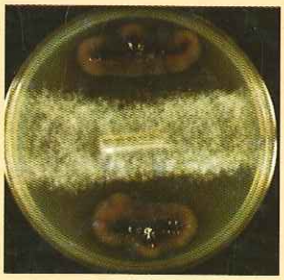
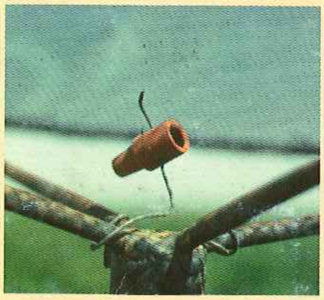
Workshop on
Semiochemicals and Microbial Antagonists: Their Role in Integrated Pest Management in Latin America





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MISTRA

Semiochemicals and Microbial Antagonists: Their Role in Integrated Pest Management in Latin America

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Compiler

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THE ROYAL SWEDISH ACADEMY OF AGRICULTURE AND FORESTRY

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Foreword

The *general background* to this workshop has to do with the need to reduce the use of chemical pesticides and other biocides in agriculture and forestry in Latin America. This need is related to environmental, food quality and human health, as well as to economic factors. More specifically, the workshop addresses the potential of making use of *semiochemicals* and *microbial antagonists* in integrated pest management (IPM). Although this potential is already well proven, the development and discovery of new and innovative ways of using such substances have increased dramatically with recent advances in genetics and biosciences.

The workshop is *organised jointly* by the *Centro Agronómico Tropical de Investigación y Enseñanza* (CATIE) in Costa Rica, the *International Foundation for Science* (IFS), and the *Royal Swedish Academy of Agriculture and Forestry* (KSLA). The initiative to organise the workshop came out of several institutional and personal linkages existing between people associated with these three organisations and with an interest in the subject area of the meeting. The workshop is funded by the *Swedish Foundation for Strategic Environment Research* (MISTRA).

CATIE is a regional research and higher education centre of excellence in the fields of agriculture, agroforestry and forestry. It has a leading position in integrated pest management in the American tropics. Its work includes studies and technology developments using pheromones, repellents, microbial antagonism, and other relevant approaches. In its recently developed strategy for the period 2003-2012, even larger emphasis is put on developing such technologies for use in small scale, market-oriented agriculture and forestry, e.g. with a focus on fruits, vegetables, organic coffee and cacao, certified timber, and other potential crops for the export market. In addressing these research and capacity building tasks, CATIE's strategy also envisages a considerable expansion of partnerships with other research and education institutions, both in the region and in Europe and North America, as well as with the private sector.

In Sweden, several major long-term research programmes addressing these potentials from a Swedish point of view are financially supported by MISTRA. The two most directly relevant programmes, both involving dozens of scientists from several institutions, are *"Pheromones and Kairomones"* and *"Microbial Antagonism against Fungi"*. For many decades, KSLA has consistently promoted and supported such work and its application in Swedish agriculture, horticulture and forestry. Today, the Academy plays an active role in the governance and supervision of the MISTRA programmes and is also taking on an active role in promoting collaboration between Swedish scientists and colleagues in other countries.

In 1996, IFS got a grant from MISTRA to establish scientific linkages between the Swedish MISTRA programmes and IFS grantees and advisers working on similar research topics in developing countries. The second and final phase of this "linking programme" came to a close at the end of 2003. Several interesting contacts have been established between Swedish scientists and institutions on the one hand and scientists in Latin America on the other. These partnerships have produced many very interesting results and have also pointed at opportunities for further collaboration between Latin America and Sweden in this field

Concerning the *aims* of the workshop, it should first of all be clearly stated that the workshop should neither have the ambition of covering the whole field of "integrated pest management", nor the more specific fields of "semiochemicals and microbial antagonists". Thus, the purpose is not to establish some form of overall state-of-current-knowledge or comprehensive lists of priorities for future work in the region in these areas. The aims, although ambitious, are more concrete and focussing on the experiences and interests of the participating institutions, viz:

1. With points of departure in the experience from the MISTRA/IFS-sponsored collaboration between Swedish and Latin American research institutions, and IFS-sponsored regional networks, explore the potential for future *"North-South and South-South collaboration"* in these fields (particularly, but not exclusively, looking at the role of Swedish institutions in such collaboration).

2. With a point of departure in CATIE's past and current work in these areas, identify the institute's future roles and priorities in regional and international research collaboration and networking.

3. Identify the regional need for building scientific capacity in these areas, and define the roles of IFS, CATIE and others in responding to such needs.

4. Define the potential for the private sector in the region to become involved in the development and use of technologies based on semiochemicals and microbial antagonists, and other IPM technologies, in agriculture and forestry.

The organizers are convinced that the very interesting group of scientists, educators and private business representatives that have come together will guarantee that these aims are competently and thoroughly addressed.

Turrialba and Stockholm. March, 2004

Pedro Ferreira	Michael Stahl	Bruno Nilsson
Director General	Director	Permanent Secretary and Director
CATIE	IFS	KSLA

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Inaugural lecture

Integrated pest management, semiochemicals, and microbial antagonists practices in Latin America: overview of issues

Luis C. Rodríguez and Hermann M. Niemeyer[1]

Integrated Pest Management

IPM involves the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment. This definition inherently considers the existence of ecological and economic thresholds, the need to adopt the socio-ecosystem as a management unit, the existence of a broad number of IPM tools, and the requisite of an interdisciplinary systems approach where many diverse fields of expertise concur in order to develop tools that may be adopted as practices. Agricultural systems in Latin America range from subsistence agriculture in common property lands of comunidades campesinas to large highly technified estates which produce crops for export. General issues which have been identified as representing obstacles for the success of IPM programs in Latin America are: insufficient technical knowledge and few active IPM researchers, poor infrastructure in both research and extension systems, a weak public sector that limits the dissemination of information, inappropriate credit and subsidy schemes, and the influence of agrochemical companies on governments and their agencies. We herein draw attention to other issues regarding the practice of IPM in Latin America.

Top-down vs bottom-up IPM research and development. IPM practitioners commonly follow a top-down scheme of research and development that goes from scientist/managers to farmers. This approach identifies a pest problem, develops an adequate practice to solve it, and transfers the practice to farmers. Alternatively, the "farmer first" approach follows a bottom-up scheme which identifies the gaps in farmers' knowledge and supplies the missing information to farmers, who finally develop their own solutions, hence adapting IPM to their needs.

We consider that in many places in Latin America, discussions regarding the top-down or bottom-up nature of IPM are of secondary importance, given the chronic lack of funds and scientists available to address at least a few of the hundreds of already identified problems potentially to be solved by IPM practices in a conventional top-down approach, on one hand, and the prevalence of traditional practices in the region such as intercropping, crop rotation, or the use of resistant cultivars, on the other. IPM approaches that promote practical, realistic, economical and achievable solutions, and systematizing and validating peasants' knowledge in order to effectively complement scientific information, might provide enough expertise and adaptability to represent a shortcut to long term and costly research, allowing the reallocation of funds to more urgent research lines or to enhance the extension system in order to properly diffuse the information to potential users, influencing the accomplishment of IPM goals.

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Complexity of IPM. Contrarily to common assumptions, IPM complexity represents a challenge for farmers all around the world and not only in developing countries where the farmers education level is expected to be lower than in developed countries. For example, the adoption of the fundamental IPM concept of economic threshold has been minimal even in the UK, allegedly due to confusion following continually changing thresholds, and loss of faith in a research system responsible for producing them. In spite of their inefficiency, fixed thresholds have been adopted because they are good enough for most British farmers as a point determining the need of a control measure. Latin American farmers often develop their own thresholds, which are usually based on empirical knowledge. Thus, IPM practitioners often need to provide solutions intermediate between strategic and tactical IPM.

Farmers' problems and needs. In many cases researchers without sufficient farmer participation develop IPM strategies which may be complex, risky, or inappropriate for farmers' needs. For example, a widespread misunderstanding is to consider that crop losses from pests represent the main production problem that smallholders face, and consequently there has been a bias towards research in biological control and resistance breeding issues over other IPM methods. Nevertheless, Latin American peasants often manifest the existence of low pest problems, while expressing their concern of crop yield reduction due to declining soil fertility or change in hydrological regimen. Hence, pest control will be relevant only if it can be shown that losses from pests represent an important production constraint relative to other factors such as soil fertility. Therefore, a close collaboration between IPM researchers/ extensionists and farmers is required to deal with unexpected and undesirable results and to develop adequate practices that address farmers' needs.

Semiochemicals and bacterial antagonists in IPM

Semiochemicals (i.e. molecules involved in communication within and between species) and bacterial antagonists (i.e. entomopathogenic organisms employed for pest control) tend to be highly specific. Although this could represent a problem in dealing with a pest complex, in general their disadvantages are mostly linked with their low persistence and potential to provide long-term control, the low speed of kill exhibited by bacterial antagonists, the need to complement semiochemicals with other control methods, and the high cost of both practices. Bacterial antagonists are biocides which may naturally exist in nature and their prevalence anthropologically increased, or they may be introduced into pest populations by inundative releases with the aim of controlling pests before economic thresholds are surpassed. These control releases are frequently done using formulated products prepared from bacterial isolates (e.g. insecticidal proteins) of naturally occurring or conjugated strains. However, bacterial antagonists are also important in prevention by minimizing the effects of insect infestations within a location through the incorporation in the plant genome of the genes responsible for production of the toxin.

Although it is recognized that the costs of these IPM products compared with chemical pesticides are not prohibitive when environmental benefits such as safety for humans and other non-target organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems are taken into account, the market rarely reflects these benefits. In the last few years the production cost of semiochemicals and bacterial antagonists has been reduced, but they are still unaffordable for most Latin American farmers, except when a transient development project or government scheme has subsidized this cost greatly. However, experience dictates that once the external support is over, the extended practices are frequently not permissible due to their real high cost, the lack of continuous product supply, and specially for semiochemicals and bacterial antagonists, the evident technical and economical incapacity of peasants to develop local alternatives thus making these practices extremely dependent of external supplies compared with other more extensible IPM practices such as cultural procedures. Considering the importance of economic matters in the use of semiochemicals and bacterial antagonists, we briefly highlight two important issues relative to their market and regulations.

Semiochemical and bacterial antagonists, an unprotected market for crop protection. IPM practices are in worldwide expansion. However, IPM farming still remains insignificant: for example, world sales of IPM products –mainly semiochemicals and bacterial antagonists– is insignificant compared to that of chemical insecticides (ca. 1%). Manufacturers of IPM products are not able to develop markets by themselves, neither seriously compete against chemical companies. Of the ca. 250 basic manufacturers of IPM products worldwide, 91 % are small or very small enterprises, with a turnover of less than USD 1 million, a portfolio of just one or two products, they do not have specific distribution networks, and additionally they must assume all marketing costs. Due to their vulnerability, it is not surprising that 72% of the companies created between 1970 and 1995 have gone bankrupt. In fact, many multinational chemical companies have acquired IPM products companies only to disinvest them and close them down.

Regulatory directives and the irregular situation of semiochemicals. Many IPM manufacturers consider that they are unprotected because regulatory directives force them to compete directly with chemical industries. Thus, in many countries, semiochemicals, although they are not biocides, by the mere fact of being chemicals have been incorporated into the class of chemical pesticides, making their registration procedure too expensive to be viable. This procedure may constrain the research and development of new semiochemicals since, although the number of semiochemicals whose structures are known is high, only an extremely small percentage of these materials will ever be considered for commercialization, and only a small percentage of those will be able to complete the current registration procedure.

Research in IPM in Latin America

Research and development have traditionally received comparatively little support in Latin American countries, where on the average less than 0.5% of GDP is devoted to RandD as compared to up to 2.2% in developed countries. This is reflected in the lack of a critical mass of scientists –and hence decreased capacity to address multidisciplinary problems such as those derived from IPM, undersized graduate training programs, and chronic shortage of funding for scientific research.

Since most programs in the national granting systems are narrowly focused and oriented towards individual researchers, multidisciplinary research has decreased opportunities to receive funding. Furthermore, since scientists are judged mainly by their capacity to publish their results in high impact factor journals, research tends to be biased towards problems that are fashionable in developed countries where main journals are published and where most citations are produced, rather than to locally relevant problems.

Most scientific research in Latin America is performed within the university system, and suffers from distractions towards extensive teaching and outreach activities, an emphasis on basic rather than applied science, and also a certain degree of instability in research programs as they depend on short-term funding and on the transient presence of students. Fortunately, most countries in Latin America have developed a system of agricultural institutes, to a large degree based on the USDA model, which perform applied agricultural research; however, they tend to be underfunded in relation to the problems they face.

Latin American IPM research. In general, IPM research and promotion have responded to two different strategies: food security and globalization. The first one is concerned with crop protection and is mainly focused on smallholder peasants, while the second one tries to fulfill the requirements of foreign markets and is concentrated on large exporters. Food security through crop protection is primarily funded by foreign donors and involve an important component of extension work for success. On the other hand, in many developing countries, government programs and subsidies have been concentrated on medium and large commercial farmers, who are able to hire personnel to do research, or to create links with external research institutions.

Donors global priorities and Latin American semiochemical and bacterial antagonist research. IPM research programs funded by international agencies may suffer from conflicts between the priorities of agencies and those of the recipient country, lack of in-depth knowledge of problems related to implementation of IPM practices, and lack of sustainability of programs. The projects usually involve collaboration between local scientists and scientists from the country of origin of the donor agency. The nature of collaborations will depend strongly on the type of farmer and crop it is geared around. For research aiming at pest control of subsistence crops, strategic alliances between local agricultural research institutions and universities should prove of value, as this will draw the farmer and his needs into the research scheme thus rooting the science in problems that need solutions, will provide access to local funds focused on basic science, and the local counterpart will be able to make a stronger case for imposing local priorities via-à-vis those of foreign aid agencies and collaborating institutions. For research on cash crops of export value, farmers associations should constitute important research partners, providing both expertise and funds to research programs.

Results and lessons learnt from ongoing and concluded research in Swedish/Latin American collaboration and other work supported by IFS in Latin America

As pheromones gain ground

Peter Witzgall[1]

Mating disruption is used to control key insects in orchards and vineyards

After four decades of pheromone research, disruption of mating by aerial dissemination of synthetic pheromone is used to control tortricid moths on ca. 75.000 ha of European orchards and vineyards. This is a very encouraging achievement which became possible through collaboration between academic institutions, extension and chemical industry.

Pheromone-mediated mating disruption is an economic and efficient method which allows control of key insects such as codling moth *Cydia pomonella*, Oriental fruit moth *Grapholita molesta* and grapevine moth *Lobesia botrana* on an area-wide scale (Waldner 1997, Kast 2001, Varner et al. 2001, Zingg 2001). Other biological methods to be used against these insects in stand-alone applications are not available. However, pheromone use is nevertheless restricted to ca. 2% of the Euopean orchard and vineyard surface.

For a more widespread use in Europe, and for a successful introduction of pheromone-based methods in South America, we must carefully analyse the reasons for failures and successes.

Technical development of mating disruption

Mating disruption must become even more reliable and more economic. This requires first of all continued investment of industries in pheromone synthesis and dispenser materials (Ogawa 1997). The goal is to achieve sufficient pheromone release throughout the season, at a reduced price for dispenser material and application. Equally important is the continued engagement of extension services and farmers' organizations to implement the commercially available dispenser materials.

Last not least, a central question remains to be solved: how to identify the best pheromone blend for mating disruption. This work relies largely on field trials, where infestation rates are the result of many factors, only one of which is the pheromone blend formulated in the dispensers.

Behavioural assays to predict the efficacy of pheromone blends need to be further improved. The fluctuations of insect populations over time and their geographic distribution should again be taken into account. A strictly empirical strategy has been employed to develop mating disruption for insects from the Northern hemisphere. Attempts to develop a 'quantitative' approach to predict and interpret the outcome of

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experiments would be particularly important for the accelerated development of mating disruption against new, South American species.

Integration of biological control methods

Behaviour-modifying chemicals, microbial pesticides, and resistant plants can be used to control insects. However, these methods are species-specific to varying degree and do not cover all insects associated with a crop. In addition, the effect of biological methods is subtle compared to insecticides, which produce a lethal effect upon contact and cover a broad spectrum of species. Single biological control methods can therefore rarely replace all insecticide treatments. All available methods should instead be developed as components of an integrated control programme, rather than as sole agents.

Microbial insecticides are likely to become an increasingly important complement of mating disruption (Moscardi 1999). A combination of mating disruption and an insect-pathogenic virus provides more efficient control of codling moth than insecticide sprays. Microbial insecticides face similar challenges as semiochemicals, in particular formulation techniques and longevity, but they are already available.

Plant compounds - direct and future use

Volatile secondary plant metabolites play a significant role in plant-pathogen and plant-insect relationships. For example, host-finding in ovipositing insects is largely guided by plant volatiles (Schoonhoven et al. 1998). A central issue is whether these plant volatiles can be used to manipulate the behaviour of egg-laying females. All insects feeding on a particular plant, such as apple, are likely attracted to apple volatiles. There is accordingly a possibility that apple volatiles can be used to manage a range of orchard insects, once we have understood how insects use plant volatiles to find their hosts.

Knowledge of plant volatile compounds will of course have implications beyond direct insect control. Plant secondary metabolites have evolved to provide defence against fungi and insects. Plant defence reactions will be studied to aid the improvement of resistance against insects and pathogens. A most exciting field emerging concerns interactions between phytophagous insects and phytophathogenic fungi (Stout et al. 1999).

Socio-economic issues

Crop protection still relies primarily on synthetic pesticides. This chemical approach to pest control is not sustainable and is therefore under increasing pressure. Dietary experts encourage us to eat a diet rich in fruit and vegetables because of beneficial health effects - while many are worried about pesticide residues. One logical strategy to market agricultural products is to increase product quality by reducing insecticide use. The consumers will reward such efforts. Attempts to reduce insecticide use is meaningful also from a wider economic perspective.

One famous example comes from South Tyrol, where growers had earned such a bad reputation for the overuse of insecticides that it became difficult to market horticultural products from this region. This negative image even had an impact on tourism. Consequent efforts to modernize the horticultural sector included attempts to cut down on insecticide use. South Tyrol became the first area in Europe to use pheromones on an area-wide scale.

It is always more difficult and demanding to use new techniques than to continue to use well-established conventional techniques. However, the growers will undoubtedly manage to adopt new, sustainable insect control strategies if only they are motivated to do so. Training of farmers includes also attempts to change their attitudes, which is perhaps the overriding goal when implementing pheromone-based control techniques.

Past, presence, and future

Research on insect pheromones has provided us with methods which are *now* available to control insects and to replace the insecticides which are being deregulated. This research has also produced the tools and concepts which make it possible to efficiently investigate the role of plant volatiles, which is currently a focus in Chemical Ecology. The potential of plant compounds for direct use in insect control is barely exploited. In addition, both genetic engineering and traditional plant breeding can be used to apply this knowledge to produce more resistant plant varieties.

Chemical Ecology has made a significant contribution to environmentally safe insect control, and will continue to provide new strategies and tools to protect food crops. The possibility to combine exciting leading-edge research on natural product chemistry, insect sensory and behavioural physiology, and plant-insect interactions with practical, industrial applications has always made this field particulary fascinating. It is particularly stimulating to witness that Chemical Ecology continues to attract students and expand in this most dynamic continent, which is South America.

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Use of semiochemicals for control of three lepidopteran species in Brazilian apple orchards

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Introduction

Apple is one of the most important fruits from temperate climate grown in Brazil, and the orchards cover ca. 32 000 ha. The most important moth pests of apple are the Brazilian apple leafroller, *Bonagota cranaodes*, and the Oriental fruit moth, *Grapholita molesta*, (Lepidoptera: Tortricidae). Recently, codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) has been detected around cities in Rio Grande do Sul and Santa Catarina States, and is expected to become a most serious threat to commercial fruit-growing in the near future.

Pheromones are used in several countries to control *G. molesta* and *C. pomonella*. Recently, a plant volatile compound attractive to codling moth males and females has been identified (Light et al. 2001). Plant compounds can even be combined with pheromone to improve the efficacy of mating disruption (Hapke et al. 2001).

However, in spite the large use of pheromones for insect control in apple orchards worldwide, in Brazilian apple orchards it remain used only for monitoring *B. cranaodes* and *G. molesta*. Therefore, the aim of this project was to provide alternative methods to replace and/or reduce the use of insecticides in apple orchards in Brazil.

Laboratory and field tests have been conducted to identify the sex pheromone and plant volatile compounds that are attractive to *B. cranaodes*, *C. pomonella*, and *G. molesta*. The methods that will be studied are: monitoring, mating disruption, and mass-trapping of adult insects by using dispensers containing synthetic sex pheromone or plant volatiles.

Methods and Materials

Larvae of *B. cranaodes* and *C. pomonella* were collected in apple orchards in Brazil and Sweden, respectively, and reared on a semi-artificial agar-based diet.

Extracts of female sex pheromone glands were used for chemical analysis and wind tunnel assays. Batch extracts were prepared by solvent extraction of ovipositors, and analysed by

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gas chromatography (GC) and combined gas chromatography - mass spectrometry (GC-MS) to identify the compounds present in the sex pheromone gland extracts. Combined gas chromatography-electroantennographic detection (GC-EAD) was used to screen for compounds eliciting antennal activity in single *B. cranaodes* and *C. pomonella* antennae.

In the laboratory, tests were done in a wind tunnel. Pheromone stimuli were delivered from a pheromone sprayer, an apparatus that allows the evaporation of known and constant amounts of gland extracts or synthetic blends, at defined chemical purity. Compounds were also formulated on red rubber septa in order to facilitate comparison with field trapping experiments. During the tests with *C. pomonella*, a flight tracker was used for behavioural observations and off-line analysis of flight tracks.

Field experiments were done to verify the results of wind tunnel studies. They were carried out in apple orchards infested with *B. cranaodes* in Rio Grande do Sul, Brazil, and in apple orchards infested with *C. pomonella* in Scania, Sweden. Tetra traps were baited with synthetic compounds formulated on red rubber septa, and were placed into apple trees. Recently, new field tests have been conducted in Brazilian orchards to verify the efficiency of *B. cranaodes* pheromone formulation for monitoring, and *G. molesta* pheromone for mating disruption.

Results and discussion

Sex pheromone of Brazilian apple leafroller

Analysis of sex pheromone glands of *B. cranaodes*, by GC-MS or GC-EAD showed the presence of fourteen structurally related acetates and alcohols of the chain length 10 to 18, including the main pheromone component, *E*3,*Z*5-12Ac. Male antennae responded to the main pheromone component *E*3,*Z*5-12Ac, its *Z*,*Z* isomer, *E*3,*Z*5-14Ac, and the monoenes *Z*5-12Ac and *Z*9-16Ac. Traps baited with a four-component blend of *E*3,*Z*5-12Ac, *Z*5-12Ac, *E*3,*Z*5-14Ac, and *Z*9-16Ac in a 100:5:5:100 ratio were significantly more attractive than the main compound alone.

As a result of this work, the four-component blend has been registered as a trap lure in Brazil to be used by the farmers for population monitoring A more complete pheromone blend is of importance also with respect to current attempts to develop mating disruption for control of this species.

Sex pheromone of codling moth

It has been a matter of controversy whether *C. pomonella* sex pheromone is multicomponent. A synergistic effect has been shown for the saturated alcohols dodecanol and tetradecanol (Einhorn et al. 1984, Bartell et al. 1988). However, saturated compounds are rarely strong pheromone synergists in moths, and a behavioural role of these compounds in codling moth could not be substantiated in a more recent study (McDonough et al. 1993).

In codling moth, wind tunnel experiments were done to investigate the behavioural effect of minor compounds produced in female sex pheromone glands. Attraction of codling moth males to the main compound codlemone, *E*8,*E*10-12OH was enhanced when blending it with dodecanol, the *E*,*Z*-isomer of codlemone *E*8,*Z*10-12OH, and codlemone acetate *E*8,*E*10-12Ac.

Blends of pheromone and attraction antagonists can be efficient mating disruptants. Wind tunnel studies confirmed that codlemone acetate *E*8,*E*10-12Ac is the most powerful attraction antagonist in codling moth. However, in mating disruption treatments in apple orchards, dispensers containing codlemone and dispensers containing a 1:1-blend of codlemone and codlemone acetate produced similar behavioural effects (Witzgall et al. 1999).

Further wind tunnel experiments showed that large amounts of *E*,*Z*-codlemone and codlemone acetate had an antagonistic effect on male attraction to codlemone, as they blocked the transition to upwind oriented flight. A 1:1-blend of codlemone and codlemone acetate strongly modified male flight behaviour and was barely attractive on its own. Adding a second, spatially separate source of codlemone almost restored the upwind flight response, and airborne males did not discriminate between attractant and antagonist pheromone sources. In the field, males are already on their wings when they encounter artificial sources of pheromone or other semiochemicals. This may provide an explanation for inconsistencies between wind tunnel and field studies.

