoxy]-pyridazin-3(2H)-one

Molecular weight: 317.3

Melting point: 73.5-78.0 °C

TOXICOLOGICAL PROFILE

Acute oral (14 days)

LD_{50} rat: 2322 mg/kg (male), 1296 mg/kg (female)
LD_{50} mouse: 982 mg/kg (male), 1355 mg/kg (female)

Acute dermal (7 days)

LD_{50} rabbit: >5000 mg/kg (female)

Mutagenicity

Ames test: negative
Micro nucleus test: negative
Rec assay: negative

Sub-chronic
(13 weeks)

LC_{50} (in diet) rat: >6000 mg/l

Aquatic organisms

LC_{50} (48 h) carp: >40 mg/l
LC_{50} (48 h) rainbow trout: >40 mg/l
LC_{50} (48 h) Daphnia: >40 mg/l

Irritation

rabbit eye: slight irritant
rabbit skin: not irritant

LABORATORY AND GREENHOUSE PERFORMANCE

Metamorphosis-inhibiting activity and ovicidal activity of NC-196

Juvenile hormone-like activity of NC-196 against *N. lugens* was evaluated in the laboratory and compared with NC-170 (Table 2).

Table 2. JH-like activities of NC-170 and NC-196 against *N. lugens* in two different types of treatment.

	LC_{50} & LD_{50} (AI)			
	metamorphosis-inhibition a)		ovicidal action b)	
	spray	drench c)	spray	drench c)
NC-170	0.075 mg/ l	>1.0 mg	>100 mg/ l	>1.0 mg
NC-196	0.003 mg/ l	0.5 µg	0.74 mg/ l	61.3 µg

a) Fourth stadium nymphs were released on treated rice plants. Abnormal metamorphosis was observed.

b) Fertile adult females were released on treated rice plants and were kept for 24 hr. to lay eggs. Hatchability of the eggs was observed.

c) AI/pot (50 cm^2), 10 µg/pot = 2 g/ha

NC-170 showed strong metamorphosis-inhibiting activity, but no ovicidal activity at practical doses; metamorphosis being the only susceptible stage to this chemical. Insecticides with such stage specific activity need a long time to suppress target populations. In addition, *N. lugens* generally attacks on lower parts of rice plants. Therefore, non-systemic chemicals such as NC-170 could not easily reach the targets when sprayed on canopies of rice plants. These negative aspects of NC-170 made its field performance unsatisfactory.

On the other hand, NC-196 showed not only metamorphosis-inhibiting activity but also ovicidal activity against *N. lugens*; NC-196 can break the life

cycle at these two different stages. This dual action may provide rather quicker population-suppressing activity in fields compared with NC-170. In addition, NC-196 showed strong systemic activity, which makes this chemical appropriate for granular application and may help the active ingredient to reach the target insects more efficiently, enhancing its growth-disturbing activity.

Residual activity of NC-196 under greenhouse conditions

Granular formulations of NC-196 provided more than three-month long persistence of metamorphosis-inhibiting activity and about four-week long persistence of ovicidal activity at doses corresponding to 1 g AI/ha and 60 g AI/ha, respectively (Table 3). Although they were obtained under closed and small-scale conditions, these results strongly suggest that granular NC-196 applications on surface water in irrigated paddies could disturb the development of *N. lugens* for a very long time.

Table 3. Residual activity of NC-196 under greenhouse conditions

Test 1. Metamorphosis-inhibiting activity

	Metamorphosis inhibition (%)					
doses (AI)[a]	7 WAT[b]	8 WAT	9 WAT	10 WAT	11 WAT	12 WAT
10.0 µg/pot	100	100	100	100	100	100
5.0 µg/pot	100	100	100	72.7	100	60.0
2.5 µg/pot	97.5	100	69.5	75.8	34.1	12.1
untreated check	0	0	0	0	0	0

a) 10 µg/pot=1 g/ha
b) WAT: weeks after treatment

Test 2. Ovicidal activity

	Unhatched eggs (%)				
doses (AI)[a]	7 DAT[b]	14 DAT	21 DAT	28 DAT	35 DAT
0.9 mg/pot	100	100	100	92.8	54.7
0.6 mg/pot	100	100	94.2	80.9	22.2
0.3 mg/pot	94.0	95.0	88.3	47.4	20.0
untreated check	34.5	24.0	19.5	33.3	27.9

a) 0.1 mg/pot = 10 g/ha
b) DAT: days after treatment

Pots of 100 cm^2 with one month-old rice plants were flooded to a water depth of 4 cm. NC-196 was formulated into 0.5-2 % granules and applied to the surface water of the pots

FIELD PERFORMANCE

Residual activity of two different formulations of NC-196 under field conditions

As mentioned previously, foliar applications of insecticides for *N. lugens* control have some disadvantages: 1) chemicals do not easily reach the target insects and eggs particularly when the canopies have developed intensively, 2) foliar applications require intensive labour and some machinery, 3) foliar applications often result in chemical drifts and waste, etc. If a chemical has systemic activity, most of these problems would be solved with a surface water application in irrigated paddies.

We formulated NC-196 into "dust" for foliar applications and "granules" for surface water applications and then evaluated the ovicidal activity provided by these two different formulations under field conditions (Table 4). The results showed that the granules have much better residual ovicidal activity than the dust, probably because the active ingredient treated as granules reached the target eggs more efficiently through the rice roots.

Table 4. Residual activity of two different formulations of NC-196 under field conditions

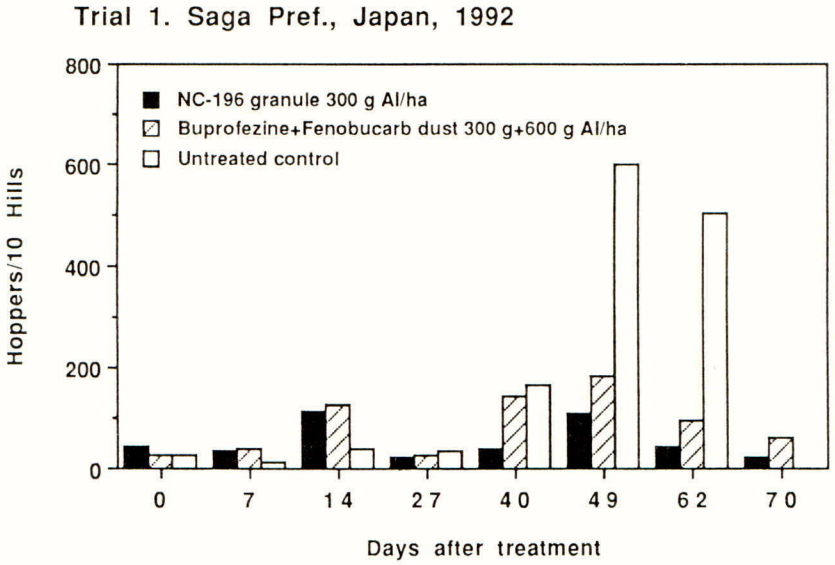
		Unhatched eggs (%)				
		7 DAT	14 DAT	21 DAT	28 DAT	35 DAT
2 % granule	600 g AI/ha	95.4	100	98.2	89.2	44.9
2 % dust	600 g AI/ha	96.8	74.2	26.4	45.5	18.8
Untreated check		20.8	15.7	33.3	40.0	25.8

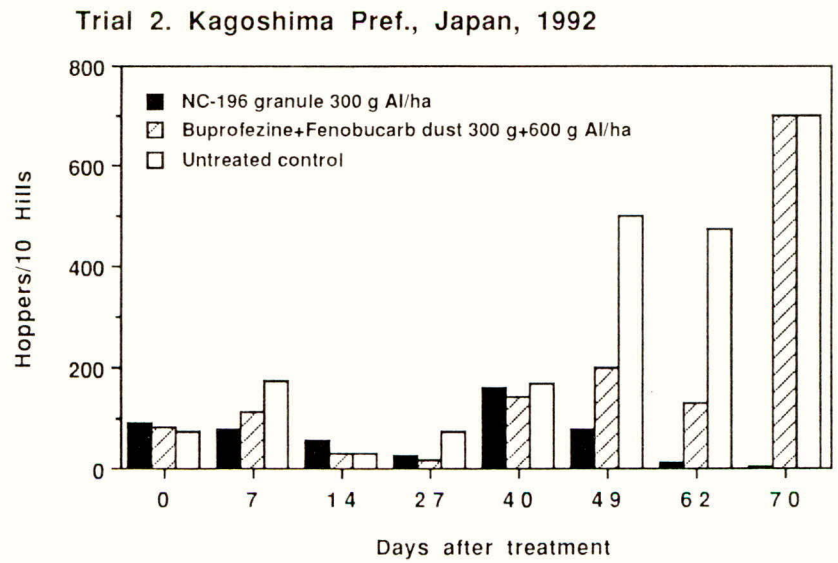
Fixed days after treatment, rice plants were brought from each plot to a greenhouse and replanted in pots. Fertile females were released on them for 24 hr. to lay eggs. The hatchability of the eggs on each rice plant was then observed.

Efficacy of NC-196 against naturally-occurring field populations of *N. lugens*

The brown planthopper, *N. lugens*, is a pest with a unique ecology as is known well: it cannot overwinter in temperate regions such as Japan, but immigrate from tropical and sub-tropical regions of southeast Asia in early summer. Although the number of immigrating insects is small, they often build up damaging population after 3 generations. In this case, the "phase" of the field populations changes from solitary to gregarious (from brachypterous to macropterous wing-forms) in the 3rd generation and thereafter the insects leave paddy fields (Kuno, 1979). On the other hand, we sometimes observe another pattern of population growth of *N. lugens* in Japan, in which the change of "phase" occurs in the 2nd generation, followed by drastic decrease of population density in the 3rd generation. We evaluated practical performance of NC-196 under these two different types of pest pressure.