Codling moth attraction to apple volatile compounds

Apple volatile compounds known to elicit an antennal response in codling moth were tested for attraction of adult moths in field trapping and wind tunnel tests. In the field, both (*E*)-β-farnesene and farnesol captured male moths Addition of other apple volatiles, including (*E*,*E*)-α-farnesene, linalool or farnesol to (*E*)-β-farnesene did not significantly augment captures. Few females were captured in traps which also captured male moths, but female captures were not significantly different from blank. In the wind tunnel, males were attracted to farnesol, but not to (*E*)-β-farnesene. Addition of (*E*,*E*)-α-farnesene to (*E*)-β-farnesene had a synergistic effect on male attraction. The male behavioural sequence elicited by plant volatiles, including upwind flight behaviour, was indistinguishable from the behaviour elicited by sex pheromone.

Continuing development of laboratory and field assays for evaluating male and female codling moth behaviour is required for a more complete understanding of which compounds guide codling moths to mating and oviposition sites. The potential of such compounds for insect management is expected to be considerable.

Recent findings

An experiment using the GC-EAD showed that *B. cranaodes* and *C. pomonella* males and females antennae responded to a similar range of plant volatile compounds.

While doing the field tests in Brazil it was possible to observe that some males of *G. molesta* were caught in traps for *B. cranaodes*, and the contrary as well. Therefore an experiment was set in six cities, where tetra traps baited with synthetic pheromone for *B. cranaodes* and *G. molesta* were placed in apple and peach orchards in each city. This experiment is showing so far that *G. molesta* is attracted towards *B. cranaodes* traps and vice-versa.

Another experiment that is going on is a mating disruption trial for *G. molesta*. Unfortunately this test started already in the middle of the season, but the results obtained until now demonstrate that it is possible to control *G. molesta* by using mating disruption technique.

Acknowledgements

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Screening, isolation and identification of metabolites from microorganisms with antifungal activity

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Introduction

Different microorganisms have a long history of use as biopreservatives for biocontrol of crops and food and feed storage. Previous research has revealed that the microorganisms can produce low-molecular-weight substances, *e.g.* phenyllactic acid, *p*-hydroxyphenyllactic acid, cyclic dipeptides, benzoic acid, methylhydantion, mevalonolactone and short-chain fatty acids with antifungal effects.

Previously, a large number of microbial strains with antifungal effects have been isolated *in vitro* from plant and soil material. In a continuous study of isolated strains with antifungal effects, procedures for isolating antifungal compounds have been devised. In this presentation methods for screening, isolation and characterization of the active metabolites are reported.

Methods and materials

The organisms were grown in 0.3-10 l liquid medium, the cells removed by centrifugation and the culture filtrates separated on C-18 (solid phase extraction, SPE). The organic fractions were separated by preparative HPLC and the separation followed by different bioassays. Active fractions were further separated by reverse phase HPLC using 96-wells plates again followed by the bioassays. Pure active compounds were analysed by NMR spectroscopy and mass spectrometry in combination with chemical methods to obtain the structures of the compounds.

Results and Discussion

The culture filtrates, obtained from microorganisms with antifungal properties grown in liquid medium, were fractionated by SPE and consecutive HPLC, both preparative and analytical scale. The separation of the active metabolites was followed by bioassays in 96-wells plates.

From this screening active compounds were isolated and their chemical structures analysed by spectroscopic techniques. Mass spectrometry using ESI or MALDI-TOF gave information on the molecular weight of the substances and also for some compounds fragments of analytical value.

Further information on the structures was obtained from NMR spectroscopy. Both one- and different two-dimensional techniques were used for assignment of signals and the ^{1}H and ^{13}C chemical shifts and coupling constants identified the structures.

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Conclusions

A procedure for separation of the metabolites from different microorganisms with antifungal properties has been worked out. By this procedure a large number of compounds with antifungal activity has been isolated and structurally characterised. Several of these compounds show specific inhibition against certain fungi and could be candidates for improved control of pathogenic fungi.

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Plant/plant communication– effects on herbivore ecology

Jan Pettersson[1], Robert Glinwood and Velemir Ninkovic

Introduction

Under average conditions the most common biotic challenge for a plant individual is that of sharing space and resources with other plants. General competitive traits such as phenology, efficiency for growth, reproduction and robustness to survive harsh or variable environmental conditions may be competitive parameters in this combat. Semiochemicals may also play an active role in interaction with neighbouring plants (allelopathy sensu Rice 1984; allelobiosis sensu Pettersson et al. 2003).

Allelobiosis is usually seen from a plant perspective but it should also include herbivores. Herbivore wounding induced release of volatiles can promote reduced herbivore acceptance of neighbouring plants and searching behaviour of natural enemies (Bruin and Dicke 2001, Pettersson et al. 1996, Ninkovic and Pettersson 2003).

It is still an open question if similar interactions may occur between undamaged plants. Support for this is given as volatile messenger substances emitted by undamaged *Artemisia* have been shown to promote, via aerial allelobiosis, induced resistance to herbivores (Farmer and Ryan 1990). In a series of investigations with a barley crop and aphids as a model system (reviewed in Pettersson et al. 2003) we have found that allelobiotic mechanisms may affect the exposed plants in different ways such as

- changes of biomass allocation favoring root growth (Ninkovic 2003)
- induced resistance to aphid establishment on the plant (Pettersson et al. 1999, Ninkovic et al. 2002, Glinwood et al. 2003, 2004)
- arresting responses of natural enemies (Ninkovic et al. 2001)

The overall objectives of ongoing studies is to contribute to a general understanding of tritrophic effects of allelobiotic mechanisms. Hypothetically these mechanisms may form a link between botanical and faunistic biodiversity, and contribute to new approaches to plant protection in line with the push/pull strategy (Pickett et al. 1998).

The operating principle for this option of aphid control means a combination of increased aphid mobility and mobilized induced plant resistance (**M**obility – **I**nduced **R**esistance = MIR). In this presentation we show how the MIR concept is taken from a hypothetic model to tests on a commercial plant protection scale.

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Methods and Materials

Insects. The bird cherry-oat aphid, *Rhopalosiphum padi* (L.), is a key pest in spring-sown cereals and was used to trace herbivore effects. *R. padi* is monophagous on its winter host plant, *Prunus padus* L. but has a broad range of grasses as summer hosts. The seven spotted ladybird *Coccinella septempunctata* L. is a common predator of *R. padi* and was chosen to represent the third trophic level.

Plant material. Test plants were exposed individually to air from another plant individual or chemical substance in a twin cage system with a one-way-airflow-system (Pettersson et al. 1999). The treatments were randomly distributed and each twin unit was regarded as a replicate.

Aphid responses to evaluate effects of plant treatment were measured as preference in host plant choice (Pettersson et al. 1999) and settling frequencies in a no choice test (Ninkovic et al. 2002). Responses to changes in odour patterns was tested in olfactometer experiments (Ninkovic 2002, Glinwood et al. 2003). *Ladybird responses* were recorded in field observations and olfactometer experiments (Ninkovic et al. 2001, Ninkovic and Pettersson 2003).

Formulation of semiochemicals. For the laboratory experiments active substances were placed in capillary tubes (Micro caps). In field experiments and glasshouses active substances were melted into paraffin pellets (Ninkovic et al. 2003).

Results and Discussion

Search for new active substances to the MIR concept

The MIR concept can be realized with different sets of substances and studies of the ecological role of methylsalicylate meant a start for this developmental project. Studies on mechanisms regulating spring migration of *Rhopalosiphum padi* (L.) from its winter host *Prunus padus* (L.) gave a lead. Methylsalicylate is formed as plant response to aphid feeding on young shoots and when applied in spring sown barley it acted as a repellent(Pettersson 1994, Glinwood et al. unpublished).

Experiments in commercial barley fields showed that methylsalicylate gives a significant aphid control. However, the effect depends on population density and current conditions and is thus to some extent unreliable (Ninkovic et al. 2003). Experiments with already known allelobiotic plant compounds showed that both the repelling effect and the inducing power could be reinforced (Glinwood et al. 2003, Glinwood et al. unpublished). Settling by *R. padi* was significantly reduced when barley plants were exposed to either root leachates from living *E. repens* plants, or to solutions containing known *E. repens* root exudates, (Glinwood et al. 2003). Barley plants exposed to root exudates also became less attractive then unexposed plants in an olfactometer, suggesting that allelobiosis changed the volatile profile.

Thistles in the genus *Cirsium* are also known to exert allelobiotic effects on other plants (Kazinczi et al. 2001). Exposure of barley to root leachates from *Cirsium vulgare* or *C.*

arvense did not affect aphid settling, however when plants were exposed to volatiles from either thistle species, settling by *R. padi* and *S. avenae* was significantly reduced (Glinwood et al. 2004).

Allelobiosis and natural enemies

Observations in a barley field showed that *C. septempunctata* was significantly aggregated to patches with *Elytrigia repens* and *Cirsium vulgare* although no obvious food resource such as pollen or aphids or other small prey insects were abundant (Ninkovic and Pettersson 2003).

In olfactometer experiments adult ladybirds showed no preference for either of the three plant species. However, a significant preference was shown for a mixture of barley odour together with each of the two weeds. The effect on barley exposed to volatiles from *E. repens* and *C. arvense* differed in that barley plants exposed to *C. arvense* remained attractive when the weed was taken away whereas those exposed to *E. repens* lost their attractiveness. This indicates that the positive effect of the barley/*E. repens* combination may merely be an effect of mixed volatiles, whereas the barley/*C. arvense* mixture is likely to represent a more complex mechanism involving allelobiosis.

Conclusions

- The results of experiments with allelobiotically active substances from E. *repens* and with barley genotypes (Ninkovic 2003a, Pettersson et al. 1999), show that allelobiotic communication may induce a significant change in aphid host plant responses.
- Olfactometer responses of *C. septempunctata* indicate that a mixture of volatiles from different plant species may release an arresting behaviour but as barley plants exposed to *C. arvense* remained attractive other mechanisms may also be operating.
- Although mixed cropping often is applied in farming the role of allelobiotic mechanisms in plant protection is still poorly understood (Vandermeer 1992). Increased knowledge should contribute to the search for more effective combinations of plants. A second contribution will be to increase our understanding of how semiochemicals can be used to manipulate induced resistance in plants and natural enemies of pests. Further knowledge might also favour breeding for cultivars that combine high yield with a capacity to allocate resources depending on prevailing conditions.

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Usage development of pheromones and biological control for Brazilian agriculture insect pests- Project FEROBIO

Evaldo Vilela and Ronaldo Reis Jr.[1]

The Project FEROBIO, sponsored by the Brazilian Agency for the Development of Science and Technology (CNPq), is a mutual initiative among Federal Government Departments (Environment, Agriculture, and Health), universities, EMBRAPA, agribusiness representatives, and the industry of agriculture supplies in Brazil. It represents an effort to diagnose and propose solutions to structural constrains which interfere or even hinder the usage of biological control agents and pheromones in the Brazilian agriculture.

The increasing concern about the deterioration of natural resources, being agriculture recognized as one of the main cause of water quality degradation, stands to the food production necessity of Brazil and the whole world, as well as the need of ensuring the farmer's profitability, constitutes the largest motivation of this project which aims to promote the sustainability of agriculture and the usage of natural resources, through the implementation of practices of biological control and pheromone technologies into the approach of integrated pest management (IPM), in Brazil.

The feeding reliability is nowadays a topic of interest to the economy of countries which are agricultural producers, such as Brazil, with direct consequences to the food exportation. In this context, the intensive utilization of chemicals to control insect pest (Brazil is the 3rd biggest worldwide consumer), has brought up serious problems to the environment, to the consumers, as well as to the producer, being able to affect the credibility of consumers of Brazilian products and, consequently, the exportations.

In Brazil, a larger application of methods of biological control and pheromones, which may definitely contribute to the feeding reliability, is limited due to the shortage of specialists in the area with knowledge view of the real problems in the field; the lack of disclosure of technologies for the producers; the scarcity of floatation and motivation for research in these areas and, the most important, the dissociation between the real demand of the productive sector and the scientific and technological research.

For the technologies of biological control and pheromones, separately or in association, to indulge the competitiveness of the agricultural products of countries in development, it is imperative to promote a larger international integration, via cooperation and partnerships, in spreading the concept of sustainable agriculture. Such activities should focus on: a) research, b) training programs and c) knowledge and technologies sharing. These approaches are necessary so that countries in development would be able to transform themselves in genuine agents in the safe usage of new technologies. Social, economical, and political barriers to a broader use of biological control and pheromones must be removed.

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For a long time, the priorities of research in agriculture have been directed towards production improvement and maximization. As a result, Brazil produces today a huge amount of food and fibers, though the challenge is not only focused on production, but also on competitiveness, environmental compatibility, and social responsibility and upon these approaches depend the chances of countries in development to move in safety. This committal should be part of the worldwide economical policy towards the adoption of new technologies, being these technologies, strategies to the countries in development.

This Project has been taking course since 1998 under my coordination, having as results progress such as:

a. Better understanding of the Brazilian laws and compatibility of rules concerning the usage and registration of pheromones and biological control agents.
b. Advances in the process of pheromone and biological control agents registration
c. Interaction of science and technology developed by public institutions with actual need for pest population control that affect the Brazilian agribusiness.

The methodology used in the project, as well as further information about the initiative, can be obtained at http://www.ferobio.ufv.br, being fundamental the participation of the community interested in the use of pheromones and biological control agents, reason why we invite everyone to visit this site and participate actively in the development of this project.

Influence of semiochemicals from aphids and their host plants on parasitoid behaviour

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Luis Carlos Rodríguez[2] and Hermann M. Niemeyer[2]

Introduction

Aphid-induced plant volatiles, acting as synomones, are important semiochemicals for parasitoid host location (Powell et al. 1998, Powell and Pickett 2003). These compounds influence innate preferences, conditioning and associative learning in adult parasitoids (Poppy et al. 1997).

This is the situation with *Aphidius ervi* Haliday, whose females are attracted toward the pea aphid (*Acyrthosiphon pisum* Harris) feeding on broad bean (*Vicia faba* L.) (Du et al. 1996, 1997, 1998). The pea aphid growing on legumes is the main host for *A. ervi*, but this parasitoid also uses the grain aphid *Sitobion avenae* F. on cereals as an important host in Chile (Starý et al. 1993).

Since molecular evidence indicates that *A. ervi* from aphids growing on legumes and cereals in Chile is composed by a single genetic strain (Daza-Bustamante et al. 2002), semiochemicals may play an important role in the spatial dynamics of this parasitoid when migrating from legumes to cereals and vice versa.

In order to evaluate whether semiochemical compounds are involved in aphid host location by *A. ervi* on legumes and cereals in Chile, we addressed: i) the attraction of alfalfa (legume) and wheat (cereal) plants, as well as the respective aphid host-plant complexes, toward naïve and oviposition experienced females of *A. ervi*.

Methods and Materials

A. ervi was obtained from mummies collected on alfalfa or wheat, and was reared in the laboratory on the same hosts they came from. Mated female parasitoids either naïve or with oviposition experience on the plant-host complex, were offered the choice between alfalfa or wheat plants (undamaged, damaged or host-plant complex) in a wind tunnel and Pettersson olfactometers, following the general methodology described by Du et al. (1997).

The behavioral variables studied in the wind tunnel were: preference of the parasitoid for either wheat or alfalfa, once it started flying, which resulted in oriented flight or landing, and response time from release into the wind tunnel until obtaining a response (Daza-Bustamante et al. 2002). Total time spent by the female parasitoid in the alfalfa or wheat

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arms was the variable studied in the case of the olfactometer experiment (Rodríguez et al. 2002).

Results and Discussion

The dual choice wind tunnel experiments with both naïve and experienced *A. ervi* from alfalfa showed a significant preference of the insects for their undamaged host plants, host-damaged plants, and host-plant complexes (Table 1). For *A. ervi* from wheat, both naïve and experienced parasitoids preferred the host-plant complexes and the host-damaged plants, but only experienced parasitoids preferred the undamaged host plants (Table 1). Results obtained with the olfactometer (Table 1) supported the pattern found in the wind tunnel, indicating that semiochemical compounds are involved in the behavioral effects observed with undamaged plants.

Our results agree with previous reports that suggest attraction by induced volatiles of host-plant complexes and of host-damaged plants in the *Vicia faba-A. pisum-A. ervi* system (Du et al., 1997; 1998). The preference and lack of preference of *A. ervi* from alfalfa and wheat, respectively, for undamaged host plants (Table 1), suggest an innate preference for alfalfa, as expected since the pea aphid-legume interaction is known as the main host-plant system at the genetic diversity center for *A. ervi* in Eurasia (Starý et al., 1993).

Table 1. Effect of oviposition experience (naïve or experienced) on behavioural patterns of *Aphidius ervi* females from different origins (alfalfa or wheat), confronted with alfalfa and wheat plants in a wind tunnel and Pettersson's olfactometers.[a,b]

Origin – Stimulus			**Wind Tunnel**			**Olfactometer**	
	Preference[c,d]		Oriented flight[d]	Landing[d]	Response time[e]	Preference[c,d]	
	naïve	exp.	naïve *vs.* exp.	naïve *vs.* exp.	naïve vs. exp.	naïve	exp.
Parasitoids from alfalfa							
Undamaged plants	**	***	E ns	E ns	E ns	**	**
Damaged plants	*	**	E ns	E ns	N ns	-	-
Host-plant complex	***	***	EN ns	E ***	E ***	-	-
Parasitoids from wheat							
Undamaged plants	ns	***	E *	E ns	E ns	ns	**
Damaged plants	**	***	E ns	E ***	E ***	-	-
Host-plant complex	**	***	E ns	E ***	E ***	-	-

[a] Levels of significance: ns= >0.05; *= <0.05; **= <0.01; ***= <0.005.

[b] Behaviours: E= attraction or lower response time by experienced female towards plant of origin; N= attraction or lower response time by naïve female towards plant of origin.

[c] Parasitoids always preferred the plant of origin.

[d] χ^2 test.

[e] U test.

Oviposition experience resulted in an increase in oriented flights (Table 1). This increase was not always statistically significant, possibly on account of the strong preferences of naïve parasitoids. Similarly, increased landing responses and reductions in the mean response times were also shown by experienced parasitoids. The effect was particularly strong towards the host-plant complexes (Table 1), which constitute the most attractive stimuli tested.

These results agree with previous work on associative learning performed on *A. ervi* by Du et al. (1997) and Guerrieri et al. (1997), providing a mechanistic explanation of the biocontrol activity observed on alfalfa and wheat crops in Chile. Semiochemicals from the host-plant complexes studied, might be useful alternatives to include within stimulo-deterrent strategies of IPM of aphid pests on these crops.

Conclusions

- Synomones are involved in host location of *A. ervi* on alfalfa (legume) and wheat (cereal) crops.
- Adult oviposition experience enhances the attraction of *A. ervi* toward volatiles from host-plant complexes.

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Integration of biological control methods for sustainable pest management in potato and vegetables

Alba Marina Cotes[1]

Rationale

Implementation of biological control as component of an IPM strategy combines natural forms of control, taking advantage of ecological relationships in the agricultural system. However, it differs from the approach used in conventional pest control. When necessary, it is combined with chemical pesticides used at lower rates that target specific pests, being less toxic to beneficial organisms.

The following factors affect the adoption of biological control: the state of the agroecology; the history of pesticide use, the farmer's access to knowledge, information, and inputs; his or her attitude toward pest management and risk avoidance; and the prices of inputs and commodities It is quite evident that purely biologically based pest management strategies often are economically viable only over the longer term, because the biological equilibrium of the soil requires time to adjust. In such cases, rationalization of pesticide use may be the first step toward making pest management more sustainable. Following Benbrook et al. (1996), the adoption of IPM can best be described as a continuum ranging from low to high use of biologically based techniques (IPM)

In recent years interest in Colombia has focused on biological control alternatives for pest management. Nevertheless, practical application of this strategy appears still limited, in part due to the small number of commercial BCAs available for using against plant pathogens and to the technical difficulties to obtain high quality biomass and adequate formulation, showing reliable effectiveness under field conditions.

Taking into account that the development of stable, cost effective and easily applied biocontrol formulations is critical for the advancement of biological control (Lisansky, 1985), the purpose of the present work was to develop biopesticides for the control of the main insect pests and pathogens affecting potato and vegetables by a multidisciplinary approach, which relies on the participation of disciplines such as phytopathology, entomology, agronomy, biology, microbiology, pharmaceutical chemistry, environmental engineering and biochemical engineering, working on different topics related to biopesticides development.

Recent findings

In order to develop efficient biopesticides that could be used as components for integrated pest management programs attention was focused in protection against foliar (gray mould and mildew), postharvest pathogens affecting vegetables, soilborne pathogens and some important insect pests affecting vegetables and potato.

For soilborne plant pathogens, attention was focused in protection of spermosphere or rhizosphere by combining seed priming with a strain of *Trichoderma koningii*, and by adding a

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suspension of the antagonist into the soil. Priming consists in a pre-sowing hydration treatment in which the germination is initiated, but stopped before radicle emergence. Solid matrix priming has been developed as an alternative to priming seeds in osmotic solutions showing results expressed in increased stand establishment, disease resistance and seedling vigour in several crops. This method has demonstrated to be ideal for the integration with biological control agents.

In a previous research we demonstrated that seed priming in the presence of a selected strain of *T. koningii* effectively protected bean and cucumber against damping-off agents (Mezui et al., 1994). Taking into account that tomato culture is severely affected in nursery and in field by plant pathogens such as *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*. This strategy obtained under natural conditions 82%, and 62% protection against diseases produced by *R. solani* in bean and tomato, respectively; and 70% protection against *F. oxysporum* f. sp. *lycopersici* in tomato.

Sclerotinia sclerotiorum and *Alternaria dauci* represent a serious threat to lettuce (*Lactuca sativa)* and coriander (*Coriandrum sativum*) crops respectively. Because in previous studies the isolate TH003 of *Trichoderma koningii* was selected by its high biocontrol activity against different soilborne plant pathogens in different crops, two biopesticide prototypes were developed based upon this strain.

The first one was formulated as dispersible granulate for soil application. This product presents a concentration of 10^9 conidia.g^{-1}, 95% of germination after 16 hours, a particle size of 4 mm and 5.2% humidity. Granulate includes in the formulation several nutrient sources in order to help the microorganism to colonize the soil. It can also be dispersed in water and used for seeds or crop applications. The second biopesticide prototype was formulated as wettable powder consisting in conidia pelletized with sunscreens in order to protect them from ultraviolet radiation. They should be suspended before application in an emulsifying concentrate which is a mixture of different vegetable oils, surfactants and suspension agents. The suspension must be emulsified in water and is recommended for foliar application. This product has a concentration of 10^{10} conidia.g^{-1}, a particle size of 100 µm, 4.9% humidity and 98% germination after 16 hours. Both biopesticide prototypes evaluated under commercial crops production efficiently controlled target diseases. In lettuce, 80.6% of *S. sclerotiorum* incidence reduction was obtained while with chemical fungicides only 36.58% disease reduction was observed. In coriander treated with biopesticide prototypes 68.3% *A. dauci* incidence reduction was obtained while with chemical fungicide only 7.3%. incidence reduction was observed. Results showed products efficacy for controlling vegetable pathogens and their high potential to reduce crops losses and heath risks caused by chemical residues in vegetables and environment. (Seed priming). Formulated as granulate for soil application, which can also be dispersed for its application as a suspension. When evaluated for *Sclerotium cepivorum* control in onion produced more than 60% protection.

Harvested fruit and vegetable are highly perishable agricultural commodities. The major limiting factor in storage of fruits and vegetables is spoilage losses unstained during production, transportation, and storage (Wilson and Wisniewski, 1989). Post-harvest rots result in lowered quality and substantial economic losses to growers. Control of post-harvest diseases of fruits and vegetables relies heavily on chemical fungicides which pose a health risk when used on products that are consumed fresh, declination of effectiveness and hazards to the environment (Cliforf and Lester, 1988). Among the alternative methods to synthetic fungicides, naturally occurring antagonistic yeasts have been the most extensively studied (Janisiewicz, 1994). Taking into account that in Colombia, approximately 30% of harvested vegetables such as tomato and onion is discarded because of spoilage due to postharvest rots, resulting in lowered quality and substantial economic losses to growers, the objective of this research was to investigate the biocontrol potential of native yeasts against *Rhizopus stolonifer* and *Botrytis allii* are important postharvest pathogens of tomato and onion, respectively.

The biological control of plant pathogens at post-harvest through the use of yeasts has shown consistent and promising results. The main objective of this work was to evaluate the biocontrol activity of native yeasts against the gray mold caused by *B. allii* in onion and the soft rot of tomato caused by *R. stolonifer*. Out of 67 native yeast isolates obtained from vegetal material and soil, 10 isolates capable of growing both at 6°C and 22°C were selected. With this yeasts protection tests, against *B. allii* in onion and *R. stolonifer* in tomato were carried out under controlled temperature conditions (6°C and 22°C). The results obtained showed that the 10 antagonistic microorganisms selected offered some protection against *B. allii* and *R. stolonifer*, which was evidenced by a lower development of disease symptoms of the infection caused by the two pathogens. Only three yeasts, Lv006, Lv027 and Lv031, identified as *Pichia onychis* in the case of bulb onion, showed a protection percentage higher than 85% at both storage temperatures (6°C and 22°C). In the case of tomato, the yeasts Lv050 (identified as *P. anomala)*, Lv031 and Lv027 both identified as *P. onychis* showed protection percentage higher than 85%, at the same temperature conditions. Therefore, the aim of the study was to produce high quality biomass quantities and to develop formulation prototypes based upon the yeast Lv027. Massive production was carried out by liquid fermentation in a 20 liters capacity airlift fermentor by using a previously standardized medium. Biomass was separated by centrifugation and used as active ingredient for developing biopesticide prototypes. Two kinds of formulations were designed, an emulsifying concentrate and a dispersible granulate. Developed emulsifying concentrate presented a viability of 4.8 x 10^9 UFC.g^{-1}, the reconstituted product has pH 6 and maintained concentration homogeneity for one hour after reconstitution.. The most appropriated granulate formulation presented a concentration of 3.0 x 10^{10} UFC.g^{-1}, immediate humectability, disintegration time of 240 seconds, and protection of 85% against *R. stolonifer* in tomato postharvest immediately after formulation.