Fig. 2. Field trials of NC-196 against *N. lugens*





In trial 1, the hopper population showed "growth for 2 generations" and the pest pressure was not so serious after all. A combination of buprofezine and fenobucarb showed good efficacy by only single treatment (Fig. 2-1). But in trial 2, the hopper population showed "growth for 3 generations" and caused severe "hopper-burn" in the end. Single application of the standard insecticide could not suppress the heavy outbreak in the 3rd generation probably because of the lack of sufficient residual activity (Fig. 2-2).

However, NC-196 successfully suppressed the population growth in the 2nd and 3rd generations of both trials, and no hopper-burn was observed.

CONCLUSIONS

We have conducted the study on JH-like activity of pyridazinone derivatives based on the following three understandings.

1) The most remarkable feature of this chemical group is their strong activity against *N. lugens*, because this insect is ecologically one of the promising targets for JHM's among economically important pests in agriculture.

2) JHM's with only metamorphosis-inhibiting activity are inherently very slow-acting. Therefore, discovering ovicidal activity as an additive must be a key to make the performance of the pyridazinones practically acceptable.

3) Considering the ecological features of *N. lugens*, systemic activity should be an important feature for control agents.

The resulting NC-196 showed excellent control activity of *N. lugens* in a number of field trials in addition to those described here and its efficacy was very stable even under high pest pressure. These results, combined with its new mode of action, the low mammalian toxicity and the minimal adverse effect against aquatic organisms, strongly emphasize the potential of the chemical for future *N. lugens* control.

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EXTRUDED STARCH CONTACT BAITS FOR THE FORMULATION OF GRASSHOPPER AND LOCUST ENTOMOPATHOGENS

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ABSTRACT

A contact bait formulation has been developed that uses aspects of feeding behaviour to achieve transfer of toxicants by prolonged contact with the bait, rather than by ingestion. A rapid, continuous and efficient production of bait carrier was achieved using a co-rotating, twin-screw food extruder. Extrusion conditions were adjusted to produce a rigid, hard and highly expanded starch extrudate. Laboratory behavioural bioassay demonstrated a high degree of acceptance of the baits by *Schistocerca gregaria* and bait shape characteristics were optimised to prolong contact. The encapsulation of sucrose during extrusion increased bait contact time. Laboratory and field bioassays demonstrated prolonged efficacy and improved weathering characteristics of extruded maize-starch contact baits relative to conventional bran-bait formulations. This formulation method may be particularly appropriate to fungal biopesticides and the incorporation of conidial suspensions of *Metarhizium flavoviride* in vegetable oil into the contact baits resulted in improved dose-mortality characteristics relative to formulation in conventional baits.

INTRODUCTION

Various carriers have been used for insecticide formulation and McGuire and Shasha (1992) classified these carriers as baits upon which the insect must feed, or inert particles which carry the active ingredient to the target site and then depend on environmental factors to release it into the feeding zone. The requirements of ingestion and of protection against adverse environmental conditions favour the formulation of chemical and many biological pesticides in baits, which are also far more efficient in terms of dose transfer than spray formulations. Bait formulations of microsporidian pathogens (*Nosema locustae*), nematodes, fungi and entomopoxviruses for grasshopper and locust control were reviewed by Caudwell (1993), and the need for further research into carrier characteristics and the use of attractants and phagostimulants was stressed.

A number of control agents have previously been encapsulated within starch matrices and successful techniques include starch retrogradation. This involves gelatinization of starch, blending of the material to be encapsulated into it, and natural retrogradation as the mixture cools and dries. Dunkle and Shasha (1988) developed a granular formulation for *Bacillus thuringiensis* Berliner using a starch retrogradation technique and McGuire *et al.*(1991) evaluated the potential for using starch retrogradation methodology for bait

formulations of an entomopoxvirus isolated from *Melanoplus sanguinipes*. Various phagostimulants and ultra-violet screens were added to enhance virus survival and to increase the degree of acceptance of the starch bait by grasshoppers. This technique is attractive for encapsulating a variety of control agents because the procedure is simple and economical, and eliminates the use of cross-linking chemicals. Carr *et al.* (1993) however stressed that many starch encapsulation techniques had a number of disadvantages, including problems associated with processing. They suggested that starch encapsulation could be accomplished rapidly, efficiently and continuously over a wide range of conditions using a co-rotating, fully intermeshing, twin-screw extruder. This paper reports an investigation into the use of starch- extrusion technology for the production of bait carriers for the formulation of grasshopper and locust entomopathogens.

MATERIALS AND METHODS

Bait production

A Clextral BC21 twin-screw extruder located at the Department of Applied Biochemistry and Food Science, Nottingham University (Sutton Bonington, UK) was used for all development work. The processing section of the extruder consisted of 8 individual barrel sections. Each barrel section had an electric heater and temperature thermocouples were located at intervals throughout the barrel. The temperature within the barrel could be regulated. Two screw units were used to feed, convey, mix and pump the raw material within the extruder barrel. For the development work, the die-head assembly was fitted with a number of dies of different shapes and sizes, and various starch-based materials were fed from a hopper into the extruder by a twin-screw volumetric feeder. Water was injected by a reciprocal co-rotating piston pump. Screw speed, motor torque, motor pressure, feeder rate, back-pressure, feed moisture content and thermic profile were measured and adjusted. The levels and combinations of these operating and processing variables were adjusted and optimised for each extrusion batch.