The insect complex called "whiteflies" includes the species *Trialeurodes vaporariorum* and *Bemisia tabaci*, which produce the highest economical impact all over the world and are considered the most important pest for agriculture at a global scale (Gerling 1990). Whiteflies cause damage not only as a direct pest, but also as vectors of viruses, which is the case in at last 23 different crops, some of which are nutritionally and economically important and conventional control approaches give rise to unsustainable production systems, in both, economic and environmental terms. For this insect control, a high quality biopesticide based upon the fungus *Verticillium lecanii* for the control of *T. vaporariorum* was developed. This formulation is a freeze-dried wettable powder for reconstituting in oil in water emulsion. In this formulation, conidia are protected from ultraviolet radiation damage. Biopesticide efficacy expressed as infection percentage for the white fly *Trialeurodes vaporariorum* when evaluated in plots on farmers in agricultural zones representative of tomato (*Lycopersicon esculentum*), and kidney bean (*Phaseolus vulgaris*) systems in Colombia was superior to 70% and increased profits, demonstrating the economical benefits were obtained. The strain and formulation technology of this product, is currently evaluated for *B. tabaci* control in crops as water melon and tomato.

Potatoes are an essential nutrition source in Latin America. This crop is severely affected by many plant pathogens and insect pests affecting negatively its production, commercialization and also decreasing the tubers quality. Among the most limitant insect pests Andean weevil *Premnotrypes vorax* (Coleoptera: Curculionidae) and *Tecia solanivora* (Lepidoptera: Gelechiidae). The last originating from Guatemala, has become a serious pest in Colombia . For the Andean grub *P. vorax* control, three biopesticide prototypes have been developed by using *Beauveria bassiana*, a granulate for direct application to the soil, a dispersible granulate for liquid application to the soil and to the plants and one emulsifying concentrate based on the use of vegetable oils and surfactants that allow to reconstitute the biopesticide in water obtaining an stable emulsion. These prototypes which produces more than 70% insect control under field conditions, are also been are also been evaluated in different potato production zones in a participation approach with farmers. Previous studies showed that among the natural enemies, viruses as a granulovirus PhopGV found in dead *P. operculella* larvae has also been efficient to control *T.solani*vora. PhopGV is used to control tuber moth infestations and is recommended by the International Potato Centre (CIP, 1995). With this isolate of *Baculovirus phtorimaea* a biopesticide was developed in Colombia for the control *T. solanivora* during the storage of potato. For its industrial production a biopesticide production Unit, with 15 ton per month production capacity was built up. This plant has been designed considering the Good Manufacture Practices and the whole manufacture process has been standardized generating the standardized operational procedures that constitute the Master Production Formula of the Baculovirus biopesticide. This product which has minimum 85% efficacy is the only management alternative for the insect control, and is sold to farmers in a low cost.

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Results and lessons learnt from ongoing and concluded research by CATIE and its partners

Bark beetles and Mahogany shoot borer: Semiochemical research in Latin America

Jorge E. Macías-Sámano[1]

Introduction

In Latin America, the major threats for pine forest and for the major tropical timber plantations are bark beetles (*Dendroctonus* spp. and *Ips* spp.) and the mahogany shoot borer (*Hypsipyla grandella*), respectively. Both pests have been extensively studied in their biology and ecology.

Bark beetle monitoring with traps lured with pheromones and kairomones is a well-established method, and used for the last 15 years in southeast United States to monitor *Dendroctonus frontalis* populations. At the time bark beetles are trapped, their predators are also caught, allowing an estimation of a prey: predator ratio, which in turn, with previous knowledge of prey-predator dynamics, provides with an inside for future bark beetle outbreaks (Billings 1988). Since *D. frontalis* is distributed from central east United States all the way to Nicaragua, is reasonable to believe that there are some variations in its dynamics and also in the associated species all across its distribution range. Formal studies on this subject are practically inexistent in Latin America and we suspect that direct implementation of this monitoring technology in our countries will result in a failure, since there is not basic knowledge on this prey- predator interactions and their chemical communications under our conditions.

In the case of *H. grandella*, there is plenty of information on biology, ecology, control and management generated mainly by a study group from CATIE in the 70's (Grijpma 1970, Whitmore 1976a,b) and more recently by Mayhew and Newton (2000). Chemical ecology studies had produced information to identify chemical composition of Meliaceae species to deter or to prevent attacks by the borer and also, very few, start elucidating pheromone composition (Macías-Sámano 2000 and literature therein).

Methods

To learn about the response, of *D. frontalis* and *Ips* spp. populations and their associated predators, to commercially available pheromones, one large experiment was set up on a pine forest in Motozintla, Chiapas, Mexico, The trial used multiple-funel traps baited with the pheromones frontalin, ipsdienol and ipsenol and the kairomone alpha-pinene (all from PheroTech). The experiment used five treatments (T1= control (unbaited trap), T2 = alpha-pinene (A), T3 = alpha-pinene + frontalin (F), T4 = alpha-pinene + ipsdienol (Ipsd), T5 = alpha-pinene + ipsenol (Ips). A random block design with ten replicates was used. Trapped insects were collected weekly through out 4 months during the main flight period. Insects were identified to species level.

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[1] El Colegio de la Frontera Sur ECOSUR, Tapachula, Chiapas, México.

An scanning electromicroscopy (SME) study was performed to situate the precise site where the pheromone gland of *H. grandella* is located. To obtain volatile components release while a female insect is calling, 3-days old virgin live females were subjected to dynamic aerations and to solid phase microextractions (SPME). Parallel, hexane washes of the abdomen's tip from insects (same physiological stage as the previous) were done. All volatile components were identified by GC analyses and their identity reconfirm by comparing their retention times with those of commercial standards. Three components, identified as potential pheromones, were tested individually and on binary combinations for attraction in a Spanish cedar plantation, using Unitrap traps and distributed in a random block design with ten replicates each treatment.

Results and Discussion

Bark beetle trapping produced very interesting results. As expected, frontalin plus alpha-pinene was the only semiochemical combination that attracted *D. frontalis*. Catches of this species were noticeably low compare to those occurred in the United States with the same semiochemicals and traps. *Temnochila chlorodia* and *T. virescens* (Coleoptera: Tenebrionidae) showed no specificity for any semiochemical (Fig. 1) proving to be a very generalist predator. An unexpected response was obtained from *Enoclerus ablusus* (Coleoptera: Cleridae), significantly responding to ipsenol and barely responding to frontalin (Fig. 2). This family is a major predator of *D. frontalis* in United States and responds preferentially to frontalin and not to Ipsenol. A previously unknown predator, *Elacatis* sp (Coleoptera: Othnidae) was captured in significant numbers to ipsenol and ipsdienol, (Fig. 3) strongly suggesting a prey specificity to *Ips* spp.

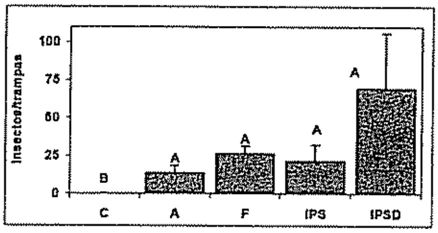
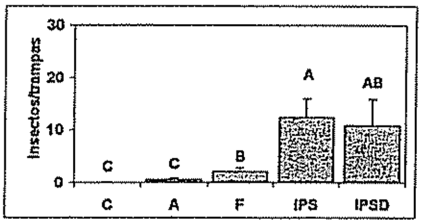


Fig. 1.



Catches of *D. frontalis*

Fig. 2. Catches of *Temnochila* spp.

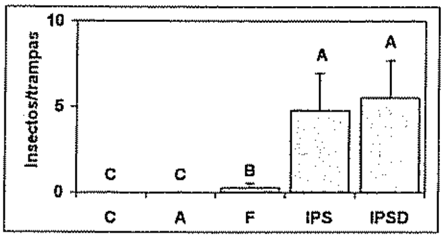


Fig 3 Catches of *Elacatis* sp.

SEM studies revealed that the pheromone gland of *H. grandella* is located ventrally in at the intersegmental membrane VIII-IX (MI VIII-IX) (Fig. 4). From the different techniques Z9-14:Ac, Z9-14:OH and Z9, E12-14:Ac were identified. All of them are well-known pheromones in different moth species. In the field the three components plus the alcohol Z9, E12-14:OH (all from ChemTica International) were tested individually in traps, without any significant differences among treatments. Z9,E12-14:Ac got the highest numerical catches and was used in the next experiment as a control.

A second experiment was done with the binary combinations of these components, with the following treatments: T1 = Z9,E12-14:Ac (control), T2 = Z9,E12-14:Ac + Z9,E12-14:OH, T3 = Z9, E12-14:Ac + Z9-14:OH, and T4 = Z9, E12-14:Ac + Z9-14:Ac. Although insect catches were not very high, this second experiment clearly showed that a 1:1 binary combination of Z9-14:Ac and Z9,E12-14:Ac got the highest number of males. Further experiments with ternary combinations were done with uncompleted results, most likely due to a very low numbers of insects flying in the plantation, therefore no statistical analysis can be done.

Fig 4. SEM photograph of the abdomen's tip of a female *H. grandella*.

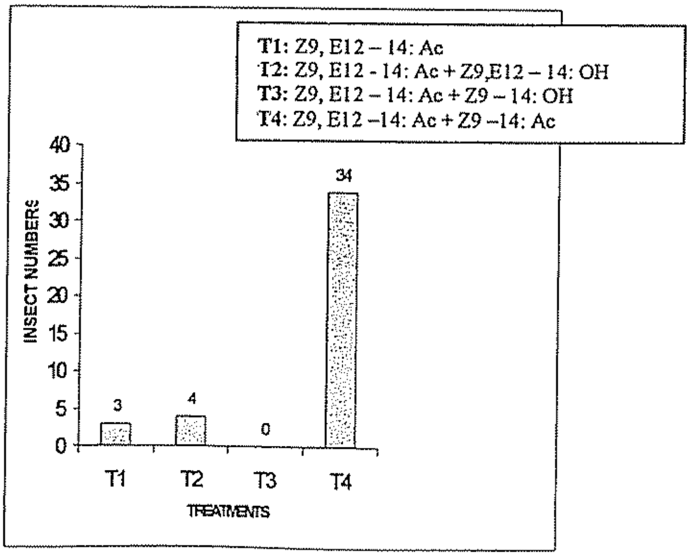


Fig 5. Catches of *H. grandella*

Conclusions

- It had became clear the necessity of testing the known commercial bark beetle semiochemicals to start generating basic knowledge on our own bark beetle populations and their associated predators. At the same time, the fact that clerids are not tuning to *D. frontalis* main pheromone under our conditions, implies to modify the actual methodology to forecast infestation trends, since is based on the dynamics between Clerids and *D. frontalis* populations and not with Tenebrionidae populations, which seem to be a more generalist predator.
- There is much to be done to elucidate the complete blend for the sexual pheromone of *H. grandella*. The activity of the two acetates Z9-14:Ac and Z9,E12-14:Ac is strong but at least a third component is missing and testing with a different trap might be important to increase the insect catches. The knowledge of this sexual pheromone will provide with an important tool to manage this serious threat to Meliaceae plantations.

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Management of tropical Coleoptera by mass trapping

A. C. Oehlschlager[1]

Trapping of *Oryctes rhinoceros* in Oil Palm in Malaysia

Commercial oil palm occupies over 4.5 million ha in Southeast Asia. At ~25 year intervals plantations must be replanted because the height of trees makes harvesting difficult. Replanting generates large volumes of dead palms that, prior to 1990, in Malaysia were routinely burned. In the early 1990's increasing population in the Malaysian peninsula forced a ban on burning and required alternative disposal techniques such as chipping. Since rotting palm trunk is an excellent environment in which *Oryctes rhinoceros* (coconut rhinoceros beetle) breed *Oryctes* populations increased steadily since burning was banned. Adults emerge in areas with an abundance of their preferred host, young palms, to feed on fronds and spears. Damage is most severe during the second and third year after planting.

In the early 1990's the author's research group in Canada identified a male-produced an aggregation pheromone for *O. rhinoceros* that was highly attractive to male and female beetles (Hallett et al. 1995). Trap and lure optimization led to the selection of a vane trap mounted above the canopy for this insect (Gait Fee Chung, Unpublished). Trials by Gait Fee Chung of Sime Darby of Malaysia determined that most populations could be lowered by over 80% in one year by 1 trap/ 2 ha. Trapping at 1 trap/ 2 ha coupled with biweekly servicing makes pheromone trapping less expensive than application of insecticide to 288 plants once every 2 weeks (Gait Fee Chung, pers. comm.).

Trapping of *Rhynchophorus palmarum* and *Metamasius hemipterus* with the same lure in Palmito Palm in Costa Rica

The heart of palmito palm (*Bactris gasipaes*, Kunth) is a delicacy in many countries. Increasing demand for dietary fiber continues to fuel demand for palmito. Areas dedicated to commercial production in Central and South America in 1996 were about 12,000 ha of which around 4,000 ha were in the Atlantic Region of Costa Rica.

Palmito palm propagates from offshoots that grow to a harvestable height of one meter in about 3 months. Harvesting discards all parts of the plant except the interior of the stem. In some plantations, competing offshoots are pruned to promote more rapid growth of the remaining offshoots to harvestable size. Harvesting and pruning provide excellent entry points for *Metamasius hemipterus* L. and *Rhynchophorus palmarum* L. Females of these weevils are attracted to and deposit eggs in cut stem bases. Larvae tunnel the lower stem and rhizome destroying maturing stems.

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[1] ChemTica Internacional. San José, Costa Rica.

Male-produced aggregation pheromones for both weevil species are known (Perez et al. 1997, Oehlschlager et al. 1992). Initial experiments conducted in Costa Rica and Honduras in 1995 led to development of a blend of the two pheromones that allowed trapping of both species in the same trap. These experiments allowed combination lure trapping of both species in palmito palm.

Commercial palmito is grown under two regimes, one involves harvesting only (non-pruning) while the other involves pruning mats before during growth to encourage the faster growth of only the strongest stems (pruning). Mass trapping in commercial palmito employing both these growing options was carried out using the combination lure for *M. hemipterus* and *R. palmarum*. Traps were set in two 1 ha plots (1 pruning and 1 non-pruning) using 4 traps/ ha.

Trapping was conducted over 1 year with several damage and yield assessments during this period. Relative to control lots the percentage yield increase attributable to trapping was 58% in plots in which pruning was conducted and 70% in plots in which pruning was not conducted (Alpízar et al. 2002).

Trapping *R. palmarum* in coconut

Trapping of *R. palmarum* in coconut palm is also effective. During 2000-2001 ChemTica conducted trapping of this weevil in 50 ha of coconut palm using traps similar to those used below in the Coto and Quepos trials. In the coconut palm plantation in which trapping was conducted direct damage by *R. palmarum* larvae was responsible for palm death.

A trap density of 1 trap/ ha was used in this trial (Oehlschlager and Gonzalez, 2004 accepted for publication). Capture rates of *R. palmarum* were initially ~11/ trap/ week and did not show a significant decline during the 11 month study. In spite of the continued high numbers of *R. palmarum* captured for the entire trapping trial the number of weevil killed palms dropped by 88%.

Trapping *R. palmarum* in oil palm

Weevils of the genus *Rhynchophorus* are also a major problem in oil palm. In Central and South America *R. palmarum* is a major economic problem. Larvae often kill trees and adults carry red ring nematode that is lethal to palms.

Prior to 1993 management of this pest was by systematic inspection of palms coupled with cutting and spraying of cut palms with insecticide. This practice did not control red ring infestation carried by the weevil. In the early 1990's ChemTica, in collaboration with ASD de Costa Rica, developed a trap for *R. palmarum* that employed the male-produced aggregation pheromone and insecticide-laden sugarcane or palm (Oehlschlager et al. 1993).

Two examples of trapping *R. palmarum* using this trap may be cited. An oil palm plantation of ~5,000 ha established near Coto, Costa Rica in the mid 1970's was mature by the late 1980's. Red ring nematode infestation (RRD) was first detected in the Coto oil palm plantation in 1989. In that year 5,171 of ~800,000 palms were diagnosed with RRD. These palms were cut and sprayed with Furadan.

During 1990 and 1991 the only measure undertaken to manage RRD was elimination of RRD infected palms. During these years the number of RRD infected palms in the plantation *approximately doubled each year*. Following trap, lure and trap density optimization trapping throughout the plantation was commenced in 1992 using a trap density of 1 trap/ 5 ha. During the first year of trapping capture rates in the plantation declined from 30 weevils/ trap/ month to 4 weevils/ trap/month, or over 80%. During the period between 1994 and 2001 monthly capture rates were no higher than 2 weevils/trap/month (Chinchilla et al. 1993, Oehlschlager et al. 2002). Red ring disease dropped by 80% during the first year of plantation-wide trapping and has remained low since that time (Oehlschlager et al. 2002).

Plantation-wide mass trapping was also conducted on 8,719 ha of commercial oil palm near Quepos, Costa Rica with similar results. Capture rates were initially high but declined to less than 4 weevil/ trap/ month by 1994. In 2001 the mean capture rate of traps in the Quepos plantation was 1.13 ± 0.16 weevils/ trap/ month (Oehlschlager et al. 2002).

Trapping *R. ferrugineus* in date palm

Mass trapping of *R. ferrugineus* is widely practiced in the Arabian Peninsula where it is a major problem in date palm. Management of *R. ferrugineus* relies on frequent inspection of palms to detect infestation, treatment of infested palms by injection of insecticide or removal, periodic spraying and trapping (Abraham et al. 1998).

A major study in the UAE between 1996 and 1998 included 1,466 farms containing >349,000 palms examined the effect of spraying alone and spraying combined with pheromone trapping. A benefit of ~30% less infestation was derived from combined use of spray and pheromone traps compared to spray alone (Ezaby et al. 1998).

In a recent UAE study in which traps were placed on 6 different farms over one year with no spraying the highest captures resulted in the greatest reduction of infestation (Kaakeh et al. 2001). Interestingly, in this study the average reduction in infestation over all 6 farms from one year to the next was 71%. This suggests that trapping *R. ferrugineus* is as effective as trapping *R. palmarum*. One can expect ~70% reduction in infestation over one year if *R. ferrugineus* traps are maintained well and infested palms are treated to prevent further breeding.

Trapping of *R. ferrugineus* in date palm over 2 years in India resulted in a 75% decrease in captures suggesting a significant decrease in population (Muralidharan et al.1999). No damage assessment was made in this study. The decrease in capture rate observed in this study was very similar to the reduction in capture rate in Costa Rica for trapping *R. palmarum* in oil palm (Chinchilla et al. 1993, Oehlschlager et al. 2002).

Conclusions

- Palm weevils are present in most plantations in relatively small numbers and have a relatively long life. These characteristics allow mass trapping to be an efficient management technique since capture of low numbers can significantly impact future populations and a significant proportion of an adult population can be captured over the long period they are susceptible to pheromone and food traps.

- Since palm weevils are strong flyers traps can be widely spaced and trapping is expected to be more efficient than spraying for weevil management.

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Development of semiochemical research at CIPRONA-UCR

Gerardo A. Mora[1]

Introduction

CIPRONA, which stands for Centro de Investigaciones en Productos Naturales, in Spanish, was established in 1979, at the University of Costa Rica (UCR), with the participation of researchers from the Department of Chemistry of the Faculty of Sciences and Letters (Liberal Arts) and the Faculty of Pharmacy.

Approximately during the first ten years, under the guidance of the late Prof. José F. Calzada (†1987), most of the work had to do with classical phytochemistry and involved only a few plants that could be called bioactive. However, the interest was purely phytochemical and not at all based on the biological activity of the plants. The interest in the applied field was started sometime in the middle of the 80's and the beginning of the 90's, thanks to the contact with Prof. Harry H. Szmant, who organized and conducted a series of workshops on the industrial utilization of natural products, sponsored by the Organization of the American States (OAS).

In fact, this marked the beginning of a transition towards a second period in the history of CIPRONA. Some work was done also with fungal diseases of agronomical interest, like the American Leaf-Spot Disease (*Mycena citricolor*, a coffee fungus) and Monilia (*Moniliophthora roreri*, a cacao fungust). Some efforts were made for the synthesis of derivatives of yodoacetamide and pheromones of agronomical interest and this marked the beginning of our involvement with semiochemical research.

Of mayor importance for this involvement was a relationship started with the Tropical Agricultural Research and Higher Education Center (CATIE) regarding non-timber forest products, due to the interest of Rafael Ocampo. Specifically, we worked with plants like *Quassia amara* and *Smilax* spp., which are important for some of the native people of the Baja Talamanca (Southern Caribbean) region of Costa Rica. The relationship with CATIE has evolved into an interesting partnership into the research of natural pest control. Other interests are directed towards the synthesis of analogs of active compounds.

Work with phytopathogenic fungi

The work with *Mycena citricolor* was done in collaboration with the Laboratory of Plant Pathology of the University of Costa Rica and the University of Alberta, Canada, sponsored by the IDRC. This work concluded with the identification of oxalic acid as the causative agent of the spots so characteristic of this disease and the design of several treatments with calcium salts to prevent the damage.

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The work with *Moniliophthora roreri* was addressed as a thesis for the Masters degree for Ana Isabel Santana, a student from Panama, guided by J. F. Calzada. The idea, following the story of *M. citricolor*, was to isolate some compound or fraction from the mycelium or the growing medium (*in vitro*) of the fungus which could cause damage in the young cocoa fruits similar to the necrosis characteristic of the disease. A poor technique for the *in vivo* testing methods and a great variability in the results did not allow for conclusiveness. Some activity against *M. citricolor* was also found in some 3-substituted-4-hydroxycoumarins synthesized as analogs of a similar compound found in the Asteraceae *Lycoseris latifolia* (Castro et al. 2000).

Synthetic efforts to obtain pheromones and kairomones

The interest in pheromones started from the chemical point of view. J. F. Calzada had been working with some procedures for the introduction of double and triple bonds, so common in many pheromones. So, the possibility of using this knowledge in an applied manner to try to solve a local problem was very appealing and an attempt was made to synthesize the sex-attractant pheromone of the Guatemalan potato moth (*Scrobipalpopsis solanivora*). The results were good and when we tried to commercialize the field traps, we experienced the first encounter with the cruel real world of competing with transnational enterprises and our attempts were crushed by Monsanto (And they most probably do not know about this!).

From this activity, came a relationship with Cam Oehlschlager, who at the time was at Simon Fraser University, in Canada[1]. He took two students from UCR under his guidance and, in this way, Jorge Cabezas[2] and Alice Pérez[3] got their Ph.D. degrees. Cabezas is not with CIPRONA anymore, but he is working on the chemistry of pheromones, improving published methods and developing new ones for the synthesis thereof. He is also working on the chemistry of new methods for the synthesis of double and triple bonds.

Alice Pérez is working, together with Guy Lamoureaux, on the chemistry of nafthoquinones, similar to several compounds found in the genus *Calciolaria*, looking for bioactive compounds, including ones with possible activity against insects such as the whitefly *Bemisia tabaci*. They have developed new procedures for the synthesis of lawsone derivatives and synthesized known and new compounds of this type, which are being tested under greenhouse conditions with the collaboration of other researchers of the University of Costa Rica.

Insect-related activity in plant extracts

This activity was started when Rafael Ocampo[4] was working at CATIE on OLAFO, a project for non-timber forest products. His initial approach was to work with <u>Quassia</u>

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[1] Cam Oehlschlager is presently co-owner and member of the board of directors of ChemTica, a firm established in Costa Rica for the synthesis and commercialization of pheromones.

[2] Jorge Cabezas is presently working at the School of Chemistry, University of Costa Rica.

[3] Alice Pérez is presently the Director of CIPRONA and Surrogate Chairperson of the School of Chemistry, University of Costa Rica.

[4] Rafael Ocampo does not work at CATIE any longer but continues his collaboration with this institution. Among other occupations, he is the President of Bougainvillea, S.A., an enterprise looking for the

amara ("hombre grande" or bitterwood) and the initial response was to reject such collaboration, based on the fact that the chemistry of this plant was so very well known. We finally gave in to his request, mainly because of the social interest of the project. The idea was to help some native population of the Baja Talamanca Region (Kekoldi) who managed the wild population of the plant for insecticidal purposes. In this way, we collaborated with CATIE for the isolation of quassin and neoquassins, to be used as standards for the HPLC quantification of these compounds in individuals of *Q. amara* collected in different parts of the country. This was part of the work for the thesis of Róger Villalobos[1], pursuing the Masters degree in CATIE.