The mechanical properties of extruded starch rendered feeding by acridoids impossible and development work therefore focused on the use of starch extrusion technology for the production of 'contact baits'. This type of formulation is defined as a bait that utilises aspects of feeding (or other) behaviour for the purpose of attraction and stimulation of the target species to maximise contact with the bait, achieving uptake of the active ingredient by contact rather than ingestion. Extrusion operating and processing variables were therefore manipulated to produce rigid, hard and expanded 'contact baits'. High temperatures and low moisture contents were required to produce highly expanded extrudates.

The extrusion process was run at high temperatures and low feeder moisture contents, using maize grits (90% starch, 8-9% protein and 1% oil. Maizecor Foods Ltd, Hull, UK) with the extruder fitted with a 2 mm ribbon die, to produce expanded strips of material (35 mm wide and 4 mm thick). The maize grits were mixed with sucrose before extrusion

(5, 10, or 20% dry weight) and the operating and processing variables adjusted to produce a rigid, hard and highly expanded extrudate.

Separate trials were undertaken to produce baits with different shape characteristics. The previous trial demonstrated that sucrose was phagostimulatory at 5% dw, and maize grits were therefore extruded with sucrose (5% dw) using a ribbon, star and wheel die to produce extruded strips, and extrudates which were star-shaped and cylindrical in cross-section.

Behavioural bioassay

A flat-bed wind tunnel was developed as a behavioural bioassay system to determine the acceptability of candidate extruded contact-bait carriers. Groups of 10 fifth instar *Schistocerca gregaria* were subjected to 2 h of food deprivation and excited by 45 s of tumbling before release into the system. Pieces of each material (0.5 g) were presented using five feeding stations equidistantly positioned within a bioassay area in the centre of the upwind end of the working section. Separate releases were undertaken for each material and the times spent in contact with it by all 10 test insects at all 5 feeding stations were summed to give the total contact time; this was defined as one replicate. Four replicates were undertaken for each material and one-way ANOVA, with Tukey multiple comparison tests, was used to assess differences in contact times.

Infectivity bioassay

The porosity resulting from the expansion and swelling of granular starch during extrusion allowed vegetable oils to be absorbed into and retained within extruded maize-starch contact baits. Wetting of the baits in the absence of oil resulted in swelling of the starch and disintegration of the bait matrix. The presence of oil on the surface of the bait repelled water and therefore enhanced bait weathering and structural characteristics. The incorporation of vegetable oils into the contact bait matrix allowed for the introduction of volatile attractants and non-volatile phagostimulants to increase the diameter of the active space of the bait and to prolong bait contact. Candidate control agents were also incorporated in this manner.

The suspension of biopesticides, particularly infective fungal conidia, into vegetable oils enhances fungal efficacy at low humidities (Bateman *et al.*, 1993) and reduces the effect of UV on them (Moore *et al.*, 1993). Oil-based suspensions of *Metarhizium flavoviride* conidia were incorporated into the extruded maize-starch contact baits after extrusion and their toxicological characteristics assessed. Infective conidia of *M. flavoviride* (isolate IMI 330189), cultured on malt extract agar at 25°C were supplied by the International Institute for Biological Control and suspended in cottonseed oil.

Concentrations of $2.5x10^6$, $2.5x10^7$, $2.5x10^8$, $2.5x10^9$ and $2.5x10^{12}$ spores/l were made up and 3ml applied to each maize-starch contact bait by pipette. A control consisted of maize-starch baits with cottonseed oil but no conidia. Oil-based conidial suspensions of $2.5x10^{12}$ spores/l were also incorporated into 1g batches of wheat-bran edible baits. The

maize-starch contact bait and wheat-bran edible bait formulations of *M. flavoviride* conidia were presented to groups of 30 fifth instar *Schistocerca gregaria* within rectangular perspex boxes. The test insects were deprived of food for 2 h prior to testing and one piece of bait (0.5 g) was placed inside each of the boxes with one insect and left for 2 h. The test insects were then fed on fresh wheat seedlings and mortality was assessed daily for 14 d after infection. Probit analysis was used to determine the dose-mortality characteristics of the maize-starch bait and the Chi square statistic used to test for differences in mortality between the maize-starch bait and wheat-bran bait.

RESULTS

The extrusion unit parameters used for the production of contact baits using maize grits mixed with various levels of sucrose are shown in Table 1. Successful extrusion was achieved with zero feed moisture content and relatively high temperatures to produce highly expanded extrudates. Using these unit parameters continuous pseudoplastic flow was achieved in the extruder barrel, blockage did not occur, and extrusion was fast and efficient. The extrusion unit parameters used for the production of contact baits with maize grits and 5% sucrose using different die types are shown in Table 2. Successful extrusion was again achieved with zero feeder moisture contents and high barrel temperatures to produce highly expanded extrudates. The results of the behavioural bioassay for acceptance of these extruded materials are shown in Figs 1 and 2. The incorporation of sucrose into maize grits resulted in a significantly greater mean contact time relative to maize grits alone, but there were no significant differences between the

Table 1. Extrusion unit parameters used for the production of contact baits with maize grits mixed with sucrose (5%, 10% or 20% dry weight).

Extrusion unit parameters	Raw material			
	Maize grits	**Maize grits + 5% sucrose**	**Maize grits + 10% sucrose**	**Maize grits + 20% sucrose**
Screw speed (mn^{-1})	250	250	300	252
Torque (Nm)	25.6	24.7	20.3	22.3
Motor pressure (W)	1375	1324	1312	1124
Feeder rate (kg/h)	5.98	5.98	5.98	5.98
Back pressure (Bar)	183	178	140	150
Feed moisture content (l/h)	0	0	0	0
Thermic profile Zone 1(°C)	38	39	39	40
Zone 2(°C)	70	69	69	69
Zone 3(°C)	99	99	99	98
Zone 4(°C)	139	133	133	154

(Use was made of a Clextral BC21 twin-screw extruder fitted with a 2mm ribbon die)

different levels of sucrose tested ($F = 80.60$, treatment $df = 3$, residual $df = 12$, $P < 0.001$***). Baits produced using the ribbon die gave significantly greater contact times than those produced using the star or wheel die ($F = 119.83$, treatment $df = 2$, residual $df = 9$, $P < 0.001$***).

Table 2. Extrusion unit parameters used for the production of contact baits with maize grits mixed with sucrose (5% dry weight).

Extrusion unit parameters	Die type		
	Ribbon	**Wheel**	**Star**
Screw speed (mn^{-1})	250	300	300
Torque (Nm)	24.7	22.6	21.5
Motor pressure (W)	1324	1419	1350
Feeder rate (kg/h)	5.98	10.95	12.0
Back pressure (Bar)	178	62	45
Feed moisture content (l/h)	0	0	0
Thermic profile Zone 1(°C)	39	40	38
Zone 2(°C)	69	68	70
Zone 3(°C)	99	98	128
Zone 4(°C)	133	142	147

Figure 1. Mean contact times (s) for maize-grit contact baits with sucrose (5%, 10% and 20% dry weight) for test groups of 10, fifth instar *Schistocerca gregaria* in 30 min bioassays (n = 4).

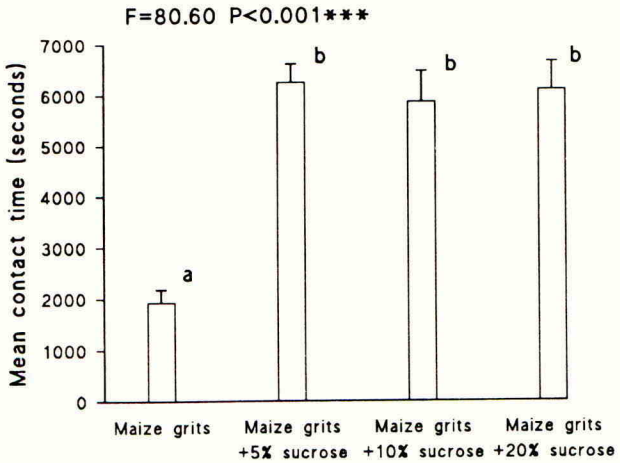
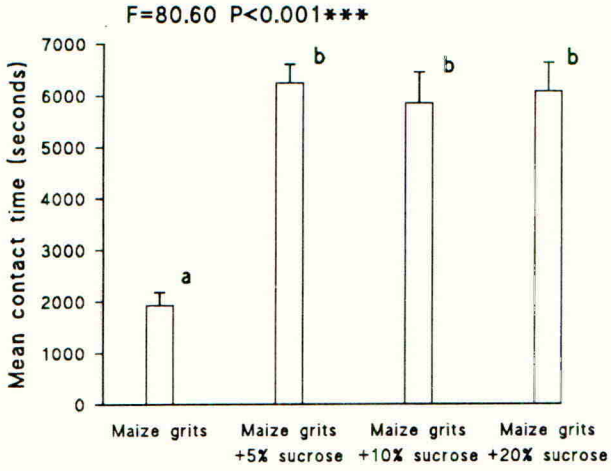


Figure 2. Mean contact times (s) for maize-grit contact baits with sucrose (5% dry weight) extruded using three different die shapes for test groups of 10, fifth instar *Schistocerca gregaria* in 30 min bioassays (n=4).



The dose-mortality characteristics for maize-starch contact bait formulation of *M. flavoviride* are shown in Table 3. Probit analysis resulted in a significant positive regression of probit mortality against log concentration and gave an LD_{50} of $1.5x10^8$ spores/l (slope=0.63). In the comparative trial, maize-starch contact bait formulations of *M. flavoviride* ($2.5x10^{12}$ spores/l) resulted in a 14 d mortality of 83.3% against groups of 30 fifth instar *S. gregaria*. Wheat-bran edible bait formulations gave a 14 d mortality of 56.7%, contact-baits therefore resulted in a significantly greater mortality after 14 d ($\chi^2=5.079$, df=1, $P<0.05$*).