Interestingly, this opened new avenues for us at CIPRONA and for the School of Pharmacy at UCR. A paper was published on the healing properties of *Q. amara* for ulcers in rats (Badilla et al. 1988). This paper received the 1998 De Girolami Award and helped put the plant again under the scrutiny of research for purposes other that insecticidal.

Also, with the guidance of José F. Cicció, of CIPRONA-UCR, and the help of Rafael Ocampo, of Bougainvillea, S.A., we are looking at the chemistry of this plant at different stages of development, as well as improving and simplifying the analysis, in order to develop quality control procedures and collaborate in the industrialization of the extract or any other products derived from it.

More recently, in collaboration with research guided by Luko Hilje[2], of CATIE, the extract of this plant has shown promising activity for managing *B. tabaci* and *Hypsipyla grandella* (Mancebo et al. 2000), as will be shown elsewhere during this workshop. As part of an ongoing program of collaboration with Luko Hilje at CATIE, other plant extracts have been tested on either of these two pests, looking for repellent effects, feeding or egg-laying deterrence, or insecticidal activity (Mancebo et al. 2001, Gómez et al. 1997, Soto 2000, Aguiar et al. 2003, Flores 2003).

Some of these are *Gliricidia sepium* (madero negro, mother of cocoa), *Sechium pittieri* (tacaco cimarrón, wild tacaco), *Ruta chalapensis* (ruda, common rue), *Allium sativum* (ajo, garlic), *Chenopodium sp.* (apazote, worm-seed), *Canavalia ensiformis* (canavalia, sword bean), *Allium cepa* (cebolla, onion), *Drymis granatensis* (chilemuelo), *Capsicum frutescens* (chile picante, hot pepper), *Cymbopogon nardus* (citronela, citronella), *Coriandrum sativum* (culantro de Castilla, coriander), *Syzygium aromaticum* (clavo de olor, clove), *Eucalyptus deglupta* (eucalipto, eucalyptus), *Neurolaena lobata* (gavilana), *Lippia graveolens* (orégano, oregano), *Momordica charantia* (sorosí, balsam pear), and *Tithonia diversifolia*) (titonia, wild sunflower). Results were compared with commercial products, including neem (*Azadirachta indica*) (Mancebo et al. 2002). All plants extracts were prepared in aqueous ethanol (or methanol) (normally 20% water) and, in some cases, the extract was freeze-dried and fractionated on a synthetic reverse-phase resin, using water, water/methanol, methanol, and ethyl acetate or diethyl ether as eluants. Only with *Quassia*

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domestication of medicinal plants and presently engaged in the production of an extract of *Q. amara* for insecticidal purposes. A pilot plant was set near Cariari, Limón.

[1] Róger Villalobos obtained his M. Sc. degree and is presently working at CATIE.

[2] Luko Hilje is working at CATIE, in the Plant Protection Unit.

amara and *Sechium pittieri* we have an idea of the chemical identity of the active principles.

Projections

Much work needs to be done to isolate and identify the active principles of the promising plant extracts and to check the activity of the alkaloids found in *Q. amara*. Also, other areas should be addressed, such as activity against vectors of tropical diseases. This is a most neglected area.

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Plant extracts as a source of deterrents/repellents against whiteflies and the mahogany shootborer

Luko Hilje[1]

Introduction

The issue of biodiversity has received a lot of attention in recent years, because of the concern about the high rates of destruction of some of the last major forest masses on Earth, many of them located in the neotropics. These forests contain many organisms not yet described, some of them potentially useful for humankind. However, rather few resources have been allocated to search for biodiversity applications to agriculture, including integrated pest management (IPM) programs. Biodiversity and IPM have been shown to be closely interrelated. As for the exploration of natural insecticides, a large number of tropical plants offer an untapped potential as insecticide sources.

Insecticides or deterrents/repellents?

Plants may contain a wide array of substances acting against insects, including repellents and deterrents, which interfere with the ability of insects to locate, feed or oviposit on their host plants. Even though there are long lists of plant species claimed to have compounds with such properties, many references are rather anecdotic. Fortunately, the interest in discovering, characterizing and exploiting natural products against insects has encouraged scientists to formally pursue the screening of crude extracts for biological activity.

However, despite the fact that the agrochemical industry has shown interest in developing commercial insecticides based upon either natural substances or their synthetic analogues, there has been little interest in repellents and deterrents. Perhaps this is because their production process is as complex as that of an insecticide, whereas their effects under field conditions do not eliminate a pest problem, but rather transfer it to neighboring fields.

Target pests

Even though the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) and the mahogany shootborer *Hypsipyla grandella* (Lepidoptera: Pyralidae) are very different insects both taxonomically and biologically, they have some commonalities in regards to their damage and possible management approaches.

On the one hand, the highly polyphagous agricultural pest *B. tabaci* acts mainly as a virus vector of several crops (tomato, bell pepper, beans, melon, cotton, etc.) in Mesoamerica, the Caribbean and other tropical regions. On the other hand, *H. grandella* attacks precious trees of the Meliaceae family, such as mahoganies (*Swietenia* spp.) and cedars (*Cedrela* spp.). Its

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[1] Plant Protection Unit, CATIE. Turrialba, Costa Rica

larva mainly bores the terminal shoots of trees, which causes forking of the stems, which has frustrated attempts to establish commercial plantations of such species in Latin America and the Caribbean.

Insecticides have generally failed to provide effective control of *B. tabaci*, not only because of the development of resistance to many compounds, but also because the damage threshold can be as low as one individual per plant. For *H. grandella*, a damage threshold as low as one larva per tree renders trees unmarketable; in this case, since damage caused by even one individual insect can result in irreversible losses, it would be worthless to kill the larva once damage is done.

Therefore, in both cases, insecticides are not very helpful. On the contrary, to deal with pests with very low damage thresholds, where a preventive management scheme is well in place, deterrents or repellents could prevent these pests from causing serious damage. Such a scheme should also be environmentally friendly and cost-effective.

Approaches and achievements

In case of eventually counting upon formulated deterrents or repellents to use against *B. tabaci* and *H. grandella* in the field, their use could be optimized by deploying them only during certain times in the crop life, aimed at minimizing contact between the insect and the host plant. For instance, since the impact of viral diseases on yields is higher at earlier stages of plant development, any management scheme for *B. tabaci* should focus on this critical period (60 days after germination). For *H. grandella*, the critical period corresponds to the first 5-8 years of tree development, depending on the region. In both cases, of course, during such a period the deterrents or repellents could be complemented with other IPM tactics

Whiteflies. So far, some 70 plant extracts, including formulated commercial products and crude (hydroalcoholic) extracts, have been tested at the Tropical Agricultural Research and Higher Education Center (CATIE). The latter, which include samples from leaves, seeds, bulbs, flower buds, fruits or essential oils, have been prepared according to standard laboratory protocols at the Centro de Investigaciones en Productos Naturales (CIPRONA, University of Costa Rica). They were selected based on ethnobotanical references, as well as on their low or nil taxonomic affinity with the most common hosts of *B. tabaci*.

Crude extracts from the following 10 species have stood out for their ability to deter adult whiteflies: bitterwood (*Quassia amara*), "chile muelo" (*Drymis granatensis*), fish bean (*Tephrosia vogelii*), mother of cocoa (*Gliricidia sepium*), neem (*Azadirachta indica*), "sorosi" (*Momordica charantia*), sword bean (*Canavalia ensiformis*), wild sunflower (*Tithonia diversifolia*), wild "tacaco" (*Sechium pittieri*), and worm-seed (*Chenopodium ambrosioides*). Their effect has been detected under greenhouse experimental conditions, at doses as low as 10 ml/l water (1% v/v) (Fig. 1). The next step has been to test fractions (methanol, water, methanol: water, and ether) of some of the promising extracts. Methanol fractions of *G. sepium* (Fig. 2) and *Q. amara*, as well as the aquaeous fraction of *S. pittieri*, caused phagodeterrence at low doses (0.1, 0.1 and 0.5%, respectively).

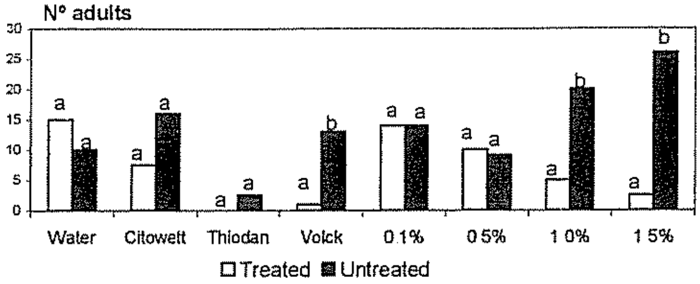

Figure 1. Average number of landed *B. tabaci* adults 48 h after the cocoa-shade (*Gliricidia sepium*) extract was applied to tomato plants, in a restricted-choice experiment. Means followed by the same letter in each pair of bars are not significatly different (P= 0.05) (Hilje and Stansly 2001)

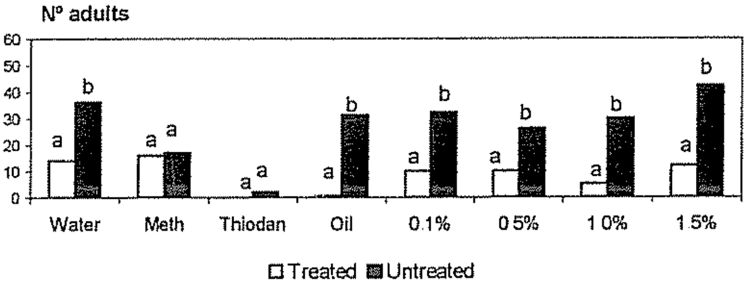

Figure 2. Average number of landed *B. tabaci* adults 48 h after the methanol fraction of the cocoa-shade (*Gliricidia sepium*) extract was applied. Means followed by the same letter in each pair of bars are not significatly different (P= 0.05) (Flores 2003)

Mahogany shootborer. Some 20 plant extracts have been tested, including some of the ones also tested for *B. tabaci*. Four of them have shown some type of biological activity against larvae: bitterwood (*Quassia amara*), common rue (*Ruta chalepensis*), wild "tacaco" (*Sechium pittieri*) and neem (*Azadirachta indica*). In fact, methanol extracts of wood of *Q. amara* (Fig. 3) and foliage of *R. chalepensis* have substances acting as feeding deterrents.

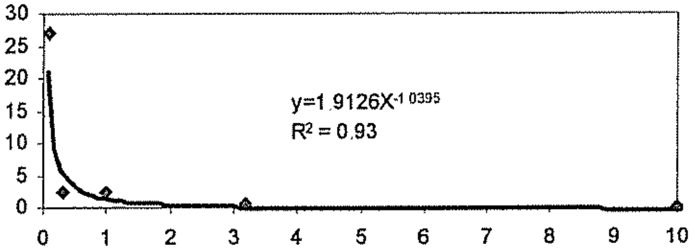

Figure 3. Cedar leaf disk consumption by third-instar *H. grandella* larvae, in response to increasing concentrations of wood extracts of bitterwood (*Quassia amara*). The continuous line depicts the predicted response curve (Mancebo et al. 2003).

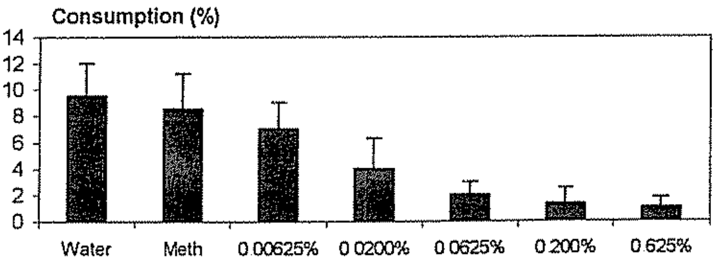

Figure 4. Average cedar leaf disk consumption (in 24 h) by third-instar *H. grandella* larvae, in response to increasing concentrations of the methanol fraction of bitterwood (*Quassia amara*) (Soto 2000).

Likewise, when fractions of of *Q. amara* were tested, methanol and ether fractions stood out as phagodeterrents. Bioassays (exposing instar III larvae to disks impregnated with increasing concentrations of the methanol fraction) showed that consumption of disks was significantly lower at doses as low as 0.0625% (Fig. 4).

Concluding remarks

- Several tropical plant crude extracts, as well as their fractions, can act as strong phagodeterrents of *B. tabaci* and *H. grandella*. It remains unknown which specific substances are responsible for causing the observed effects.
- Recently, CATIE's work on deterrents/repellents was expanded to test pure substances of botanical origin manufactured by ChemTica International.

Acknowledgements

To Gerardo Mora and Juan C. Brenes (CIPRONA), for his permanent collaboration in these endeavors. To my students (Fernando Mancebo, Francisco Soto, Guillermo Flores, Alana Aguiar and Paul Gómez) and assistants (Manuel Carballo, Douglas Cubillo, Guido Sanabria and Arturo Ramírez) who have supported these efforts.

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Neem as a source of semiochemicals against several insect pests

Anne Kathrina Gruber[1]

Introduction

Neem (*Azadirachta indica* A. Juss, Meliaceae) is a rapid growing, medium high tree from southeast Asia (India, Sri Lanka, Burma), well adapted to tropical dry climates and introduced to Central America from 1975 on. In India it was long known as a medicinal plant, but also as a source of repellents against pests of stored grains.

In the 1970's real insecticidal properties were observed, too. And in the 1980's the main responsible substance, azadirachtin, was isolated from the seeds and its molecular structure elucidated (Broughton et al. 1987). In the following 15 years the rest of the different active substances were isolated from seeds, leafs, bark and roots of the tree, all belonging to the biochemical group of the triterpenoids (limonoids).

Since then more than 340 species of insects and nematodes have been tested as positively responding to these substances. The worldwide interest on neem as a natural source of alternative products for pest control results from the non-toxicity, the target specificity and very reduced effect on natural enemies, the complete biodegradability and the relatively cheap and easy way of production.

The active biochemical substances of the neem tree and there mode of action

The neem tree contains an astonishingly high amount of secondary plant substances. The main group consists of more than 60 different triterpenoids synthesized by the tree in the so called isoprenoid pathway. Most of them are identified in the leaves and in the seeds, some others in the bark, the roots or the wood, none in the flowers. Many of them have a more or less repellent activity against insects, being the strongest ones in the group of nimbines and nimbidines. Others have an antifeedant effect, being the salannins the best investigated. Even some specific changes in the behavior of adult insects could be attributed to neem substances.

But only the 13 isomers of *azadirachtin* extracted from the seeds are able to exert a real insecticidal effect upon insects. The repellent substances of neem (nimbin and others) sprayed on the plants do signal danger or bad flavor to the insects and are received by their chemoreceptors. The antifeedant substances (salannin and others) must be ingested and seem to inhibit the normal gust movements, so that the insect will reduce digestion, reducing their appetite as a feed-back reaction. The azadirachtins must be ingested, too. As real insecticidal agents their mode of action upon the metamorphosis and egg maturing is the best investigated by endocrinologists, having allowed at the same time a far better knowledge of the complex hormonal system of the insects. The target molecule of

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[1] Investigaciones Orgánicas S A. Managua, Nicaragua

azadirachtin is not yet known but it seems to be proved that these substances were transported to the insect's neurosecretory cells of the brain, able to inhibit or reduce drastically the PTTH (prothoracicotropic hormone) production, which is necessary to regulate the biosynthesis and release of ecdysone and/or juvenil hormones responsible for the normal progress of metamorphosis and gonadotropic cicles (Rembold 1995). The ingestion of azadirachtins by immature stages acts like a growth regulator and conducts to death. The azadirachtins have also an effect upon longevity of the adult stages of several insect species and upon the fecundity and oviposition rate of females. They can be called "nature's soft chemicals" (Schmutterer, ed. 1995). Neem seeds contain about 48% of oil consisting of various fatty acids, which contribute to the pest control.

The main pests controlled by neem substances

Up to 1995 there where 324 species of insect pests and nematodes investigated to show some reaction to applications of different neem preparations (Schmutterer ed. 1995). Some new species are put to this list every year. The main group consists of the biting insects (larvae of Lepidoptera, immature and adult stages of some Coleoptera, immature stages of Orthoptera and larvae of some Diptera), all leafminers are included. All these insects need to be treated with water or alcoholic extracts of the ground neem seed or of the ground cake (a residue of the oil pressing) at a concentration of at least 30 to 50 ppm of azadirachtins in the sprayed liquid. The second group are the sucking insects (Homoptera). Their immature stages are very susceptible to the neem oil and need far less azadirachtins to react. So they can be controlled by spraying an emulsifiable neem oil with no more than 10 to 15 ppm of azadirachtins in the sprayed liquid. Also insect and nematode pests in the soil can be controlled applying the ground neem cake directly to the soil.

So nearly all economically interesting pests of cultivated plants and forest trees can be controlled by some neem product with the exception of those who are born deep within the plant tissues or reach to introduce themselves very rapidly after eclosion. Common insect pest in the tropics controlled by neem are: *Spodoptera* spp., *Helicoverpa zea, Heliothis virescens, Manduca sexta, Feltia subterranea, Plutella xylostella, Diabrotica* spp., *Cerotoma* spp., *Liriomyza* spp., *Leptinotarsa decemlineata, Phyllocnistis citrella, Thecla basalides, Hypothenemus hampei, Hypsipyla grandella, Bemisia tabaci, Thrips tabaci, Nezara viridula, Leptoglossus zonatus, Phyllophaga* spp. and the nematodes *Meloidogyne* spp. and *Pratylenchus* spp., as the best investigated examples.

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Participatory research on biocontrol of frosty pod (*Crinipellis roreri*) and black pod (*Phytophthora* spp.) of cocoa (*Theobroma cacao*) using mycoparasites

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Claudio Arroyo[1], Johnny García[1], Armando Portuguez[1], Manuel Palacios[3],
Octavio Menocal[4], Vilmár Sánchez[5], Robert Mack[6] and Hans Grebe[7]

Introduction

Frosty pod, caused by *Crinipellis* (=*Moniliophthora*) *roreri*, and black pod, caused by *Phytophthora* spp., are the leading cocoa (*Theobroma cacao*) diseases in Central America. Under traditional management, combined losses generally exceed 80% with *C. roreri* being most damaging. Due to the certified organic status of most of the regional production, cultural and biological control are the only disease management options available to the indigenous smallholders, who are affiliated with the co-operatives. Resistant germplasm is not yet available to growers.

Biological control of frosty pod and black pod by broad host-range mycoparasites has previously shown great promise in Peru, especially with antagonist mixtures (Krauss and Soberanis 2001, 2002). Our objective was the participatory evaluation and optimisation of mixed inocula, consisting of native and Peruvian antagonists, for the simultaneous, effective and economical biocontrol of frosty pod and black pod under conditions of farmers' fields in multilocational trials in Central America.

Materials and Methods

Scientists, rural developers, extension staff and smallholder producers jointly designed these participatory trials in Nicaragua, Costa Rica and Panama. These were supported by on-station trials in La Lola and laboratory experimentation at CATIE. Antagonist mixtures consisted of *Clonostachys* and *Trichoderma* spp. We chose the randomised block design throughout and, when needed, arranged treatments in a factorial manner. We made no attempt to minimise edge effects or high within- and between-field variability because these phenomena are a smallholder reality.

Results and Discussion

In Panama, four out of seven mixtures of native mycoparasites reduced black pod significantly. No inoculum reduced frosty pod incidence, but five treatments reduced inoculum production by the pathogen. All but one treatment improved yield by up to 27% (average improvement 19%). In Nicaragua, a different Central American antagonist mixture performed best, also with a 27% yield improvement. However, this treatment was

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mixture performed best, also with a 27% yield improvement. However, this treatment was ineffective in Costa Rica where Peruvian antagonists, which were ineffective in Nicaragua, excelled.

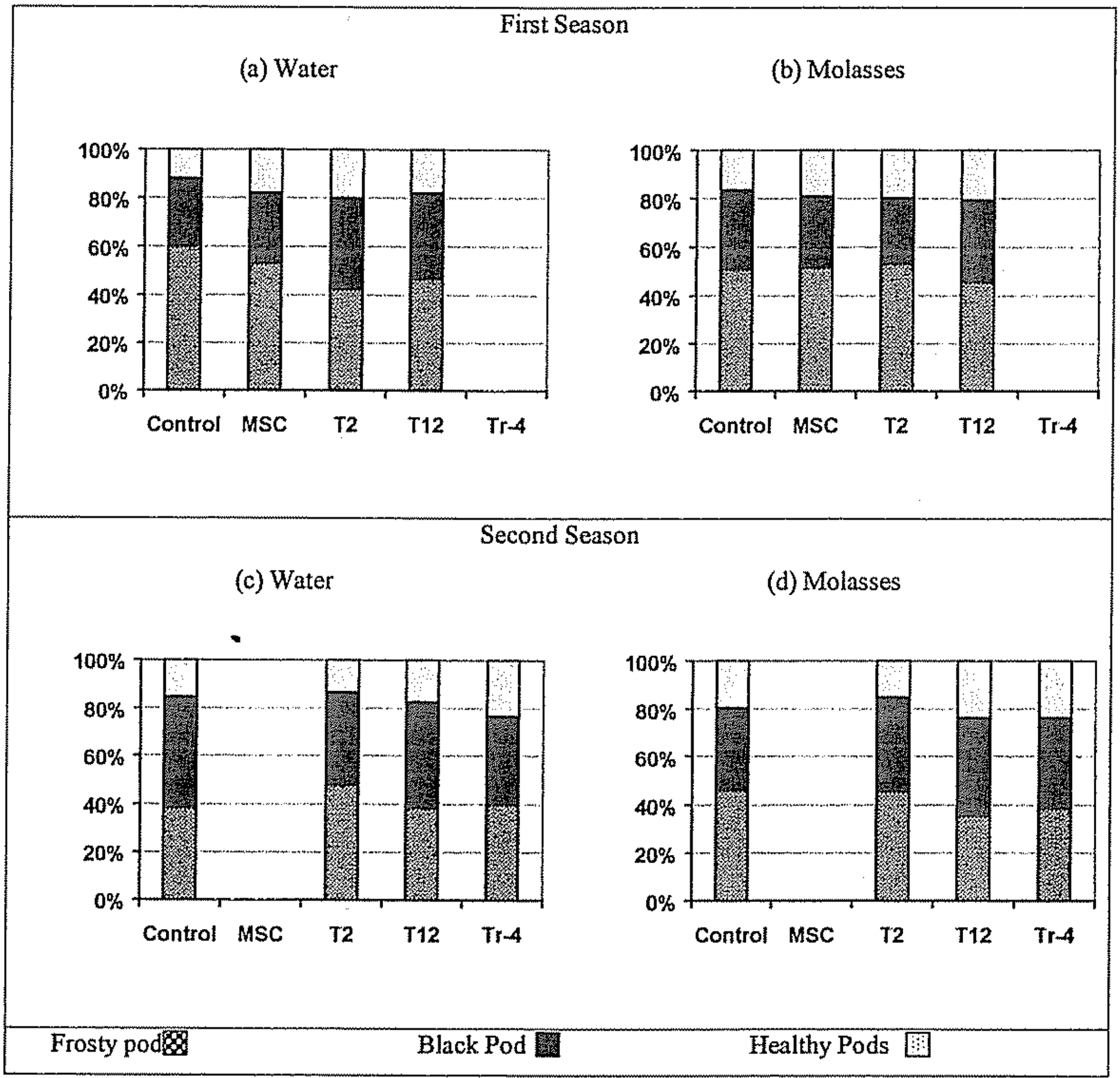

Fig 1. Disease incidence and percentage healthy pods in participatory biocontrol trials in Panama testing four inocula and two formulations (water and 3% v/v molasses) over two growing seasons.

In follow-up studies, the four most promising treatments (three local mixes and one Peruvian single strain: *Trichoderma* sp. (Tr-4) were tested in two formulations, water and 3% molasses, over a two-year period. In the first year, all treatments improved the percentage of healthy pods (Figure 1). The water formulations were effective against frosty pod. Amended with molasses, two treatments effected black pod control, a different one frosty pod control. In the second year, only *Trichoderma* Tr-4 increased the percentage of healthy pods. In contrast to the first year, two water formulations reduced black pod, but not frosty pod, whereas two treatments formulated in molasses reduced frosty pod. Only Tr-4 reduced both diseases but in different formulations (Figure 1).

Black pod behaved opposite to frosty pod, i.e. successful biocontrol of frosty pod was accompanied by increased black pod and *vice versa*. Young pods (< 2 months) are particularly susceptible to frosty pod (Evans 1981). Under high disease pressure, as observed here, few pods escape early infection by *C. roreri*. When protected by biocontrol, however, more pods reach a larger size and, thus, become available for infection by *Phytophthora* spp. unless the BCA shields them against both diseases. In Peru, where Krauss and Soberanis (2001 , 2002) did their work, black pod is of lesser importance than in Panama, where both diseases are perilous.

The effect of molasses was complex. This cheap agricultural by-product improved yield, mostly due to a beneficial effect on black pod control. However, biocontrol agents reacted differently to molasses in different years. Therefore, it would be premature to issue a generalised recommendation. Nevertheless, molasses amendments may be an economic additive for improved biocontrol in many cases and merit further testing.