Table 3. Dose-mortality characteristics for maize-starch contact bait formulation of *Metarhizium flavoviride*.

Concentration spores/l	14 day mortality
0	1
$2.5x10^6$	6
$2.5x10^7$	10
$2.5x10^8$	19
$2.5x10^9$	24
$2.5x10^{12}$	30

Probit regression analysis $LD_{50}=1.05x10^8$ spores/l (Slope=0.63)
Groups of 30 mid-fifth instar *Schistocerca gregaria* were exposed to 0.5 g pieces of bait for 2 h within bioassay boxes and mortality assessed over 14 d.

DISCUSSION

Contact 'baits' in the form of insecticide-impregnated cloth targets have been used in applied entomology, particularly for tsetse control (Vale, 1993). Bait formulations that achieve dose-transfer by contact rather than ingestion have not however been considered for grasshopper and locust control, and this approach may be particularly appropriate for the field delivery of fungal biopesticides that infect by contact rather than ingestion. Non-volatile phagostimulants and water-based solutions of control agents can be incorporated into the baits during extrusion. For example, Carr *et al.* (1993) encapsulated the herbicide atrazine within maize grits during extrusion at temperatures up to 100°C with no subsequent loss of activity. Alternatively vegetable oil solutions or suspensions of behaviour-modifying chemicals and control agents can be incorporated into the baits after extrusion.

Grasshopper and locust baits could be made more effective by increasing the efficiency of individual particles and by reducing rates of application. The optimal-size characteristics for baits in the field is a function of the diameter of the active space of each bait particle and will also depend upon logistical considerations. Using bait particles with a large active space, resulting from increased visual apparency and/or the incorporation of volatile attractants, may reduce application rates. For example, Boppre and Fischer (1993) reported that *Zonocerus elegans* was attracted to pure pyrrolizidine alkaloids and these strong and species-specific attractants could be used in baits for the control of this species.

Applied insect pathology has a long history of largely unfulfilled potential, and this is probably because previous work has concentrated on the active ingredient, the pathogen. But enhanced shelf-life, persistence, efficacy and field-targeting of biopesticides can only be achieved by improved formulation. The use of starch-extrusion technology for contact-bait formulation of biopesticides has not been considered in this context and therefore represents an innovative approach. In a separate study in the USA, the feasibility of the use of starch extrusion for the formulation of herbicides has been investigated. Patents are currently being sought in the USA for the use of the twin-screw extrusion process for this purpose and several agrochemical companies have applied for licences to use the system (Carr *et al.*, 1993). However its use for the delivery of insecticides or entomopathogens has not previously been proposed. Detailed toxicological investigations of extruded maize-starch contact baits have been undertaken in our laboratory and the performance of the baits assessed in field trials against grasshoppers in West Africa (Caudwell, 1994).

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SZI 121 - CHEMICAL AND BIOLOGICAL EVALUATION OF A NEW ACARICIDE

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ABSTRACT

SZI-121, a tetrazine type acaricide, has been improved by Chinoin. The compound is very active against phytophagous mites and has good toxicological and environmental properties. The chemical structure was designed to get improvements in mode of action and efficacy simultaneously. SZI-121 acts not only as a contact ovicide but due to very good translaminar activity it can enter mites via ingestion. SZI-121 possesses transovarian activity and it is also able to stop the development of mites at chrysalis stages. Thus it can be used with good results against both overwintering and summer eggs as well as populations.

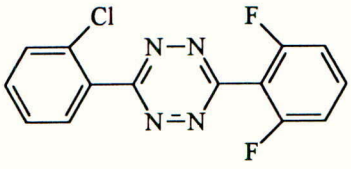
In field trials SZI-121 provides long lasting control at application rates as low as 80 g Al/ha against *Panonychus, Tetranychus , Aculus and Calipitrimerus* spp. in apple and vine. It is harmless to beneficial insects and has low impact on predatory mites. SZI-121 can be easily fitted into integrated mite management.

INTRODUCTION

Because of the growing economic importance of phytophagous mites new potent and selective acaricides are continuously needed for mite management. The tetrazine type acaricides having specific ovicide action seems to meet these requirements. For this reason a large number of structure-analogues have been synthesised and patented (EP 0 005912, EP 0 029657, EP 0 248 466). One of these is the 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine which is marketed under the common name of clofentezine. From an efficacy point of view the major limitation with clofentezine is the lack of translaminar and transovarian activity at normal application rate (Brooker *et al.*, 1987). Moreover it has no vapour effect.

In the late 1980s Chinoin began synthetic and biological research directed at overcoming the major limitations with clofentezine. Large numbers of structure analogues have been synthesised and screened to clarify the structure-activity relationship. We recognised that the asymmetric halophenyl substitutions in the 3 and 6 position of tetrazines offer higher activity than any other symmetric ones. The results suggested that the 2,6-difluoro substitution is essential for improvement of the monohalo-substituted tetrazines such as clofentezine. From the large number of synthetised derivates a difluoro-analogue, SZI-121, was chosen for further development. This paper describes the biological activity of SZI-121.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name:	3-(2-chlorophenyl)-6-(2,6-difluorophenyl)- -1,2,4,5-tetrazine CA: 1,2,4,5-Tetrazine., 3-(2-chlorophenyl)-6- (2,6-difluorophenyl)
Empirical formula:	$C_{14}H_7N_4ClF_2$
Molecular weight:	304.7
Structural formula:	

Appearance:	magenta crystal	
Melting point:	183-185°C	
Solubility (25°C):	water	<10 mg/l
	acetone	21.4 g/l
	chloroform	50.0 g/l
	hexane	0.3 g/l
	acetonitrile	1.0 g/l
	hexane	0.3 g/l
Stability:	stable to light and air; stable under acidic conditions, but hydrolysed at pH>7	
Formulation:	20 % water based suspension concentrate particle distribution: 98% < 5 µm;	

TOXICOLOGY

Acute oral toxicity study in rats	male	: 979.09 mg/kg
	female	: 594.02 mg/kg
	male + female	: 752.67 mg/kg
Acute dermal toxicity study in rats	male	: >2000 mg/kg
	female	: >2000 mg/kg
Acute eye irritation study in rabbits		: the highest irritation index was 15
Acute dermal irritation study in rabbits		: combined irritation index was zero
Acute inhalation study in rats	male	: >5000 mg/m³
	female	: >5000 mg/m³
Skin sensitization study in guinea pigs		: not sensitizer
28 days repeated dose oral toxicity study in rats NOEL		: 50 mg/kg/day
28 days repeated dose dermal toxicity study in rats N0EL		: 500 mg/kg
Micronucleus test		: inactive
AMES test		: inactive

LABORATORY EVALUATION

Methods

A sensitive strain of two-spotted spider mite *(Tetranychus urticae)* was used for testing. The strain originated from the Plant Protection Institute, Hungarian Academy of Science, Budapest and has been reared in our laboratory for 6 years. Clofentezine and hexythiazox were obtained from Riedel-de Haen.

1. Contact ovicidal activity by dipping

French bean *(Phaseolus* sp.) leaf discs of 15 mm diameter infested with 0-24 h-old eggs were dipped into test solutions for a period of 5 s. For active ingredients acetone was used as solvent. The treated discs were kept under controlled conditions of 25±2° C, 60 % RH, and a photoperiod of 14:10 (L:D) h. Moisture was provided through filter paper. The control was treated with water and/or solvent only. Egg hatching was assessed after 7 days.

2. Contact ovicidal activity by Potter-tower

Leaves infested with 0-24-h-old eggs were sprayed by Potter-tower (Potter, 1952). Post-treatment conditions and assessment were the same as used for the dip method.

3. Translaminar effect on adult ability to lay viable eggs

Upper surface of leaves was treated with appropriate test solution for 5 s. After 24 h incubation the lower surface of leaves were infested with female mites to lay eggs for a period of 24 h. Post-treatment conditions and assessments were the same as used for the dip method.

4. Translaminar effect on egg hatch

Upper surface of leaves was treated with appropriate test solution for 5 s. After 24 h incubation the lower surface of leaves were infested with 0-24 h-old eggs. Post-treatment conditions and assessment were the same as used for the dip method.

5. Sterilant/transovarian activity

Female mites fed on treated leaves for 48 h were transferred to untreated leaf discs to lay eggs. Post-treatment conditions and assessment were the same as used for the dip method.

6. Effect on chrysalis stage

Treated leaf discs were infested with just hatched larvae. The motile and chrysalis forms were recorded every day until the 95% of control group had become adults. Post-treatment conditions and assessments were the same as used for the dip method. Total mortality was expressed as a proportion of mites which died in chrysalis form.

7. Effect via vapour action

Untreated leaves infested with 0-24 h-old eggs were kept at a distance of 55 mm from the test solutions containing 100 mg/l active ingredient. Post-treatment conditions and assessments were the same as used for the dip method.

8. Persistence

Leaves of intact plants were treated by dipping for 5 s with tests solutions. Leaf discs were cut from leaves weekly and infested with adult female mites to lay eggs for a period of 24 h. Post-treatment conditions and assessments were the same as used for the dip method.

Results

The results of laboratory studies demonstrate that SZI-121 compared to clofentezine has higher inherent ovicidal activity both as the active ingredient (Table 1. Method 1) and in formulation (Table 2. Methods I and 2). Similarly SZI-121 is more effective on chrysalis forms than clofentezine (Table 2. Method 6). The Methods 3, 4 and 5 reveal unique differences between clofentezine and SZI-121. While clofentezine has no translaminar (Methods 3 and 4) and sterilant (Method 5) activity in the highest tested concentration (500 mg/1), the activity of SZI-121 is excellent via these routes (Table 2.). Longer residual activity (Method 8) was also observed for SZI-121.

TABLE 1. Activity of SZI-121 compared with clofentezine. Tests with active ingredients.

Exposure technique	Mite stage exposed	clofentezine	SZI-121
		LC50 (mg AI/l)	
1. Leaf dipping	eggs	0.23	0.11
3. Translaminar effect	adults	>500	9.23
5. Transovarian effect	adults	>500	50

TABLE 2. Activity of SZI-121 compared with clofentezine. Tests with formulations.