Disease incidence with cultural control applied in fortnightly intervals (controls in Figure 1) was exceedingly high. About half of the crop was lost due to *C. roreri* alone. Between 30% and 40% were lost to *Phytophthora* spp., resulting in combined losses of over 80%. Soberanis et al. (1999) showed that weekly phytosanitation is needed to control frosty pod in Peru because *C. roreri* sporulates within one week after the appearance of brown lesions on the pods, the symptom most likely to be recognised by the farmers during phytosanitary inspection. Leach et al. (2002) suggested that this recommendation holds under conditions in Talamanca, Costa Rica. Our results are in agreement with their assessment.

The main effect of biocontrol agents on *C. roreri* was a reduction in spore production by over 40% on average. Although we identified several biocontrol agents that significantly improved the control of frosty pod or of black pod compared with cultural control alone, current control levels are only moderate. The much larger effect on the number of sporulating pods than on disease incidence suggests frosty pod control is mediated via a reduction in sporulation, i.e. reduction of pathogen inoculum. The experimental unit in our trials was only 20 trees. If weekly cultural control and biocontrol application were practised on a larger scale, less inoculum pressure would almost certainly further decrease frosty pod incidence.

The participatory research methodology employed here proved highly productive. Feedback between researchers, extensionists and growers led to a learning process and enabled us to design trials adapted to specific questions of interest to all parties, such as the effect of applied nutrients on biocontrol efficacy. Some flexibility on both sides was required, i.e. in mutual agreement, MSC was replaced by Tr-4 after one year. Although such decisions increase statistical variability, the randomised block design is well suited to accommodate these situations, as well as marked biophysical and man-made differences between farms. The latter is important because our aim was to select treatments that are effective on a multitude of smallholdings with different microclimates, germplasm and management practices. In contrast to results obtained on research stations under much more controlled conditions and the management of scientists, treatments that prove effective under on-farm conditions can be assumed to be very robust.

Conclusions

Combined losses due to frosty pod and black pod of over 80% in plots with fortnightly phytosanitation could be reduced significantly by several biocontrol agents in farmers' fields. This is a highly promising finding. Biocontrol of frosty pod appeared to be mediated through a reduction in inoculum production by *C. roreri*. Therefore, larger validation areas are needed. Black pod showed a trend opposite to frosty pod. Thus, the design of a mixed inoculum against both diseases is not yet satisfactory. Molasses was beneficial to black pod control, but its effect was complex and it would be premature to issue a generalised recommendation. The participatory methodology provided mutual feedback and drove the trial design. This requires flexibility of all participants and increases variability, but does not necessarily compromise statistical analysis. Rather, it generates robust and well-adopted recommendations.

Acknowledgements

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Naturally occurring insect pathogenic fungi and the influence of management practices

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Introduction

Insect pathogenic fungi are important naturally occurring mortality factors of plant pest insects and mites. About 750 different fungal species are known to infect insects, and most fungal species that infects insects or mites beong to the class Hyphomycetes or the order Entomophthorales (Hajek 1997). Species belonging to the class Hyphomycetes mainly causes natural infection and epizootics in soil dwelling pest insects and mites. Fungal species belonging to Entomophthorales mainly cause natural infection in foliar insect and mites (Pell et al. 2001).

Biological control of pest insects and mites by using insect pathogenic fungi is well known, and there are several approaches: Conservation biological control, classical biological control, inoculation biological control, and inundation biological control (Eilenberg et al. 2001). Many hyphomycetous fungi grow easily on several different substrates. They are therefore often massproduced and used in inundative and inoculative biological control. Most Entomophthorales, however, are difficult to grow on artificial media, and the conservation approach is more relevant for fungi in this group.

Management practices (pesticide spraying, use of fertilizer, shading etc) are important factors for the natural occurrence and prevalence of insect- and mite pathogenic fungi on pest insects and mites in soil, and on the plant. There are many reports on the natural occurrence of insect- and mite pathogenic fungi in Central America, and it is quite common to use hyphomycetous fungi for inundative biological control pest insects and mites. Only few studies have, however, focused on the effect of cropping system on natural occurrence and prevalence of insect- and mite pathogenic fungi. In this paper we will therefore present conducted, ongoing or planned studies from Nicaragua, Costa Rica and Norway that aim to clarify this.

Hyphomycetes in soil and on pest insects

Fungi from organic and conventional soil

Comparison between the occurrence of insect pathogenic fungi in organically versus conventionally farmed soil has to our knowledge been undertaken only on a minor scale. In a study conducted in Norway, the aim was therefore to compare the abundance of insect pathogenic fungi in organically versus conventionally farmed soil. A method for baiting

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soil samples with *Delia floralis* larvae was developed, and a systematic survey was conducted on soils from northern Norway for insect pathogenic fungi, using *D. floralis* and *Galleria mellonella* larvae as bait.

The occurrence of insect pathogenic fungi in soils from arable fields and adjacent field margins of conventionally and organically managed farms was compared. The study showed a significantly higher occurrence of insect pathogenic fungi in soils from arable fields of organically managed farms. No significant differences in the occurrence of insect pathogenic fungi were, however, found between the field margins of the two cropping systems.

Fungal species identified in the study were *Beauveria bassiana*, *Fusarium merismoides*, *Metarhizium anisopliae* and *Tolypocladium cylindrosporum*. *T. cylindrosporum* was found more frequently when using *D. floralis* as the bait insect than when using *G. mellonella* (Klingen et al. 2002).

System experiments in coffee in Nicaragua and Costa Rica

In coffee systems, shade management and pest control practices are directly related to the occurrence of arthropods and microorganisms in general, because such practices alter the environmental conditions in the system. Particularly the level of chemical pesticides and fertilizer used in the system can affect the occurrence of natural enemies, including insect pathogenic fungi.

The insect pathogenic fungi *B. bassiana* is used as a microbial control agent against coffee berry borer *Hypothenemus hampei*, in several countries in Central America, and it has also been reported to infect the coffee leaf miner *Leucoptera coffeella*. This fungus is always present as a natural infection wherever *H. hampei* is present, and can be particularly common in humid conditions and where *H. hampei* infestations are severe (Baker 1999). Only few studies have, however, focused on the natural infection level and the effect of shade and pest management on the dynamic of *B. bassiana* or other insect pathogenic fungi on key coffee pests. By understanding the effect of different coffee growing systems on the dynamics of *B. bassiana* in the field is it possible to favour its natural occurrence (conservation microbial control) and to enhance the efficacy of *B. bassiana* as an the inundative/ inoculative microbial control agent.

In a master thesis conducted in Nicaragua the natural occurrence of insect pathogenic Hyphomycetes in the coffee leaf miner *L. coffeella* was studied, showing a higher *B. bassiana* infection level with increasing shading (Simonsen et al. 2001). More studies are, however, needed to confirm this trend.

In an ongoing PhD project at CATIE Nicaragua, naturally occurring insect pathogenic fungi on key coffee pests in Nicaragua and the influence of management practices on these fungi are therefore studied. The main objectives of this PhD project are to obtain knowledge about the dynamics and impact of naturally occurring insect pathogenic fungi on key coffee pests in Nicaragua, and obtain knowledge about the influence of management practices on these fungi.

In addition a master study on the occurrence and prevalence of insect pathogenic fungi in soil from a high chemical input system versus a low input organic system have been conducted at CATIE, Managua, Nicaragua and CATIE, Turrialba, Čosta Rica. Two different bait insects were used in this study in addition to counting of colony forming units (CFUs) on selective artificial media (data analysis still in process).

***Neozygites floridana* (Entomophthorales) and cropping system**

Neozygites floridana is a fungus in the order Entomophthorales that infects and kills the two-spotted spider mite, *Tetranychus urticae*. *N. floridana* is the key regulator factor of *T. urticae* on maize and soybean. In the mid-western and southeastern USA farmers are encouraged to adapt their fungicide spray programmes to avoid suppressing the fungus (Cross et al. 1999). To our knowledge few systematic studies have been conducted on *N. floridana* as a mortality factor of *T. urticae* in strawberry fields. A cropping system that enhances the prevalence of *N. floridana* might therefore be important for the reduction of *T. urticae* in strawberry. In a study conducted in Norwegian strawberry fields we therefore aim to clarify the effect of different strawberry growing systems on the infection level of *N. floridana*.

Preliminary results show that *N. floridana* infected and killed *T. urticae* in all strawberry fields studied. Infections from 0 to 19% were registered, and the highest infection rates were observed late in the season from the second sampling. Our preliminary results do not show any relationship between occurrence of *N. floridana* and strawberry cropping systems, but this needs to be more thoroughly analyzed.

A preliminary laboratory study was conducted to examine the killing capacity of *N. floridana* to *T. urticae* after *T. urticae* had been fed on leaves treated with different chemical pesticides. The pesticides tested were three fungicides; Euparen (tolylfluanid), Teldor (fenhexamide), Switch (cyprodinil + fludioksinil) and one acaricide: Mesurol (mercaptodimetur). The experiment indicates that Euparen and Switch do not affect the *N. floridana* killing capacity, but both Teldor and Mesurol do.

Conclusions

- Insect pathogenic fungi are important naturally occurring mortality factors of pest insects and mites.
- Few studies have been made on natural occurrence of insect pathogenic fungi and the effect of management system in Central America.
- One study from Norway confirms that management system (organic compared to conventional) is important to the occurrence and prevalence of insect pathogenic fungi in soil in arable fields.
- Another study from Norway indicates the importance of pesticide use on the killing capacity of the mite pathogenic fungi *N. floridana* to *T. urticae*.
- A master study from Nicaragua showed a higher *B. bassiana* infection level in *L. coffeella* with increasing shading more studies are, however, needed to confirm this trend.

- In a PhD project at CATIE Nicaragua, naturally occurring insect pathogenic fungi on key coffee pests and the effect of crop management practices on these fungi is under study.
- An ongoing master study will reveal the occurrence of insect pathogenic fungi in soil from different coffee growing systems in Nicaragua and Costa Rica.

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Compatibility of entomopathogens and mycoparasites

Ulrike Krauss, Eduardo Hidalgo and Claudio Arroyo[1]

Introduction

Organic production systems require the simultaneous management of all pests and diseases with non-chemical means (IFOAM, 2002) and a lot of hope is placed in biocontrol. Under field conditions, a crop is commonly attacked by several pests and diseases. There is, therefore, an urgent need to determine which biocontrol agents are compatible for simultaneous employment in IPM systems. The objective of the current study was to evaluate the compatibility of important entomopathogens (*Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces fumosoroseus*) and broad host-range mycoparasites (*Clonostachys* spp. (= *Gliocladium* spp.) and *Trichoderma harzianum*).

Materials and Methods

The *in vitro* susceptibility of entomopathogens (*Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces fumosoroseus*) to broad host-range, necrotrophic mycoparasites (*Clonostachys byssicola, Clonostachys rosea, Lecanicillium lecanii* and *Trichoderma harzianum*) was tested using a host-range assay as described by Krauss et al. (1998) for all possible combinations. *In vivo* interactions on insects were tested for the mycoparasites *C. rosea* and *T. harzianum* against the entomopathogens *M. anisopliae* and *B. bassiana* only. Three insect species, *Cosmopolites sordidus, Diatraea saccharalis* and *Sitophilus oryzae,* were treated simultaneously with suspensions of one entomopathogen and/or one mycoparasite. Ten replicate insects per three replicate containers per treatment were sprayed with $0.5\text{-}1.0\times10^8$ conidia ml^{-1} of each fungus. After some insect mortality in response to the mycoparasites was detected in early experiments, a copper hydroxide fungicide (7.5 g L^{-1} a.i.) was included for comparison.

The growth rates of the host-range assay were compared by ANOVA. Mortality rates of insects were analysed by sigmoid regression (Gompertz function). Significant Gompertz parameters were compared by ANOVA for the different treatments.

Results and Discussion

B. bassiana was most susceptible to *C. rosea* (Fig. 1). *C. byssicola* and *L. lecanii* accomplished limited overgrowth. *T. harzianum* was unable to invade *B. bassiana.* No significant differences between the two *B. bassiana* strains were observed. *M. anisopliae* was attacked by all mycoparasites and supported their fastest overgrowth. Strain 5\89 was more susceptible to *C. byssicola* AMR0055, *C. rosea* APP0023 and to *L. lecanii* CATIE than *M. anisopliae* strain GTE-15. *P. fumosoroseus* was immune to mycoparasitism *in vitro* (not shown in Fig. 1).

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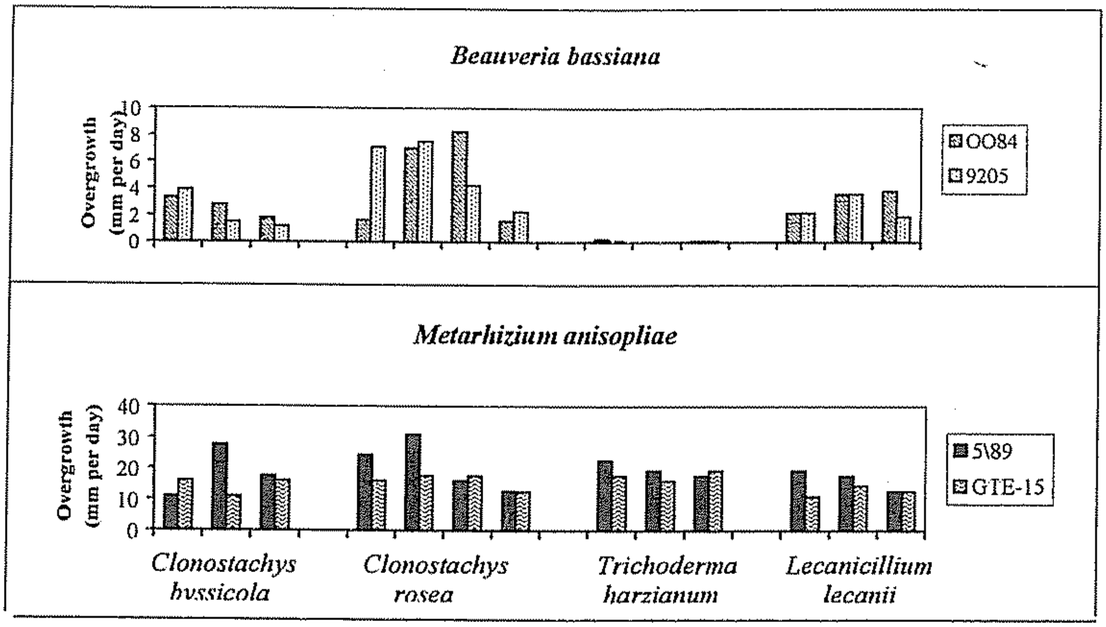

Fig. 1. Overgrowth (mm day^{-1}) of entomopathogens by several isolates of four mycoparasite species.

In insect bioassays, no difference between entomopathogen strains was observed and these were pooled. *B. bassiana* efficiently killed all three insect species. Both mycoparasites killed *D. saccharalis*, with *C. rosea* being more aggressive, but less so than the entomopathogens. Only *C. rosea* affected *C. sordidus* and *S. oryzae*. Coapplication of mycoparasites had no significant influence on mortality of any insect pest, except maybe for a slightly delayed death of *S. oryzae* (Fig. 2).

M. anisopliae caused only low mortality in *C. sordidus*. The mycoparasites alone as well as copper hydroxide were innocuous. In the other two insect species, *M. anisopliae* achieved virtually complete kill. Coapplication of mycoparasites with *M. anisopliae* had no significant effect on insect mortality. The copper fungicide caused a slow but marked death, exceeding that caused by mycoparasites (Fig 2).

The use of broad host-range mycoparasites, such as *Clonostachys* and *Trichoderma*, raises the question as to which extent non-target species, in our case entomopathogens, are also affected. Our results parallel those obtained for mycorrhizae (Rousseau et al. 1996). While parasitism is observed *in vitro*, under non-sterile conditions *in vivo*, mycoparasites seem to have a limited non-target effect. Even the entomopathogenicity of the highly susceptible *M. anisopliae* was not seriously impeded by mycoparasites in mortality assays.

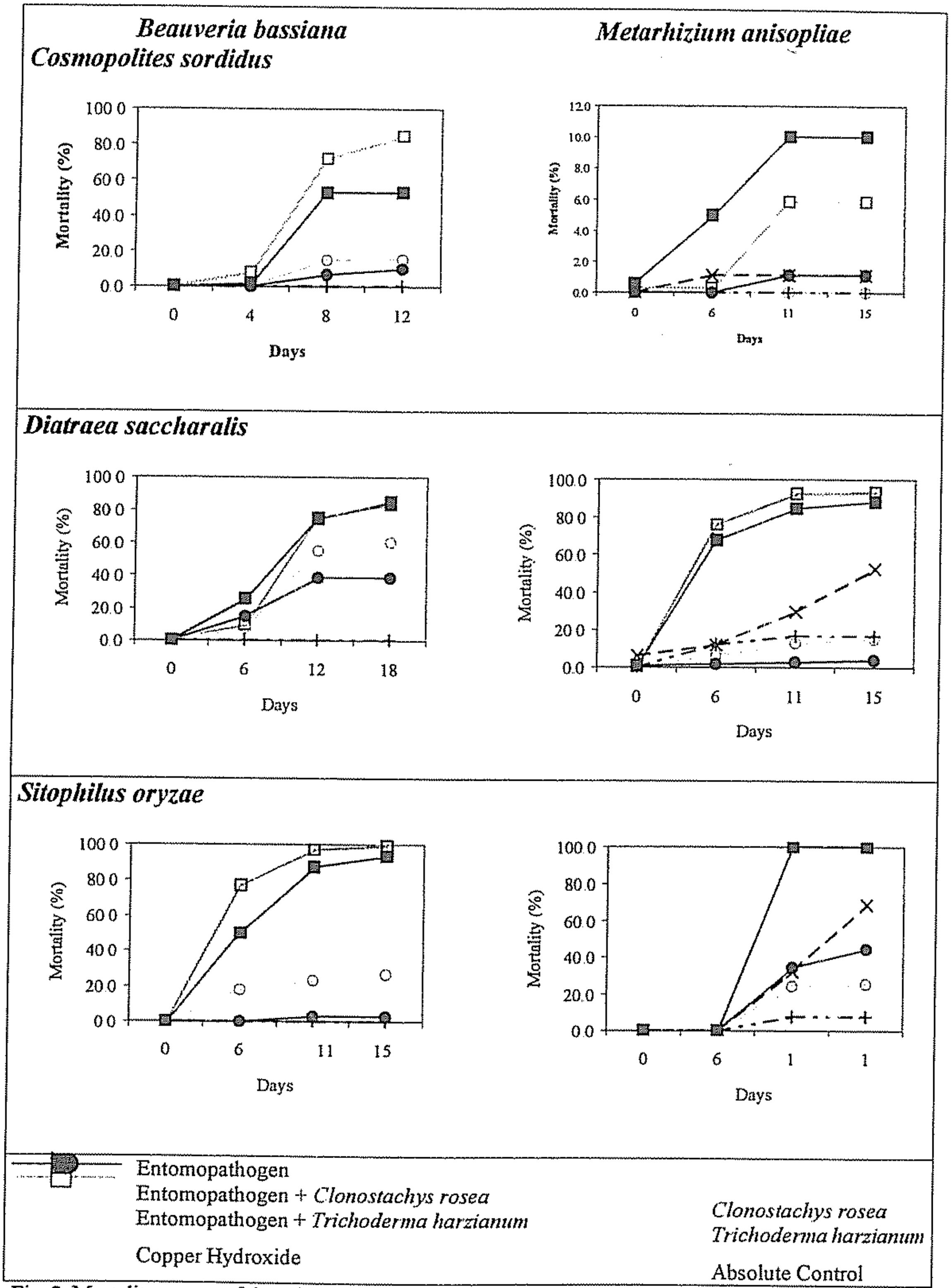

Fig. 2. Mortality curves of three insect species treated with the entomopathogens *Beauveria bassiana* (left) or *Metarhizium anisopliae* (right) (mean of two strains) and with the mycoparasites *Clonostachys rosea* or *Trichoderma harzianum*.

Mycoparasites caused some insect mortality at inoculum levels of 10^8 conidia ml^{-1}. The same has been observed for *Trichoderma lignorum* applied to nests of *Atta cephalotes* (Ortiz et al. 1999). Typical field application rates of 10^6 conidia ml^{-1}, however, were harmless to the leaf-cutter ant. A powdery formulation of up to 10^9 cfu g^{-1} of *C. rosea*, the more harmful of the mycoparasites tested here, was innocuous to honey bees (*Apis mellifera*) employed as vectors (Sutton et al. 1997). Copper hydroxide, a fungicide still permissible in organic agriculture, was consistently more harmful to insects than the mycofungicides.

Conclusions

Coapplication of mycoparasites with entomopathogens did not affect their biocontrol efficacy, although at least *M. anisopliae* was highly susceptible to mycoparasites *in vitro*. We conclude that the entomopathogens and mycoparasites tested here are compatible at standard concentrations and can probably be used simultaneously in integrated pest management. *In vivo* tests are preferable to *in vitro* screens as the latter tends to overestimate the host-range of biocontrol agents.

Acknowledgements

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Results and lessons learnt from ongoing and concluded research by other institutions

Developing new semiochemical-based strategies for pest management: solving the problems of sustainable production, registration and delivery

John A. Pickett[1]

Introduction

Semiochemicals can be useful for controlling pests directly, for exploiting beneficial organisms such as predators and parasites of pests, and even in the conservation of endangered species. The term is generic for chemicals that act as signals in triggering behavioral or developmental changes. In animals, semiochemicals interact with sensory neurons, but semiochemicals can also act as signals for plants, with as yet undefined mechanisms.

Where the target is crop protection, use of semiochemicals requires integration with other control methods and should include a system for direct reduction of pest populations.

Pheromones, the specific group of semiochemicals where communication is within one species, have achieved success when used in "lure and kill" strategies, i.e. attracting the pest to a site of destruction such as a pesticide or sticky trap.

More sophisticated approaches are now being devised, involving attractant and repellent semiochemicals in a "push-pull" or stimulo-deterrent diversionary strategy, with a pathogen or highly specific pesticide deployed in an attractive trap or trap crop (Agelopoulos et al. 1999).

Production: General

Semiochemicals are frequently small molecular weight lipophilic compounds and often highly unstable, so formulation development has been an important process for their practical use.

Because of isomeric purity requirements, semiochemicals can be expensive to produce and also fall within a difficult range with regard to commercial synthesis, with costs similar to pharmaceutical synthesis. However, in agricultural, horticultural and human health situations, unit cost per treatment must be low.

One approach achieving considerable success is to generate semiochemicals from plants. This can be done by growing repellent intercrops or attractive trap crops so that the active components are released directly from living plants, a strategy particularly suited to organic or Third World subsistence farming. An alternative is to produce semiochemicals in plants grown for extraction, followed by chemical elaboration and formulation as would have

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been the case for fully synthetic materials. Examples will be given of progress with these different options.

Natural release from companion crops

In Kenya, as part of a programme in collaboration with the International Centre of Insect Physiology and Ecology on habitat management in subsistence cereal crops, control of stem borers and the witchweed *Striga hermonthica* has been achieved by semiochemical release from intercrop and trap crop plants which are also useful as cattle forage (Khan et al. 2000, 2002).

One of the intercrop plants, the molasses grass *Melinis minutiflora*, releases chemicals such as 4,8-dimethylnona-1,3,7-triene which, in addition to repelling adult stem borers, act as foraging stimuli for wasps parasitising these pests (Khan et al. 1997).

Production by new industrial crops

Sex pheromones currently being developed for direct control of aphids, and more particularly for the attraction of wasps parasitising aphid pests, comprise specific isomers of nepetalactone and nepetalactol.

One of the nepetalactones is now being produced on a large scale with commercial collaborators, including Botanix Ltd., by growing the herb *Nepeta cataria* (Lamiaceae) as a new industrial crop and extracting and formulating the product for slow release, using a novel polymeric rope system developed by AgriSense BCS Ltd. (Birkett and Pickett 2003).

A similar approach to the development of the oviposition pheromone of *Culex* spp. mosquitoes and to the male-produced sex pheromones of the South American sandfly, *Lutzomyia longipalpis*, is being developed. For the *Culex* pheromone, Δ^5-hexedecenoic acid is produced in various plants, including *Kochia scoparia* (Chenopodiaceae) (Olagbemiro et al. 1999). For one particular sandfly pheromone, the germacrane skeleton is provided as germacrone from the essential oil of *Geranium macrorrhizum* (Geraniaceae) (Hamilton et al., 1999).

Plant stress related signals to switch on plant defences

The most exciting development, however, relates to the fact that, when plants are damaged by pests, adjacent plants can become repellent to incoming pests and attractive to parasitoids and predators.

Two natural products are known to have such effects and comprise volatile compounds emitted by plants under a range of damaging processes, including pest feeding. These are the volatile methyl esters of jasmonic and salicylic acids.

Using the herbivorous and parasitic insects as detector systems, we have recently discovered a new signal which has a much longer term impact on the target plant than the effects previously observed. This compound, *cis*-jasmone, although well known as a plant component, had not previously been reported as a plant stress signal (Birkett et al. 2000).

Field studies with *cis*-jasmone over three years on cereal crops in the UK have shown reductions in aphid colonisation.