Exposure technique	Mite stage exposed	clofentezine 500 SC	SZI-121 200 SC
		LC50 (mg AI/l)	
1. Leaf dipping	eggs	0.23	0.05
2. Spraying by Potter	eggs	7.25	1.87
3. Translaminar effect	adults	>500	5.11
4. Translaminar effect	eggs	>500	18.66
5. Transovarian effect	adults	>500	≈10
6. Effect on chrysalis stage	larvae	>20	0.39
8. Persistence	adults	>20	≈2*

* mortality > 80 % two weeks after treatment

An other outstanding difference between clofentezine and SZI-121 is illustrated by the effect via vapour (Method 7). Contrary to both clofentezine and hexythiazox, the SZI-121 has excellent efficacy via vapour (Table 3.).

TABLE 3. Activity of SZI- 1 21 via vapour action on inhibition of egg hatch

Formulation	Hatching inhibition	
	%*	SEM**
clofentezine 500 SC	13.1	5.5
hexythiazox 10 WP	11.2	2.1
SZI-121 200 SC	91.2	9.4

* average of three experiments

* * standard error of mean

FIELD EVALUATION

Small and large plot field trials were conducted with 200 g/l SC formulation of SZI-121 in apple and vine. Two timings of treatments, spring and summer were made.

Results

The results of field studies are presented in Tables 4-8. The efficacy of SZI-121 at 60 g Al/ha was higher than that of the label rates of clofentezine and hexythiazox in spring treatments. Comparing to the conventional adult acaricides, such as amitraz and propargite, SZI-121 showed the same or higher efficacy in summer treatments. During two years of field tests SZI-121 has not shown any adverse effect on bees, beneficial insects or predatory mites.

Spring treatment

TABLE 4. Control of overwintering eggs of *Panonychus ulmi* on vine in Gyor-Sopron-Moson, 1994

	Dose	Number of mites/leaf DAT		
Chemical	(mg Al/1)	0	14	28
clofentezine 500 SC	250	15.9	7.2	7.1
hexythiazox 10 WP	50	15.8	3.8	4.7
SZI-121 200 SC	60	14.2	1.6	1.7
SZI-121 200 SC	100	16.7	1.0	1.2
untreated		15.5	16.0	17.9

Treatment: April 27 (small leaf stage); spray volume: 1000 1/ha; plot size: 0.12 ha; replicates: 2.

TABLE 5. Control of *Tetranychus urticae* on apple in Gyor-Sopron-Moson, 1994

	Dose	Number of mites/leaf - DAT		
Chemical	(mg Al/1)	0	14	28
clofentezine 500 SC	250	23.4	6.8	8.2
hexythiazox 10 WP	50	22.8	7.6	9.7
SZI-121 200 SC	60	24.2	1.1	1.7
SZI-121 200 SC	100	24.7	0.4	0.7
untreated		22.3	28.0	23.4

Treatment: April 20 (mouse-ear stage); spray volume: 1000 1/ha; plot size: 0.3 ha; replicates: 2.

TABLE 6. Control of *Calipitrimerus vitis* on vine in Veszprem, 1994

Chemical	Dose (mg AI/1)	Number of mites/leaf 14 DAT
clofentezine 500 SC	250	13.0
pyridaben 20 WP	200	28.0
SZI-121 200 SC	80	0.0
untreated		146.5

Treatment: May 2 (2-3 leaf stage); spray volume: I 000 1/ha; plot size: 0. I ha; replicates: 2.

Summer treatment

TABLE 7. Control of *Tetranychus urticae* on vine in Gyor-Sopron-Moson, 1993

	Dose	Number of mites/leaf - DAT				
Chemical	(mg Al/1)	0	7	14	21	28
clofentezine 500 SC	200	17.9	7.9	8.6	8.9	10.9
SZI-121 200 SC	80	17.4	1.7	1.9	3.5	5.0
SZI-121 200 SC	100	17.8	0.7	1.0	1.4	2.6
untreated		17.1	17.3	17.0	17.8	16.8

Treatment: August 26 (ripening); spray volume: 1000 1/ha; plot size: 10 stock; replicates: 4.

TABLE 8. Control of *Aculus schlechtendali* on apple in Zala, 1994

	Dose	Number of mites/leaf - DAT			
Chemical	(mg Al/1)	0	4	14	21
propargite 570 E	1140	76.9	4.0	5.1	16.2
amitraz 20 EC	1000	74.5	6.9	9.9	8.7
SZI-121 200 SC	100	76.1	8.1	4.7	7.9
untreated		75.4	85.1	95.0	104.4

Treatment: June 20; spray volume: 1000 1/ha; plot size: 0.1 ha; replicates: 2.

RESIDUE ANALYSIS

Residues were determined in grape and apple samples by HPLC after second (summer) treatments with a dose of 0.6 l/ha SZI-121 200 SC. The average recovery and the limit of determination were 91.9 % and 0.02 mg/kg, respectively.