These types of signals and their analogues present a new type of crop protection agent for the longer term future (Birkett et al. 2001, Chamberlain et al. 2001, Pickett et al. 2003). Already, a compound is marketed that, under the trade name Bion, acts as a signal, but this is a purely synthetic compound and does not have the advantage of semiochemical status, which should bring with it registration advantages (Matthes et al. 2003, Bruce et al. 2003).

These approaches are also applicable to control of insects that act as vectors of pathogens causing livestock and human diseases (Costantini et al. 2001).

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Mating disruption of several lepidopterous pests using high emission rate, low point source density dispensers

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Introduction

The concept of using widely spaced, high-emission-rate pheromone dispensers for disrupting mating communication in moths has been tested (Shorey and Gerber, 1996a,b; Fadamiro et al. 1998, 1999) and such dispensers have been in commercial use for many years in both field and fruit-tree crops. The Metered Semiochemical Timed Release System ("MSTRS") started out in 1993 as a timed, metered aerosol device, but gradually evolved away from the clock-timer 'active' release of pheromone to now become an entirely 'passive' release system while still retaining its high-emission-rate low-point-source-density attributes.

European Cornborer and Blackheaded Fireworm

In 1996 we conducted pheromone disruption experiments using MSTRS metered aerosol devices against the most destructive pest of corn in the midwestern United States, the European corn borer, *Ostrinia nubilalis*. Experiments were conducted in Iowa corn fields containing significant proportions of grassy borders and waterways in the fields' interiors that serve as mating aggregation sites (Showers et al. 1976). We also conducted MSTRS disruption experiments against the blackheaded fireworm, *Rhopobota naevana*, a pest of cranberry, in Wisconsin cranberry marshes. These experiments showed much promise in disrupting males' ability to locate synthetic pheromone sources or calling females by up to 99% for both the first and second flight periods of both species.

In 1997 our goal was to increase our understanding of MSTRS efficacy by using larger plots for both species and measuring the mating success of females that we captured in the plots' interiors and examined for the presence and number of spermatophores. Analyses were made of the bursa copulatrices of more than 2,400 feral females that were captured by hand-netting during the daylight hours as they were flushed from their grassy aggregation areas.

During the first flight of the European corn borer, we were able to reduce the percentage of feral females mating in grassy aggregation areas by up to 50% early in the flight. However, the mating success of females in these plots eventually reached 100% during the ensuing weeks as the flight proceeded. They attained this 100%-mated status more slowly than did females in the check plots, which were 100% mated beginning at Day 1 Fadamiro et al., 1999a). These levels of mating disruption were similar to those achieved in plots treated with Shin-Etsu twist-tie polyethylene tubes using higher levels of pheromone per hectare. What was interesting was that the disruption treatments only seemed to delay females' ability to mate, not prevent mating entirely.

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Analysis of the frequency of mating by the European corn borer females captured in the disruptant-treated plots versus those from the check plots showed that throughout the entire flight females from the disruption plots were attaining matings at a significantly lower rate than those from the check plots (Fadamiro et al. 1999b). The mating disruptant was impairing the ability of females to attract and mate with males on a constant, daily basis but it didn't completely eliminate mating. The mating disruption results in cranberry marshes against the blackheaded fireworm also showed significant reduction of mating by free-flying females of that species (Fadamiro et al. 1998).

A follow-up study by Fadamiro and Baker (1999) investigated the effects of several manipulations on the timing and frequency of European corn borer females' matings during their lifetimes. With regard to delaying the first mating of these females, a delay of first mating to Day 4 as compared to Day 1 produced a greater than 30% overall reduction in the number of fertile eggs laid during their 16-day lifetimes. Further delaying first mating until Day 7 resulted in no fertile eggs laid at all, even though these females lived for 16 days total.

Fadamiro and Baker (1999) also investigated the effects of reducing multiple mating, because the majority of females from the check plots in the field experiments had mated two or more times (two or more spermatophores), implying that there is an advantage for feral females to obtain a second mating. Therefore another set of experiments were performed to examine the effects of allowing females to mate more than once (Fadamiro and Baker 1999).

Females allowed to mate only once at Day 2 laid only 50% as many fertile eggs during their lifetimes as those that had been allowed to mate twice. Thus, one mechanism by which mating disruption of this species can help suppress populations is by significantly reducing fertile egg production by delaying the first mating of females, as well as by impeding their opportunity to achieve a second mating. When one considers other factors such as mortality of females over time due to predation and other natural causes, these factors could conceivably help augment these delayed-mating-produced fertility effects and produce even more profound levels of population reduction.

Thus, as demonstrated in studies on the oriental fruit moth (Rice and Kirsch 1990) and codling moth (Knight 1997), mating disruption success does not require keeping the population of females virgin, but rather just needs to impede females' ability to attract males and attain their first or even subsequent matings. In other words, we must now expect to only retard, not eliminate mating by females as a benchmark for successful population suppression through the application of mating disruption formulations.

In subsequent years we developed the formulation into a completely passively emitting polyethylene bag that is hung on bamboo gardening stakes in grassy areas in and around corn fields. The application of this MSTRS formulation, containing 0.86 grams of the 2-component pheromone per bag, has been shown to reduce damage to corn by an average of 50% in various trials. In 2001, for instance, the grassy areas in and around three 40 ha corn fields were treated with the MSTRS bags (Baker, unpublished data), and three check fields of the same cultivar located 1 mile away from the treated fields with which they were paired, served as controls. Eight-hundred corn plants were sampled on random transects through the fields and each plant was checked for evidence of characteristic European corn borer shot-hole feeding by larvae. A plant showing signs of any shot-hole damage at all anywhere on the plant caused that plant to be labeled 'damaged'. From Table 1, it is clear that these widely spaced, high emission rate mating disruption dispensers reduced damage in all three plots resulting in an average reduction in damage of approximately 50%. This is estimated to increase yields by an average of 20 bushels of corn per hectare (approx. US $46.00). The density of bags needed to be deployed is only 25 bags per 10 hectares of corn

Table 1. European Corn Borer Mating Disruption Plots, 2001. Eight hundred corn plants in each plot were sampled for damage (evidence of shot hole feeding by corn borer larvae).

	% Damaged Plants
Check Field #1	24.1%
Disruption Field #1	8.8%
Check Field #2	34.0%
Disruption Field #2	19.6%
Check Field #3	23.4%
Disruption Field #3	15.6%
Total Average % Damage in Check Fields	27.2%
Total Average % Damage in Disruption Fields	14.7%

Oriental Fruit Moth

We have used the same polyethylene bag high-emission-rate MSTRS dispenser system for mating disruption targeting the Oriental fruit moth (OFM) in California peach and almond orchards. In 2000, dispensers were placed in the upper 1/3 of almond trees with a 25 m grid spacing on three 3-ha plots. Three 3-ha conventionally treated plots served as checks. Standard OFM commercial monitoring traps, three traps per plot, monitored the ability of the widely spaced disruption dispensers to suppress trap catch of males.

Throughout the 8-week period that the traps were monitored, the widely-spaced dispenser system effectively reduced trap capture in the disruption plots to near-zero (Fig. 1). A similar nearly 100% trap shutdown was achieved on three 3-ha blocks of peaches during that same summer. We feell that widely spaced mating disruption dispenser systems such as this have the potential to be effective against the Oriental fruit moth in the U.S. and elsewhere and in becoming incorporated into pest management systems on peaches, almonds, and apples.

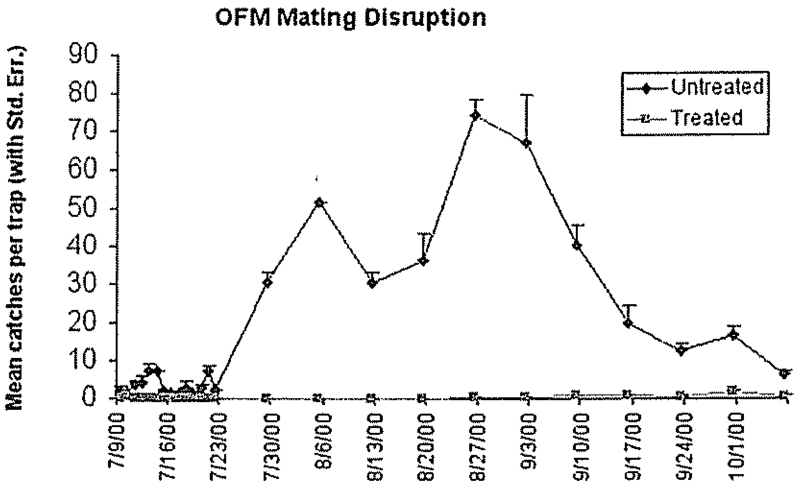

Figure 1. Disruption of Oriental fruit moth in California almond orchard in 2000, using MSTRS high-emission-rate, polyethylene bags deployed on a 25 m grid spacing. Mean trap capture of males in three, 3-ha mating-disruption-treated ("treated") plots with three monitoring traps per plot was reduced to near-zero compared to check plots.

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Plant odours as a source of aphid repellents

J. Hans Visser[1]

Introduction

The control of insect pests is mainly based on the toxicity of plant-derived or newly synthesized chemicals. The specific interaction of insects and plants, i.e., the behavioural steps leading to the selection and acceptance of plants for feeding and oviposition, is hardly a topic in the development of new pest control strategies. Insects make use of a variety of semiochemicals for vital decisions in their selection of food and mates. Although the market potential of these semio-chemicals, that is, the amount of money a few large pesticide industries will make, is relatively limited compared to convential insecticides, human societies largely depend on ecologically sound solutions like those offered by semiochemicals. Our efforts to find new semiochemicals from plant odours will be shortly presented in this paper. As aphids transmit plant viruses and are important pests of most crops in The Netherlands, they were selected as targets for repellents.

Odours and Aphids

A lot of plant odour components and essential oils are commercially available as they are widely used by the food industry. The line of research we followed was strongly affected by the presumption that we needed a screening programme in order to evaluate these plant odour components rapidly for their potential to repel aphids and, thus, to prevent aphids to land and to transmit viruses. As we wished to develop general aphid repellents, several aphid species were studied at the same time: the vetch aphid *Megoura viciae* Buckton, the cabbage aphid *Brevicoryne brassicae* (L.), the peach-potato aphid *Myzus persicae* (Sulz.), and the black bean aphid *Aphis fabae* Scop.. Aphids have a complicated life cycle involving asexual and sexual forms, they possess wings or are wingless, and can be host alternating, *i.e.*, they select different host plants in autumn and spring. All these different aphid forms were included in the first screening of plant odour compounds.

Screening on Sensory Responses

We developed methods to record electroantennograms (EAGs) from these small insects (Visser and Piron 1995) and screened a collection of plant odour components on aphids' olfactory receptors. Although such EAG data do not tell us whether chemicals are repellent or attractant, the response profiles obtained differentiate plant odour components in: (1) compounds hardly perceived at the moderate concentration tested, and (2) compounds strongly perceived by the particular aphid species and morph under study. We could appreciate aphid species and morph specific trends in the perception of plant odour components (Visser et al. 1996), and, thus, listed the compounds being interesting for further evaluations in behavioural studies. Detailed analyses of the EAG shapes (Visser and Piron 1994), which represent different phases in the transduction process in the olfactory receptors, such as the transport of odour molecules to receptor sites and the interaction of

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odour molecules with receptor sites followed by their deactivation, did not reveal extra criteria to select compounds.

Behavioural Studies

We first developed an open Y-track olfactometer in order to test the attraction of aphids towards host plant odours (Visser and Piron 1998). This olfactometer is very simple and easy to run as individual aphids placed on the vertical stem walked easily towards the light source and chose between a clean air flow or a flow carrying odour in a few minutes time. The study was focussed on testing compounds and essential oils for their repellent characteristics. For that purpose the Y-track was replaced by a T-bar of similar size. Odour dilutions in paraffin oil were applied on filter paper circles positioned left and right at 15 mm from the junction of the T-bar. In this way, individual aphids walked upwards and at the junction had to choose between the odour side or the control side with just paraffin oil, and moved towards one of the filter paper circles. When touching the surface of the filter paper the aphids made their second choice. A number of compounds and essential oils showed strong repellent characteristics and were further studied in a leaf disk bioassay where aphids could choose to settle on clean or treated leaf disks. All kind of formulations of repellent compounds were tested and the formulations used had an extreme impact on repellency.

Perspectives

We tested a number of formulations and repellent compounds in potato fields for their action to diminish virus transmission. Aphid populations and virus presence were, however, too small to conclude anything from these field studies. For spray applications of repellents, the legal registration like an insecticide is needed. Although a range of strong aphid repellents has become available from the present study, the costs of registration of these compounds still prevent their application by the biopesticide industry.

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Important role of mating disruption in IPM

Kinya Ogawa[1] and Keiko Sasakura[2]

Introduction

We have been developing and selling dispensers for mating disruption (MD) for more than 20 years. From these 20 years of experience, methods on how to keep a stable efficacy of MD and its practical use have been established. With the establishment of the system of MD, it has increased its importance in IPM (integrated pest management) and now MD's role is essential for a successful IPM. The importance of MD has become larger year by year, and in 2002 the areas with MD have increased up to 600,000 ha (Table 1).

Table 1. Worldwide importance of mating disruption (MD) by year 2002.

PEST NAME	PLANT/CROPS	AREA (ha)	COUNTRIES
Lymantria dispar	**Forest**	**230,000**	**USA**
Cydia pomonella	**Apple, Pear, Almond**	**120,000**	**USA, EU, South America, Africa**
Pectinophora gossypiella	**Cotton**	**55,000**	**USA, Israel, South America, EU**
Grapholitha molesta	**Peach, Nectarine, Apple, Pear**	**50,000**	**EU, USA, South America, South Africa**
Lobesia botrana	**Grape**	**41,000**	**EU**
Europecilia ambiguella	**Grape**	**32,000**	**EU**
Leaf Rollers	**Tea, Pear, Apple, Peach, Grape**	**24,000**	**Japan, Australia, USA, N.Z**
Keiferia lycopersicella	**Tomato**	**10,000**	**Mexico, USA**
Synanthedon spp.	**Apricot, Black currant, Peach**	**5,000**	**Japan, USA, N.Z**
Chilo suppressalis	**Rice**	**4,000**	**EU**
Zeuzera pyrina	**Pear, Olive**	**2,000**	**EU**
Plutera xylostella	**Cabbage**	**2,000**	**Japan**
Endopiza viteana	**Grape**	**1,000**	**Canada, USA**
Others	**Turf, Vegetable, Apple**	**27,000**	**Japan, USA**
Total		**603,000**	

The role of MD in IPM

For more than 20 years a good IPM system has been sought by many growers and researchers. However, a lot of the efforts ended in failure. Two reasons could be raised. One reason could be the existence of the pests that live inside the plant at the larval stage. These pests cannot be well controlled by natural predators and parasites (natural enemies, NE). The second reason could be because of the lack of soft and mild insecticides to the NE.

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The most important policy in IPM is to use NE at their maximum ability. Co-work between these NE and MD is a promising method for a successful IPM. MD can control the type of pests that live inside the plant at the larval stage by disrupting the mating activity of the pests (Table 2) while preserving NE as well.

Table 2. Pests that are suitable target for mating disruption.

Pest		**Place where the larva lives in**
Cydia pomonella	**Codling moth**	**In a pome fruit**
Lobesia botrana	**European grapevine moth**	**In a bud**
Grapholita molesta	**Oriental fruit moth**	**In a fruit**
Pectinophora gossypiella	**Pink bollworm**	**In a bud, flower and boll**
Homona magnanima	**Oriental tea tortrix**	**In a webbed tubular nest by leaf**
Adoxophyes orana	**Apple tortrix**	**In a webbed tubular nest by leaf**
Adoxophyes honmai	**Tea tortrix**	**In a webbed tubular nest by leaf**
Synanthedon hector	**Cherry tree borer**	**In a plant trunk**

For example in Japan, MD is being applied in peach orchards, where in one area, MD is being used in an IPM system which is using only soft chemicals to NE. For the last three years, they have reduced insecticide sprays into half and still could control the leafrollers, *Carposina niponensis*, *Lyonetia clerkella L.*, and *Grapholita molesta* very well. In contrary, in another area, MD was being used in addition to the conventional control using pyrethroids. In this area, they did not reduce insecticide sprays, and as a result, in 2002, serious damages were found, caused by leafrollers. In 2003, people suffered damages caused by leafminers still with the combination of conventional insecticides. Judging from these two cases, a successful MD could be realized only in IPM.

Conclusion

In order to obtain a good efficacy of MD, the following conditions should be kept:

- To choose a good dispenser system
- To maintain the pheromone concentration necessary for MD
- To apply in a large field where the pheromone concentration can be kept
- To apply the dispensers at a proper timing (before the pests emerge)
- To avoid use of broad-spectrum insecticides and use natural enemies' ability to control the target pests.

Monitoring of Stored Product Insects by Pheromone Trap and it's Role in Integrated Pest Management

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Introduction

Stored product insects bring two serious economic damages: Post harvest loss and contamination in products. In food industry, the insect contamination is usually more important than post harvest loss. This is the reason why the contamination of one insect requires a company huge compensation for the damage caused by the contamination, even if the contamination is rare case. Therefore, acceptable economic injury level in integrated pest management (IPM) is zero in a food and tobacco factory. This is the most different point between IPM in food factory and that in agricultural field. However, it is true that many stored product insects are in a food factory. Most of them are in other places of the manufacturing lines. They fly and/or crawl into the manufacturing lines and contaminate food products. Some of them have so strong mandible that they bore packing film into the products (Cline 1978). Furthermore, some moth full grown larvae disperse and pupate on the packing film and/or in the products (Shinoda et al. 1990)

To this end, it is the most important for food companies to detect early occurrences of the stored product insects and to conduct some early action for their control. Then pheromone traps for stored product insects have bean used in order to detect the early occurrences in many food and tobacco factory and to find out their source of infestation (Levinson and Buchelos 1988, Ryan 1999). On the other hand, standard methods on evaluation of trap capturing efficacies, placement-replacement and analysis of monitoring data obtained by the pheromone traps as the number of catches have not established enough. In the present study, then, we refer to evaluation of the trap efficacy and trap placement-replacement, and discuss the analytical methods on the monitoring data.

Factors affecting total capturing efficacy of a pheromone trap

Most of pheromone traps are sticky type and their attractant lure, trap shapes and sticky materials affect total capturing efficacy. The capturing efficacy of the lure consists of active ages of adult insects to the lure, its active distance, active life and validity. Trap shape and sticker also have important role on the capturing efficacy, because bad shape and sticky materials decrease the number of catches, even if the same number of individuals is attracted by the lure.

Active ages of the tobacco beetle, *Lasioderma serricorne*, to the lure containing "Serricornin" as its sex pheromone (Chuman et al. 1979). Fifty males of different age groups were released in each plastic case (50x50x50 cm, 20 ml of fresh air was introduced

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per minute) with "New Serrico" trap for the tobacco beetles. Then, the number of catches was recorded in 72 h. after their release.

No activity of unmated male was observed until the 2nd days of adult age (Fig. 1). Then, the activity increased rapidly and reached a peak on the 5th days. They were attracted until they died. Furthermore, mated males were also attracted until they died. The activity of mated males to the pheromone lure is likely to depend on their mating behavior that they mate with female multiply (Coffelt 1975). The highest activity on the 5th days indicates that the 5th days old males should be used in the experiments on capturing efficacy of the pheromone trap.

Active distance, active life and validity. To test the active distance of the lure, 50 unmated or mated males of the 5th days old were released at the distance of 1.5, 3.0, 4.5, 6.0 or 7.5 m from a pheromone trap in a strait line in a dark room (8.0 m x 6.0 m wide, 2.4 m high). Then the number of catches was recorded in 3 days after their release. The active life was tested by the following methods. A new pheromone trap with a new lure was placed in the same plastic case as shown in Fig. 1 and 50 unmated males of the 5th days old were released there. Then, the number of catches was counted in 24 h after release. Thereafter, the lure was replaced on a new trap in 1-5 weeks repeatedly and the same experiments were conducted. The validity of the pheromone trap, which were stored under constant conditions (25°C, in dark), was tested in 6 and 12 months after its manufacturing. Forgoing results are summarized in Table 1 and detail data was presented by Shinoda (2002). Capturing efficacy decreased linearly as the distance between the trap and releasing point increased. Then maximum active distance was estimated to be 13 m. The active life also decreased linearly as the day elapsed after the trap was placed. These results are very important for trap user in order to determine the distance among traps placed in a space and the interval of trap replacement. However, these linear relationships give user no evidence on the distance and the interval. The evidences are likely to be given by the optimization of trap placement and replacement as Shinoda (2002) pointed out. Validity and storage conditions are also important for users to realize a stable capturing efficacy. In the present study, the stable capturing efficacy was kept for one year under 25°C in dark. Furthermore, when the traps are stored under room conditions (10-40°C), they keep their stable capturing efficacy (unpublished data).

Trap shape and sticky materials

Trap shape and sticky material also affects the capturing efficacy of a pheromone trap. To evaluate their effect on the capturing efficacy, we conducted the following experiments. Two types of trap with the same sticky surface (4.5 x 32 cm) were prepared. Each trap was placed in the same plastic case as mentioned before and 50 unmated males of 5 days old were released in the case. Then, the number of catches was counted in 24 h after release. To evaluate stickers, 3 types of sticker with the same size (4 x 4 cm) were prepared: Specially designed sticker for the tobacco beetle, sticker for the cockroach and Sticky tape. Each sticker with a sex pheromone lure was introduced into a plastic share (9 cm inside diameter, 2 cm high) with 10 unmated males of 5th days old. Then, the number of catches was counted in 24 h after the introduction.

Percentage of males captured by U-shape trap was significantly higher than that by Flat-shape trap (Table 2). About 50% of males were captured in the bottom area of U-shape trap. This result indicates that U-shape increase the capturing efficacy of a pheromone trap; 90% of males were captured by specially designed sticker for the tobacco beetles, whereas the sticker for cockroach is stronger than specially designed sticker (Fig. 2). Many males were captured at the center of the specially designed sticker. On the other hand, strong sticker captured males at the edge of the sticker. These males worked as a barrier for the following males, so that total number of catches decreased. Sticky tape was not available for the tobacco beetles, because most of males walked on the surface and escape from the sticky tape.

Analysis of monitoring data

Monitoring data in a food factory gives us the following direct information: seasonal prevalence, distribution and source of infestation, and evaluation of some control. In a food factory, however, it is very important for IPM to detect unusual occurrences as earliest as we can. Many trap users have detected the unusual occurrences, by comparing the present monitoring data with the past accumulated monitoring data. But this takes so much time and cost that some users loose the chance of timely action for control.

Therefore, it is necessary to establish some parameters indicating unusual occurrences, which parameters are calculated from the monitoring data. Furthermore, a new monitoring system including these parameters with a computer may make it possible for users to detect unusual occurrences earlier than before and to get the timely action for the pest control in IPM system.

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Table 1 Capturing efficacy (Y) of each factors of a pheromone trap* for the tobacco beetle

Factors	Capturing efficacy	
Active distance (x m)	$Y=109.34-8.02x$	$R=0.9629$
Active life (x days)	$Y=65.50-7.12x$	$R=0.9336$
Validity	Y > 85 % for 12 months	

* " New Serrico" was used as a pheromone trap.

Table 2 Effect of trap shape on capturing efficacy

Shape	□ of catches at each area		
U-shape	84	Lure area	32.1
		Opposite lure area	20.4
		Bottom area	47.5
Flat shape	61		

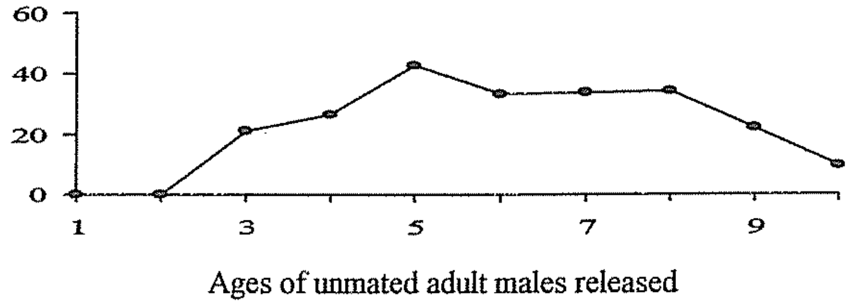

Fig. 1. Age-specific activity of unmated male tobacco beetles for a sex pheromone lure for the beetle

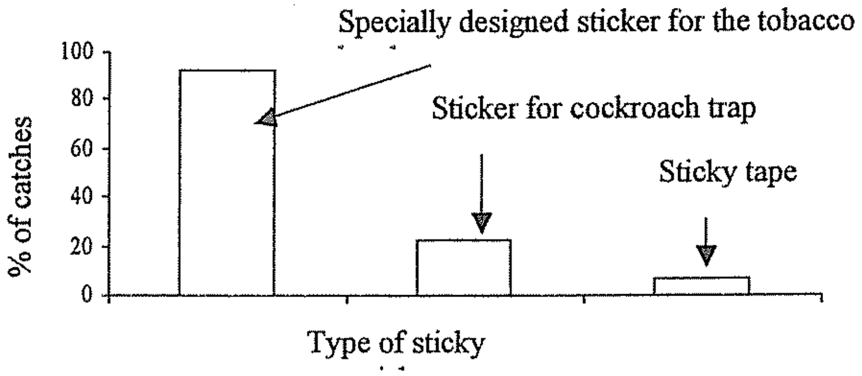

Fig. 2. Effects of sticky materials on capturing efficacy of a pheromone trap.

EMBRAPA's Work on Semiochemicals

Miguel Borges[1], Carmen Pires[1], Edson Ryoiti Sujii[1],
Maria Carolina Blassioli Moraes[1] and Raul Alberto Laumann[1]

Introduction

The Brazilian Agricultural Research Corporation (Embrapa-http://www.embrapa.br/) develops special programs and projects concerning areas such as food safety, small-scale agriculture, natural resources, advanced technology and agribusiness, and acts as a partner in several others.

Embrapa coordinates the National Agricultural Research System, responsible for development and implementation of agricultural research in different Brazilian regions or fields of scientific knowledge. Embrapa, comprising 40 research units around the country and extends its actions to North America and Europe as well, where it maintains two laboratories of technological prospecting and institutional exchange (Labex) in the United States and France. Based on mutual interests and benefits, Labex aimed at the strengthening cooperation in research and development to enhance agricultural research and capitalize on opportunities and research trends with potential mutual benefits for agribusiness between the countries.

The work on insect semiochemical identification at Embrapa started during the middle of 80ths reporting the pheromone of the fitophagous pentatomid *Nezara viridula* (southern green sting bug) (Baker et al. 1987, Borges et al. 1987). Since then, several other pheromones identification have been reported for other members of the soybean stink bug complex, such as *Euschistus heros* and *Piezodorus guildinii* (Aldrich et al. 1994, Borges and Aldrich 1994). Recently, some lepidopterans pheromone were considered, as well as the direct and indirect defenses of plants were taken into account by few Embrapa's scientists (Piubelli et al. 2003) and work on allelopathy (Souza Filho and Alves, 2000).

However, these efforts were carried out isolated by the scientists fact that have made it more laboriously and some times with not a practical applications of the results attained. With the knowledgement that research on semiochemicals has produced clean and more efficient tools for the integrated pest management programs, Embrapa, in 2001, organized a home based network for chemical ecology studies. This net should integrated most of their research units, taking the advantage of the different researchers profiles Embrapa has among its researchers scientists, integrating this with other research institutions inside Brazil and overseas.

Embrapa truly believe that the results of the chemical ecology studies may be used for biological control of insects, weeds and various plant diseases, for enhanced agronomic

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[1] Embrapa Genetic Resources and Biotechnology. Brasília - DF, Brazil

plant characteristic such as increased drought tolerances and nitrogen efficiency, for bio herbicides and for pharmaceutical agents.

Scientists Profiles

Embrapa`s scientists have built different expertise backgrounds that habilitate them to work with the different aspects of the chemical ecology in the main areas such as pheromones, allelochemicals, allelopathy, plants direct and indirect defences and endophytic microorganisms. However the area of semiochemical identification and organic synthesis at Embrapa research units count with only few scientists. To solve this problem it is necessary to train the existing employees and expand the cooperative projects with another institutions for that purpose.

Examples of Projects on Semiochemicals Undertaken by Embrapa

1. **Investigation of insects producing chemical molecules for the integrated pest management use** – Embrapa Genetic Resources and Biotechnology

Target Insects: *Tibraca limbativentris*, *Oebalus poecilus*, and *Piezodorus guildinii* (Heteroptera), *Conotrachelus humeropictus* (Coleoptera) and *Elasmopalpus lignosellus* (Lepidoptera).
Main Objectives: Identification and synthesis of volatiles compounds involved for the different species and to carry out bioassays and behavioural studies for evaluation of the biological activity of the compounds under study.

2. **Investigation of chemicals compounds production by forage plants with potential for production of bio herbicide** - Embrapa Eastern Amazon.

Target Organisms: Different species of weeds
Main Objectives: Emphasize the search for new molecules in forage plants and to select chemical substances with bioherbicidal potential in the following species: *Brachiaria brizantha*; *Brachiaria humidicola* and *Pueraria phaseoloides*.

3. **Identification and potential of utilization of chemicals from plants and microorganisms for the control of pests in the soybean-maize sorghum production system** – Embrapa Soybean.

Target Organisms: The seed feeder *Sternechus subsignatus*, the stink bug complex (Pentatomids), beneficial insects such as *Microcharops bimaculata*, *Podisus* sp. e *Calosoma granulatum* and beneficial microorganisms such us *Baculovirus anticarsia* e *Nomureae rileyi*.
Main Objectives: Emphasize the search for new molecules and to test the biological activity of the chemical compounds with potential use in the integrated pest management of pest insects, fungi, nematodes and weeds

4. **Evaluation of the potential of plant features for pest control in the small-scale agriculture** – Embrapa Mid-North

Target Organism: *Spodoptera frugiperda* (Lepidoptera).

Main Objectives: Emphasize the search for new molecules in *Albizia niopoides, Anadenanthera macrocarpa, Piper frutescens* and *Petiveria alliarcea*.

5. **Investigation of chemicals compounds production by the Amazon plants and Brazilian savannas (flora) aiming the production of bio defensives** - Embrapa Eastern Amazon, Embrapa Acre, Embrapa Beef Cattle and Embrapa Corn and Sorghum.

Target Organisms: Different species of weeds, Acaridae and insects including the horn fly and beneficial insects.
Main Objectives: Emphasize the search for new molecules in *Virola surinamensis, Virola michelii, Canavalia ensiformis, Calopogonium mucunoides, Sclerolobium paniculatum, Triplaris surinamensis, Cimbopogon citreodora, Toona ciliata, Simarouba versicolor, Protium heptaphyllum, Annona dioicaamong,* among others.

6. **Semiochemicals for monitoring and control of temperate fruit pests in south Brazil** - Embrapa Grape and Wine.

Target Organisms: South American Fruit Fly - Anastrepha fraterculus; Oriental fruit moth - Grapholita molesta; Leafrollers - Argyrotaenia sphaleropa and Bonagota cranaode.
Main Objectives: Development of pheromones and food lures for field monitoring and Evaluation of mating disruption as a practical approach for pest control

7. **Diversity of endophytic microorganisms and their biotecnologic potencial** – Embrapa Environment.

Target Organisms: maize, soybeans, citrus, coffee, cassava, *Brachiaria* and *Dicksonia* (xaxim)
Main Objectives: This project proposes to study the biological diversity of endophytic microorganisms (considering both number of species and the partition of abundance) from important plant species growing in Sao Paulo State

Concluding Remarks

The building of the chemical ecology network with Embrapa and partner institutions have allowed working on the different steps necessary in the chemical ecology studies, such as insect rearing, semiochemicals collection, secondary metabolites, chemical identification, organic synthesis, behavioural studies and field evaluation.

During the process of building the chemical ecology network the establishment of a reference laboratory for identification and synthesis of compounds associated to a reference laboratory for bioassays and behavioural studies is essential for the establishment of the chemical ecology studies at Embrapa.

These two reference laboratories working integrated with the other Embrapa's research units for product, such as soybean, rice and bean, corn and sorghum, among others, is essential for the standardization of the procedures. Also another benefits should be: prospecting new interactions with potential for application in the field of pest management, training of students and other professionals in the field of chemical ecology. Additionally, this interaction among different units/institutions would accomplish the success of the integration of the different activities.

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The work of CBC (EUROPE) Ltd.

Vittorio Veronelli[1]

Introduction

CBC (EUROPE) Ltd. is based in Milan (Italy) as the Italian branch of CBC Co. Ltd. Tokyo - Japan who develops and distributes in several countries around the world the pheromones MD products produced by SHIN-ETSU Chemical Co. Ltd - Japan.

In the '80 several trials to commercially apply pheromones for mating disruption (MD) failed in Italy. The pheromones commercial application for pest control of fruit orchards in Italy begun in the early '90 when South Tyrol faced resistance to Chitin inhibitors and Trentino started to implement a pheromone MD based IPM system in vineyards.

The key factors to apply pheromone MD and integrate this technology into the orchard and vineyard management are discussed.

Conclusions

- In 14 years the application of pheromone MD expanded in Italy from the initial 100 ha of apple orchards to more than 35,000 ha of apple, pear, peach and vines.
- A key factor is the field technical support to growers and the willing to cooperate for area wide applications.
- The interdisciplinary knowledge is extremely important for the deep understanding of this technology and the relevant transfer of the technique to field.
- MD efficiency is generally high, however improvements are still possible, for which more field and basic research to reach a fine tuning of pheromones applications are needed.
- Pheromones are species specific and their wide application on single pest will increase risks for other similar species of insects, developments of multi-species MD technology and strategy is the new challenge.

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[1] Managing Director for the Italian branch of CBC Co. Ltd.

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Current examples and potential role of North-South and South-South collaboration

Involvement of the Swedish Life Science University (SLU) in N-S research collaboration on IPM supported by IFS/MISTRA

Jan Pettersson[1]

Introduction- crossing borders in scientific collaboration

An important trait in creative science means crossing existing borders usually interpreted in terms of scientific borderlines i.e. between scientific disciplines, different methods, etc. In perspective of North-South interactions mobility of sophisticated equipment and technologies often plays a decisive role. This usually means heavy economical investments in equipment, high costs for maintenance and if appropriately taken in regular use it also means long bterm development of scientific competence among users. All these factors contribute to make these projects visible for all parties involved. However, crossing geographic borders may also have other less visible but still very important positive effects for development of new perspectives on old problems and the scientific basis for conceptual approaches. These effects are often less visible and recognised as positive outputs from collaborative projects. They are also resistant to different kinds of evaluation processes.

The efforts to apply an integrated approach to pest control are a success story for an international movement to find alternatives to conventional pesticides. The integrated pest management (IPM) concept was gradually developed during the 70ties. The over all principle is to integrate all possible steps in the crop ecosystem to prevent economical consequences of pests. The crop perspective is sometimes stressed to an integrated crop management (ICM) perspective but combines different methods such as the label push-pull strategy (PPS), when semiochemicals are used to manipulate pests and their natural enemies. In principle research aiming at an integrated pest control system can be seen in two different dimensions. One dimension is efforts made to bring different pieces of existing knowledge together and to implement it at the farming level. The other dimension is to develop further means to improve the system. In between these two dimensions is a qualified process of analysis of current applied techniques and identification of how to best possible find and use existing knowledge and current techniques. Although there are outstanding examples of how new knowledge has had a dramatic and immediate effect on cropping systems such as the control of the weed Striga control by mixed cropping it can be expected that is via dof the pieces in the system and to.

SLU as a N-S research organisation

Swedish Life Science University has with support from different aid authorities been involved in projects in different scientific fields and with different aims. According to official statistics for 2003 the SIDA support was 5.7 % of all categories of SLU project support to be compared to 19 % from FORMAS (the major sponsor in SLU:s principal research fields) and 10.9% from MISTRA.

The SLU activities cover a broad field, such as courses in veterinary science, support to development of scientific competence, PhD training programs, specific bilateral collaborative

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projects such as control of pest insects etc. Most projects have been long term commitments and few have been shorter than 2x3 years. Common components have been exchange of senior scientists and students, joint course arrangements, coordinated research efforts and joint publishing.

With a Latin American focus it is appropriate to mention a long term commitment between SLU and Universidad Nacional Agraria, Nicaragua. This bilateral project started 1981 and is still running with different kinds of activities. MSc-degree has been completed by 22 of UNA's teachers and two PhDs got their degrees in plant husbandry at SLU during 2001 and 10 UNA teachers are PhDs students at SLU.

Beside training and exchange programs the collaboration has also meant investements in scientific prerequisites for qualified research. Thus an advanced laboratory for molecular biology has recently been opened at UNA and library facilities reinforced. Financial support from SIDA chanelled via SLU has also been used to raise the housing standards for student and training laboratories

The MISTRA/IFS program

- A MISTRA/IFS funded program for collaboration with Latin America 199n-2004 was affiliated to the Swedish MISTRA –program *Pheromones and kairomones for insect control*. The scientific structure, content and achievements have been described under other sessions in this conference. Principal components of the IFS mediated part have been exchange of scientists and sandwich PhD programs. Two students have completed their PhDs at the aphid group, SLU in Uppsala and the fruit orchard pest group, SLU in Alnarp. One Brazilian student is just about to complete her studies at the chemistry group at KTH Stockholm.
- The PhDs from the program have been links between Latin American and Swedish groups. As a contribution to strengthen the N-S contacts at the student level a series of courses for graduate students from Europe and Latin America on chemical ecology was initiated (IFS Annual Report 2001). The course was a jointly organised activity between Dept. of Entomology, SLU Uppsala and Laboratorio de Química Ecológica, Universidad de Chile (Prof. Hermann Niemeyer). Also, a workshop focused on pheromones for control of fruit orchard pests was organised in Brazil autumn 2004.

Comments and conclusions

- The IFS/MISTRA activities on semiochemicals were built on previously established personal contacts. These long term relations between individual scientists shortened the planning time and formed a basis for efficient use of project time. The administrative support is nevertheless of utmost importance. The social and bureaucratic support from units like IFS and International Science Program at University of Uppsala for visitors is invaluable as it allows the hosting research groups to focus on the basic objective for the visit i.e. the scientific program for visits. Experienced and qualified units of this type cannot be formed within the ordinary departmental structures.
- The importance of long term commitments can never be stressed to much personal relations between senior scientists reduces practical problems and support a sound scientific development with the projects.
- Creative science breaks borders. To do that it is necessary to be able to recognise also intellectual borderlines the passage of which may broaden the minds and open for scientific and applied development.

Green networks within the German Development Cooperation

Ulrich Roettger[1]

Introduction

The *German Development Cooperation* is oriented especially towards the North-South Cooperation. A number of institutions are involved, providing services and information: GOs, NGOs, church organizations and foundations. They are dedicated to a wide variety of topics, not only management of natural rescores and rural development. All of them have their own network(s) and electronic information system.

In the first part of the presentation, the mayor players are presented with their orientation and web address. This is important to understand the full scope of German Development Cooperation and the options involved. In the second part of the presentation, information on networks within the Deutsche Gesellschaft fuer Technuische Zusammenarbeit (GTZ) is provided with special emphasis to management of natural resources.

Institutions of German Development Cooperation (DC)

The *umbrella institution* for (nearly) all of German DC is the Federal Ministry of Economic Cooperation and Development (BMZ, www.bmz.de). The mayor part of funding for DC activities is provided by BMZ. The next level consists of *implementing agencies*.

Government organizations are the main implementing agencies:

- Kreditanstalt für Wiederaufbau (KFW) www.kfw.de is a bank and provides credits for development projects and is the lead agency for German financial cooperation (FC).
- Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) www.gtz.de and Deutscher Entwicklungsdienst (DED) www.ded.de are institutions dedicated in the implementation of German Technical Cooperation (TC) proyects and programms.
- Inwent, http://www.inwent.org (formerly DSE) is providing training for students and professionals from developing countries.

Church organizations are basically involved in poverty alleviation and education

- Brot für die Welt www.brot-fuer-die-welt.de
- Misereor www.misereor.de

Political foundations are mainly oriented towards the development of a humane society

- Friedrich-Ebert-Foundation, www.fes.de
- Konrad-Adenauer-Foundation, www.kas.de

Quite a number of *NGOs* are involved in German DC. Their focus is very wide, but humanitarian aid in problematic areas is a main topic. Two mayor players are:

- CARE Deutschland e.V. care.de www

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[1] CATIE/GTZ, Project of Promotion of Non Synthetic Phytosanitary Products Costa Rica

- Terre des Hommes Deutschland e.V. www.tdh.de

Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) is the main German implementation agency for Technical Cooperation (TC). Their main tools are projects and programs in development countries and with the coordination of the German contribution to UN Institutions (FAO, UNIDO, etc.).

Main focus of GTZ is on poverty alleviation, environmental protection and conservation of natural resources, education and training. GTZ is working within a number of multilateral networks like the United Nations www.un.org International financing institutions (World Bank, regional development banks, International non-governmental organizations (NGOs), WWF, PAN, Greenpeace, CropLife and the European Union, europa.eu.org

GTZ web side www.gtz.de gives you a wide range of information on the work of the institution in more than 100 countries. It provides insight into actual topics on DC and TC as well as access to GTZ information on all projects/ programs in execution. Through this page you can access GTZ offices and service centers all around the world.

Within the GTZ information system are embedded and accessible a number of Regional Networks (Fachverbuende) oriented towards *green topics*.

- **SOWAS,** www.gtz.de/newtwork/sowas, Services on Water and Sanitation Africa
- **SNRD Africa,** www.gtz.de/snrd, Sector Network Rural Development, Africa
- **SNRD Asia,** www.gtz-asia-online.org, Sector Network Rural Development, Asia
- **MEN-REM,** www.gtz.de/network/men.rem, Mediterranean Environmental Network
- **GATE,** www5.gtz.de/gate, German Appropriate Technology and Ecoefficiency Programme
- **BIOPESTICIDES,** www.biopesticidas.org: Information system on biopesticides

GTZ was involved in an important information system, now running under the coordination of WHO:

- **INFOCAP,** www.who.int.ifcs/infocap, information Exchange Network on Capacity Building for the Sound Management of Chemicals,

All of these information systems are open to the public and generally in German and English. They not only provide valuable information for the network members but also represent a tool to discuss new topics and positions between projects / programs (representing the view in the field) with the groups attached in the GTZ headquarter.

Building research capacity in the fields of IPM in general and semiochemicals and microbial antagonists in particular in the Latin American region

An overview of CATIE's Postgraduate Program: Contributions to date and opportunities for future cooperation

Glenn Galloway[1]

Introduction

Established in 1946, CATIE's Postgraduate Program is the oldest in Latin America devoted to tropical agriculture and disciplines related to the management and conservation of natural resources. Over 1,700 professionals from over 40 countries have earned their Masters degree from CATIE's Graduate School, most from less developed countries in tropical regions.

Mission and Scope

CATIE's graduate education is strongly influenced by the Institutional Mission and philosophy. CATIE's Mission statement is as follows: *"CATIE will strive to be instrumental in poverty reduction and rural development in the American tropics, by promoting diversified and competitive agriculture and sustainable management of natural resources".*

As the Mission statement implies, CATIE is concerned with not only the technical and ecological aspects of tropical agriculture and natural resource management, but also socioeconomic, institutional and policy concerns that influence the viability of sustainable management practices.

Although CATIE's headquarters is located in Turrialba, Costa Rica, the scope of the institution is much greater. CATIE is characterized by the activities developed in its member countries which now includes 13 countries, including all of Central America, plus Mexico, Colombia, Venezuela, Bolivia, Paraguay and the Dominican Republic. The bulk of CATIE's research and outreach activities are carried out in member and non-member countries in close association with a wide host of partners from the public and private sectors, educational institutions and community base organizations.

CATIE's principal strength lies in the close articulation among its research, outreach and higher education activities. Most thesis research is carried out in conjunction with research and development projects in a host of countries across tropical America.

Research is oriented to respond to critical information needs in these countries, and the knowledge generated from this research is incorporated directly into CATIE's graduate program. This direct link between field activities in a wide host of countries and CATIE's graduate education program, ensures that our students are exposed to and take part in initiatives relevant to their countries of origin.

CATIE has a long tradition in working with North American and European partners in the planning and execution of research projects in tropical America. At present, CATIE is executing over 70 research projects in genetic resources, integrated pest management,

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[1] Director of the Education Program. Dean of the Graduate School. CATIE.

agroforestry systems, forests and biodiversity and socioeconomic topics. CATIE's graduate students make important contributions to many of these research projects through their thesis research.

CATIE's Academic Program Structure

CATIE's Masters Program is structured into five main academic areas:

- Ecological agriculture
- Tropical agroforestry
- Management and conservation of tropical forests and biodiversity
- Integrated watershed management
- Environmental socioeconomics

Overtime the specific topics of interest within each academic area have evolved. Apart from traditional interest in productivity and ecological aspects, more attention is now given to topics such as restoration of degraded lands, environmental services and retribution for these services, organic production, biodiversity issues, commercialization and managerial aspects of small eco-enterprises, rural livelihoods, policy issues, among others.

In 1996, after 50 years of postgraduate education, CATIE began its doctoral program. Students earn their Doctorate in Philosophy (PhD) degrees under different schemes designed to strengthen the overall program.

Currently, two joint doctoral programs are offered, one with the University of Idaho (United States), the other with the University of Wales (United Kingdom). Students may also opt for the CATIE doctoral program (not a joint program), but even under this program students are required to take courses in institutions other than CATIE. To fulfill this requirement, many doctoral students take courses in universities in the United States and Europe with which CATIE has ongoing agreements. It is important to note that the official language of the doctoral program is English Currently, this young Doctoral Program has students admitted or graduated from 23 countries, indicating great demand for opportunities to conduct doctoral level research in the American tropics.

CATIE and IPM

With regards to integrated pest management (IPM), in the 1970's and 1980's, there were important educational efforts initiated involving CATIE. These efforts involved sending abroad faculty for graduate training, as well as the inclusion of IPM topics in regular courses. CATIE's largest IPM project was launched in 1984, an initiative promoted by the Consortium for International Crop Protection (CICP) and funded by the U.S. Agency for International Development (USAID).

This project developed a formal graduate *Magister Scientiae* program and provided short-term in-service training to several young scientists at CATIE, as well as demand-driven short courses in the region. Contributions of this project were truly remarkable, not only by endorsing and legitimizing IPM as a feasible alternative for crop protection in Mesoamerica, but also by giving rise to an endurable tradition on IPM in this region and accomplishing its institutionalization at CATIE. About 100 IPM-major graduates from CATIE's M.Sc. program have made a significant contribution in this regard in the Region.

Roles of CATIE´s Graduates

A recent study of a sample of CATIE's graduates (242 persons that graduated between 1990 and 2001) yielded the following results:

- Most graduates return to the countries of origin where they work as professors, researchers, project coordinators, extensionists and managers in private firms.
- A significant percentage of CATIE's graduates maintain active in education and research (at least 60% of those sampled). In the context of countries with limited expenditures for research, this finding was quite significant.
- Several graduates presently occupy important leadership posts, examples include:
  - M.Sc. Patricia Panting, Minister of Environment of Honduras, Class 1999-2000
  - Dr. Miguel Santiago Campos, Agricultural Secretary of Argentina, Class 1987-1989
  - M.Sc. Sergio García, Permanent Secretary, MAF, Belize, Class 1995-1996
  - M.Sc. Lorena San Roman, Earth Council, Costa Rica, Class 1980-1982
  - M.Sc. Yemel Ortega, Director Monsanto, Central America, Dominican Republic, Class 1988-1990
  - M.Sc. Sergio Abarca, Director "Sanidad Agropecuaria", MAG, Costa Rica, Class 1986-1988

In addition to the persons in this list, there are many graduates occupying important positions in their respective institutions, for example, coordinators of academic programs, associate dean of one faculty, managers of diverse firms, directors of research centers, directors of operations, heads and coordinators of national research programs in at least two countries.

These graduates have the opportunity to put into practice their knowledge and influence the philosophies and technical orientations of the institutions they represent. In many cases, these institutions are linked to development projects and other initiatives directed to diverse end-users.

About 5% of the graduates sampled are currently involved in doctoral programs. Most of these professionals will pursue careers in higher education and research.

In the last few years, CATIE has devoted considerable efforts to form and consolidate active alumni associations in the countries and a blanket regional organization representing all CATIE graduates. An increasing effort will be made to provide graduates with alternatives to update their knowledge and make them aware of employment and funding opportunities.

CATIE´s dynamic research, outreach and higher education programs and the considerable network of competent graduates throughout tropical America makes CATIE an ideal partner for European and North American research and higher education centers with interest in pursuing work in the neotropics. Indeed, CATIE should be viewed as node or hub for cooperative endeavors, which seek to alleviate poverty through sustainable agricultural practices and sound management of natural resources.

Chemical Ecology: Future development in Costa Rica– the University perspective

Alice L. Pérez[1]

Introduction

The development of Chemical Ecology as a field of research has been slow in Costa Rica, in spite the rich biodiversity in our country. For many years, the research at our universities and other institutions has involved the study of natural products and their applications, without having an integrated view or awareness of the mediation of chemical signals *per se*. In the specific field of semiochemicals and their use in integrated pest management strategies, the situation is not much different.

The purpose of this presentation is to illustrate some of the work in which the University of Costa Rica has been involved, to show the potential of being part of a more ambitious Chemical Ecology program and to show also some of its limitations.

Discussion

The University of Costa Rica has several well-established research centers that deal with: a) Crop protection, b) General agricultural studies, c) Molecular biology, d) Food technology, e) Grain and seeds, f) Natural products, g) Environmental studies, h) Harvesting and storage, i) Microorganism, j) Entomology, and k) Economics.

Moreover, there are at least seven research stations located at different places in the country that are devoted to the study of agricultural systems and conservation.

Having all these advantages, an integrated view of the field of Chemical Ecology has still been wanting. For example, studies about semiochemicals have been limited in most of the cases to fieldwork observations using very few examples. The laboratory work needed for the leap from casual observation to a more serious program is missing.

More recently, with the opening of the Organic Crop Production Program, the use of natural product extracts to control insect populations and different diseases has increased. However, there is not a systematic cooperation or study to determine the chemical basis of such uses (or more properly, the chemical language is missing) sometimes because of the lack of resources and sometimes because it is preferable to use more traditional approaches.

This situation may change if a program for the development and study of semiochemicals in Costa Rica can be developed, first through the establishment of a local network and then through international cooperation. This consortium may allow discussion of common ideas and problems and allow us to seek a basis for the development of weak areas like behavioral entomology, insect rearing or electrophysiology.

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Conclusions

- All the resources for the development of a chemical ecology program are present in Costa Rica, what is missing is the organization and effort to put a multi-disciplinary program together.
- The Universities can become the focal point of this program because of their combined experience.
- The establishment of a Chemical Ecology program should be a priority for the funding agencies and more information should be provided to show the advantages of such a program.

Building capacity in semiochemical research in Chile

Hermann M. Niemeyer[1]

This paper describes the model used for setting up a multidisciplinary laboratory studying plant and insect semiochemicals at Universidad de Chile, and the efforts to develop a similar model in Peru.

The isolation of a semiochemical (signal chemical) is a multidisciplinary endeavor. Within the context of integrated management of pest insects, this research requires a certain degree of expertise in various disciplines: *ecology* and *behavior* in order to define the problem, i.e. studies ascertaining the occurrence of a semiochemical mediating the interaction (plant-plant, plant-insect, insect-insect, plant-insect-insect) studied; *biology* addressing life history traits necessary to optimize the conditions for isolation of the semiochemical; *analytical chemistry* necessary to isolate, quantify, and determine the structure of the compound – most likely coupled with *organic synthesis* in order to test different alternative structures if the compound is isolated in minute amounts, as is usually the case with insect pheromones and most particularly when chirality is involved; *plant and insect physiology and biochemistry* in order to define the molecular mechanisms of production of the semiochemical and its mode of action on the insect; and *genetics* in order to address the mechanisms controlling of the interaction, which may eventually lead to the manipulation of the system so as to warrant the use of the semiochemical in an integrated pest management scheme.

In the late 1970's, research on insect-plant semiochemicals in Chile was virtually non-existent. The development of a Chemical Ecology Laboratory (CEL) at University of Chile through collaboration with Chilean experts from relevant disciplines was considered unfeasible mainly because those scientists were focused on other type of problems. For example, while ecologists were studying general ecological processes, particularly in relation to plant conservation, plant physiologists the effects of stress on plant biochemistry, and entomologists mainly taxonomy, synthetic organic chemists were doing semisynthesis of plant natural products and organic polymers, and structural organic chemists were gathering data on plant constituents, with a strong specialization on taxa or type of compounds. Additionally, most colleagues did not show willingness to embark in a multidisciplinary effort

Hence, collaboration with foreign experts was considered. Although this would doubtless give a greater depth and increased efficiency to whatever studies were to be undertaken, it would also limit or postpone the building up of a wide-base infrastructure at Universidad de Chile, and would involve a certain degree of loss of academic independency, not to speak of eventual quarantine problems arising from work on pest insects. The strategy finally chosen was based on building up a multidisciplinary laboratory with strong links to several

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wise and generous colleagues abroad whose laboratories covered a wide range of disciplines.
The initial problem addressed at the CEL -differential resistance of wheat cultivars to cereal aphids- led to a wide range of studies, for example, analysis of wheat allelochemicals and synthesis of chemical analogues, and their effects on aphids at population, behavioral, physiological and biochemical levels; induced plant defenses; learning in aphids; tritrophic interactions; population genetics; aphid semiochemicals, among others. Each topic was undertaken by a different graduate student at the CEL, whose study program usually included one or more visits to the collaborating laboratories abroad for different periods of time in order to acquire the relevant expertise, participation in national as well as international meetings, writing up of grant proposals, either their own or CEL's, and also supervision of Latin American fellows visiting the CEL. By the end of their Ph.D. studies, these graduate students were fully mature scientists with strong international connections, and with experience on the full range of activities of a university research laboratory.

The model was very successful, as measured by the insertion of most students graduated from the CEL into the university system in Chile, by their capacity to generate active research laboratories of their own, and by their willingness to collaborate between each other, in spite of being at universities in different towns.

An attempt was made to develop a multidisciplinary laboratory studying plant and insect semiochemicals in Peru using the same model as in Chile. Several Peruvian fellows were trained at the CEL in projects related to semiochemicals. Most of them went back to Peru and either remained in teaching or research jobs at their home universities, or went abroad for further training. Simultaneously, a strong leader was selected among the Peruvian students at the CEL with the hope he would return to Peru and gather around him former CEL fellows, thus creating a multidisciplinary group on semiochemicals which would nourish not only from the scarcely available local funds but also from the international contacts he had had a chance to create while a student at CEL, and from the CEL itself. The program evolved differently from the original plan, because the chosen Peruvian leader decided to develop his scientific career in Chile. On the other hand, he has been able to get support not only to bring to his laboratory in Chile other Peruvian fellows, but also to further train former CEL Peruvian fellows. It is hoped that this "deviation" from the original planning represents only a delay in the building up of research capacity on semiochemicals in Peru.

Acknowledgements. My deepest appreciation to former Chilean graduate students (H.R. Bravo, P.P. Caballero, C.A. Escobar, C.C. Figueroa, E. Fuentes-Contreras, A. Givovich, J.M. Jerez, V. Leighton, F.J. Pérez, A. Quiroz and C.C. Ramírez), Peruvian fellows at the CEL (S. Beltrán, H. Collantes, L. Córdova, C. Costa-Arbulú, P. Daza-Bustamante, L. Gavilano, E. Gianoli, W. Gonzáles, M. Guerra, R. Loayza-Muro, G. Manrique, A. Martos, R. Reátegui, J. Ríos and L.C. Rodríguez), and foreign colleagues (J. Pettersson, J.A. Pickett, W.F. Tjallingii, S.D. Wratten); and to granting institutions (IPICS, SAREC, CEC, Cátedra Presidencial en Ciencias, FONDECYT, FIA, Iniciativa Científica Milenio), without whose generous intellectual support and material input, respectively, the CEL would have remained but a dream.

University of Idaho and CATIE collaboration for semiochemical discovery and application

Sanford Eigenbrode[1]

Introduction

The University of Idaho and CATIE are developing collaboration for teaching and research that addresses the commonalities of our two missions. Both institutions are dedicated to finding novel, appropriate and effective solutions to problems in agriculture and forestry. Our collaboration recognizes that these problems and their solutions transcend the boundaries of ecoregions and economies.

Together, we now offer graduate student education in tropical forestry and agriculture that includes fieldwork at CATIE, or sites in its member nations throughout Latin America, combined with coursework at both of our institutions in the latest applications of technology. In our new Joint Doctoral Program, students will have graduate committees comprised of faculty members of both institutions and fully coordinated programs. A formal exchange program in development will facilitate shorter-term cross-institutional opportunities for other students at each institution.

Chemical Ecology at the University of Idaho

Chemical ecology is one of the areas in which collaborative research and training will occur between the University of Idaho and CATIE. In the Division of Entomology at the University of Idaho, we have an active and growing program in Chemical Ecology, with infrastructure and expertise that can complement field-based research at CATIE to develop integrated approaches to semiochemical research for the tropics. Principal areas of research at the University of Idaho have been the role of plant surface waxes in insect plant interactions; pheromone discovery; and the semiochemical dimension of plant-virus-vector interactions.

Plant Waxes and Insects

The aerial, primary surfaces of all terrestrial plants are covered with a layer of hydrocarbons and their parent fatty acids, and related primary and secondary alcohols and ketones, wax esters, and other components including triterpenoids. Among these components are potentially stimulants and deterrents influencing host plant selection by herbivores. Our work in this area has identified triterpenoid phagodeterrents to the diamondback moth, *Plutella xylostella,* in waxes of resistant genotypes of *B. oleracea* that could contribute to improved management of this serious cosmopolitan pest (Eigenbrode et al. 1991, Eigenbrode and Pillai 1998). We have also found evidence for oviposition stimulants in wheat waxes for Hessian fly (Cervantes et al. 2002).

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Our work on waxes also concerns their physical and chemical effects on insect attachment to plant surfaces. Pure wax components differ in the attachment forces insects can generate on them (Eigenbrode and Jetter 2002), evidently because of the interactions between these components and the wetting agents of insect tarsi. Crystallized waxes severely disrupt insect attachment, but the effects differ among species of herbivore and carnivore. These effects of plant surface waxes on insect attachment open the possibility for developing insect resistant and biological control-compatible crop plants (Eigenbrode et al. 2000, Eigenbrode 2004).

Pheromones

At the University of Idaho we have investigated potential pheromones of insects important for human health and agricultural security. The sugarbeet root maggot fly, *Tetanops myopaeformis* (Otitidae), is the most important pest of sugarbeets in North America. We have found that males of this fly exhibit lekking behavior associated with the production of a blend of 9 and 10-carbon alcohols and a ketone. This behavior could be exploited to improve monitoring of the fly (Emmert 2002, Eigenbrode et al. unpublished).

Several cuticular hydrocarbons of *Aedes aegypti* and *Anopheles gambiae* females and males change after mating and are implicated in a syndrome of reduced subsequent mating by these females (Polerstock et al. 2002, Chauvin et al. unpublished). Understanding the mating biology of these mosquitoes is important for developing new ways to manage these vectors of human disease-causing organisms.

Semiochemicals in plant-virus-vector pathosystems?

Plants infected by aphid-vectored viruses are sometimes colonized preferentially by aphid vectors of these viruses (Macias and Mink 1969, Castle et al. 1998), but the basis of such discrimination is not fully understood. We have shown that volatile organic compounds (VOC) from potato plants infected with *Potato leafroll virus* (PLRV) differ from those of virus-free plants or plants infected with *Potato virus X* (PVX) or *Potato virus Y* (PVY), and that PLRV-infection-induced The virus-infection induced VOC (VIV) from PLRV-infected plants attract or arrest the green peach aphid, *Myzus persicae,* the principal vector of this PLRV (Eigenbrode et al. 2002).

Vector attractive VIV also are produced in response to infection of *Solanum sarrachoides* by PLRV and in wheat by *Barley yellow dwarf virus* (BYDV) infection (Jiménez et al., in review). Although the adaptive significance of VIV is not known, we hypothesize that VIV are a mechanism by which certain viruses manipulate the behavior of their vectors to promote virus acquisition and spread. Luteoviridae, in which we have documented the phenomenon so far, are obligately transmitted by their aphid vectors, lending credence to this idea. Driven by the hypothesis that VIV are viral adaptations, we are examining the molecular and biochemical mechanisms and ecological implications of this interaction.

Chemical Ecology in the University of Idaho/CATIE Collaboration

At this writing, there are 12 students in the University of Idaho/CATIE Joint Doctoral program. Although none has chemical ecology at the center of his or her research plan, there will be chemical ecology components to some of them.

Edgar Varón is studying the leaf-cutting ant *Atta cephalotes* in coffee plantations, to determine ecological approaches to assessing its pest status and to evaluate the efficacy of several botanical formulations for its control.

Francisco Soto is examining the effects of tree plantation composition on the level of attack by *Hypsipyla grandella,* the mahogany shoot tip borer. Part of his work has been to examine the hypothesis that *H. grandella* employs an oviposition deterrent pheromone that could form the basis of methods to reduce its level of attack.

Julián Pérez-Flores is also working on *H. grandella,* examining the induction of resistance in scions of susceptible genotypes grafted to resistant rootstock. He will use chemical comparisons of resistant and susceptible scions of the same genetic material to examine the mechanisms. Julián spent a year at the University of Idaho during which he took a 6-credit-hour course in chemical ecology methods.

Ruth Dahlquist is working with the banana weevil, *Cosmopolites sordidus,* in small-scale organic banana production in the Talamanca region of Costa Rica. She plans to examine the effectiveness of traps baited with Cosmolure + ® (ChemTica International, San José Costa Rica) in different vegetative contexts.

Conclusions

Although we are just beginning our CATIE-University of Idaho Joint Program, we are excited about the potential to for collaborative work in chemical ecology as an important component because it can build effectively on the strengths of our institutions.

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The private sector and the market place

Strategies for commercial development of pheromones

A. C. Oehlschlager[1]

Introduction

The pheromone industry is estimated to be currently US$100,000,000/ year, about 1% of the world's insecticide market. Sales volumes of pheromone-related products are growing at an annual rate of ~10%/year, while insecticide sales are stagnate on a world-wide basis. Sales of pheromone-based products are greater than all biological alternatives (viruses, fungi, predators, nematodes) combined. Mating disruption products are the strongest component of world-wide pheromone sales accounting for about 66% of sales volume. Remaining sales are of monitoring and attract and kill (trapping) strategies. It is estimated that approximately 400,000-500,000 Ha are currently under control by mating disruption while 120,000-150,000 ha are under control by attract and kill (trapping).

Revenue sources

Revenue sources of pheromone companies are highly variable. In some instances emerging as well as established companies receive up to 100% of revenue from governmental sources. In other instances revenue is entirely derived from sales. While the pheromone industry has some vertically integrated companies that prepare pheromones, formulate them and market them most are horizontally integrated and outsource one or more of these functions. All pheromone enterprises that have been in existence for more than 10 years have some component dedicated to new product development.

Strategies for growth

The principal activity that has led to growth within the pheromone industry has been the formation of regional enterprises that target regional problems. Within each company strategies for growth include both development of new products and penetration of existing markets with improved products. While the first strategy offers the prospects for the highest margins the second offers an existing clientele of pheromone users.

Slow Release Strategies

There are several slow release strategies available for pheromones. Because of the ease of manufacture the most popular release devices for pheromones are those in which the pheromone is mixed with a flexible polymer (matrix). The pheromone is mixed with a plastic that is either already polymerized or polymerized after mixing. The more popular polymers used in this strategy are rubber, silicones, polyurethanes, polyvinyl chloride and wax.

Because of the physical interaction of pheromones with flexible polymers the release kinetics of these devices are invariably exponential. Most of the pheromone is lost in the

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[1] ChemTica Internacional San José, Costa Rica.

early stages of field use and low amounts are available during the latter part of the active field life of such lures. The release rates of matrix lures increases at higher temperatures.

A second strategy for slow release of pheromones is enclosure in semipermeable membranes. In this strategy the release is regulated by the rate at which the pheromone permeates through the membrane. Release from membrane lures remains rather constant until the internal reservoir of the lure is depleted. Release kinetics of membrane lures is therefore more linear than from matrix based lures. The major problem with membrane lures is that at high temperatures the permeability of pheromones through membranes can become very rapid and pheromone can be lost in a very short period of time. This shortens the field life of the device.

A modification of membrane release technology is microencapsulation. Many variations are available but none have had a major impact on their major market target: mating disruption. The primary problem with microencapsulation pheromone delivery is that pheromone evaporation is not significantly restricted by microcapsules available up to this point in time.

Electronically driven release devices are also available. Most of these devices depend on battery power to activate a release mechanism of a pressurized container containing pheromone. These devices have been developed primarily for the mating disruption market. Their advantage is that pheromone can be released only during the calling period. These devices compete directly with matrix and membrane devices and to date have been adopted by very few users. An economic analysis of these devices reveals that although matrix and membrane devices emit pheromone constantly that the cost of pheromone emitted by these passive devices during periods in which females are not calling is less than the cost of the electronics and associated mechanical devices required to emit pheromone only during calling periods.

A modification of the electronically driven device based on slow delivery of antibiotics to animals has been adopted to delivery of pheromones. In this strategy the pheromone is enclosed in a plastic bag that is then placed adjacent to a second bag. The second bag is then pressurized slowly by gas produced by an electrolytic process. This method of delivery of pheromones is very constant and may find use for extremely expensive pheromones that are required to be delivered very accurately.

Future Prospects

Although the governments of most countries are under public pressure to limit the application of insecticides on food crops significant barriers still exist for implementation of alternative pest control strategies including use of pheromones. The greatest progress has been made for introduction of pheromone-based pest control strategies in those countries with the lowest regulatory hurdles for registration of pheromones. Since pheromone-based technologies are usually implemented in "niche" markets this industry can expected to continue to be dominated by regional enterprises with unique market opportunities.

Regulations for registration and use of semiochemicals in Central America. A first draft.

Ulrich Roettger and Manuel Carballo[1]

Introduction

The pheromones and semiochemicals that affect the behaviour of insects are an important tool in integrated pest management (IPM), mainly because of their advantages over synthetic pesticides, for example, high specificity, mode of action as insects behaviour regulating rather than killing, and the possibility to be use in fixed location as lures or traps, and for these reasons it is expected that most semiochemical products pose lower potential risk to human health and the environment than conventional pesticides.

Nowadays, this type of products in Central America are registered to be used with the same regulations used to register chemical pesticides. The CATIE/GTZ Project of Promotion of Non Synthetic Phytosanitary products has been promoting the development of specific regulations to the registration of microbial and botanical biopesticides and has a lot of interest in the development of protocols to register pheromones and other semiochemicals as exits in North America and Europe.

The necessity to develop an specific regulation

Among the purposes to have specific regulations to register these products is to facilitate the development, registration, commercialization and their use, in order to control of pests and consolidate an harmonized regulation at the Central American level.

Semiochemicals that do and do not require registration

In North America, the regulations indicate that the semiochemicals that do not require registration are semiochemicals that are used in fixed-location lures with the purpose of attracting and monitoring pests and have a minimal impact on either the environment or human health. Meanwhile, semiochemicals that require registration are products contained in solid-matrix dispensers that are placed in large numbers by hand or machine with the purpose of controlling pest and those that are sprayed on the field.

Data requirements to registration

The require information to obtain the registration should include information about the characteristics or the active ingredient and the formulated product. This information includes the chemical composition, chemical and physical properties and the manufacturing process. In order to estimate the environmental and human health risk, the toxicological information should be included, for example, acute oral, acute dermal, acute inhalation toxicity and chronic toxicity studies or long term studies. The effects on non target organisms that include wild birds, bees, terrestrial invertebrates, fish and studies about food residues and finally information to estimate the efficacy of the product including the mode of action, description of he pest problem and efficacy trials.

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[1] CATIE/GTZ, Project of Promotion of Non Synthetic Phytosanitary Products. Costa Rica.

Data requirements for semiochemicals and pheromones.

Level	Title
Specifications for active ingredient	
Characteristics	Common name
	Chemical name
	Identity of components
	Impurities
Manufacturing methods	Process description
	Starting materials
Chemical and physical properties	Water solubility
	Solvent solubility
	Color
	Physical state
	Odor
	Density
	Stability
Specification for formulated product	
Product identification	Trade name
Formulation process	Starting materials
	Formulation process
Chemical and physical properties	Color
	Physical state
	Odor
	Density
	Stability
	Formulation type
Toxicology	
Acute studies	Acute oral
	Acute dermal
	Acute inhalation
Short term studies	Rat development studies and others
Environment toxicology	For non-target terrestrial invertebrates and freshwater invertebrates, fish and wild birds
Efficacy studies	Efficacy trials

Opportunities and constraints in the commercial development of semiochemicals- Regulatory aspects

Iain Weatherston[1]

Introduction

Although the structure of the first pheromone was elucidated in 1959, the first commercial application of a regulatory approved pheromone product for crop protection did not take place until 1978, when Conrel aerially applied their hollow fiber formulation on Arizona cotton to control the pink bollworm.

This long development time has been variously blamed on such reasons as a) an oversell of the potential of pheromones and other semiochemicals; b) skepticism by the agrochemical industry; c) that commercialization was and generally still is undertaken by small under financed companies; d) premature introduction of products into the market place; e) regulatory issues; f) lack of proprietary protection and patentability; and g) other perceived issues such as target specificity, small markets, user education, lack of robustness, etc.

At this time, the role played by regulatory issues in the development of semiochemical products is discussed in light of opportunities and constraints, using the regulatory system employed in the United States as a model.

Types of Uses for Semiochemical Products

Of all of the different types of semiochemical products (pheromones, attractants, floral emissions, repellents, etc.) it is the pheromones, and principally sex pheromones, which have been commercialized. These pheromones have been used in two ways: a) in traps, as monitoring agents for detection, timing of control measures or quarantine purposes; and b) as direct control agents in disruption, attract and kill, attract and trap and bio-irritation strategies.

Regulatory Issues

There are opposing viewpoints as to whether semiochemicals should be regulated and require registration (government marketing approval) before being allowed into the market place. On one hand there are those who argue that these biochemicals are found in nature (or are synthetic replicates), are used in very small amounts in comparison to conventional pesticides and act by non-toxic modes of action and hence should not be regulated. The opposing view held by the proponents of the Precautionary Principle maintain that, amongst other things, the manufacturers/vendors of pheromones and other semiochemicals must accept the "burden of proof of harmlessness."

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[1] Technology Sciences Group Inc. Goodyear, AZ 85338, USA.

Although there may be differences between the reasoning and requirements of the regulatory authorities in different countries regarding pest control products, it can be generally considered that regulatory approval is granted once an applicant has shown that the product is safe to humans and the environment, and efficacious.

Globally, registration of some semiochemical products is required in most countries but the degree of regulation varies greatly. For small entrepreneurial companies, the issues translate into cost and time, two resources usually in short supply.

Semiochemical Registration

Using the U.S. EPA system as a model the role of regulatory issues in semiochemical commercialization can be discussed.

As mentioned above, the first EPA registration for a pheromone product for area-wide dissemination was granted to the Conrel Division of Albany International for the pink bollworm control product Gossyplure HF, the cost of this registration was in excess of $1 million. Shortly thereafter the cost of registering a lepidopteran pheromone with the EPA was estimated to be $1.34 million.

In 1979 there were discussions at the Advanced Research Institute on Chemical Ecology, one of the recommendations pertaining to the commercial development of behavior modifying chemicals that came out of the discussions was that "*regulatory agencies in all countries should publish special guidelines during 1979 for the registration of semiochemicals for pest control.*"

Twelve countries had participated in the Advanced Institute, but only four of them Britain, France, the Netherlands and the United States agreed to participate in a NATO sponsored study, and of those only the United States at that time recognized the need for, and developed specific requirements and guidelines for the registration of pheromones and other biorational insecticides. About this time France also recognized the need to regulate "unconventional pesticides" differently from conventional pesticides. In the French guidelines it was proposed that the use of pheromones in traps would not be regulated, and for control only basic toxicological data would be required if the semiochemical product was not applied to the edible part of a plant.

While in 1982 regulatory requirements were still viewed as a major disincentive to semiochemical commercialization, the tide had turned and the EPA recognized the unique nature of what they called "biorational pesticides." At an international conference sponsored by IOBC/WPRS, again in Holland one of the recommendations which issued detailed that the registration procedures for biocontrol agents were unclear and too time consuming, and that legislative measures should be adopted that would result in an easier and less expensive approach to the registration of biopesticides.

Further progress was made between 1988 and 1996, when the EPA:

a) Issued a regulation that products not containing any toxicants, intended only to attract pests for survey or detection purposes and labeled accordingly are not considered to be pesticides and therefore do not require registration.
b) Issued a regulation that pheromones labeled for use in pheromone traps, and the pheromone traps in which these pheromones are the sole active ingredient are exempt from regulation.
c) Issued a blanket exemption from the requirement of tolerance for lepidopteran pheromones, which they define as having an unbranched aliphatic chain (9 – 18 carbons) ending in an acetate, alcohol or aldehyde functional group containing up to three double bonds and used on growing crops at a rate of no more than 150 grams active ingredient/acre/year.
d) Expanded the minimum acreage to 250 acres from 10 acres for the testing of arthropod pheromones contained in polymeric matrix dispensers when deployed at a rate not exceeding 150 grams active ingredient/ acre/ year without the need to obtain an experimental use permit (EUP).
e) Broadened the scope of this regulation to include broadcast applications and sprayable formulations of non-food use arthropod pheromones.
f) Further broadened the scope of the regulation to include food uses of broadcast and sprayable formulations of lepidopteran pheromones. This only applies to products where the lepidopteran pheromone is the only active ingredient. Any pheromone/insecticide combination product and non-lepidopteran pheromone products still require an EUP if tested on more that 10 acres in a non-retrievable matrix type formulation.
g) Issued a regulation designating certain materials as "minimum risk pesticides" which when used alone, or in combination with other minimum risk materials and in combination with certain inert ingredients ("minimal risk inerts") are exempt from regulation under the Federal Insecticide, Fungicide and Rodenticide Act.

In the last several years Canada and the OECD have also made strides to ameliorate the requirements for semiochemical registration, but primarily in regards to straight chain lepidopteran pheromones. Albert Minks as far back as 1990 opined that the EPA guidelines for semiochemical regulation should form the basis on which the regulatory authorities in all other countries should develop their registration requirements and guidelines.

Summary of current regulatory status of semiochemicals

In this summary the status is presented in two ways: a) based on the type of formulation and use; and b) based on the type of semiochemical. For each type, the current situation is:

Formulation type

Monitoring/Survey: arthropod pheromones, attractants and "minimum risk pesticides" are exempt from registration.
Mass trapping: arthropod pheromones when the sole active are exempt from registration.

Kairomonal use: a semiochemical used in control strategies as a kairomone to mitigate parasites and/or predators is exempt from registration.
Disruption: all pheromones irrespective of formulation require to be registered, but when used on food or feed crops lepidopteran pheromones (SCLP) are exempt from the requirement of a tolerance.
Attract and Kill: pheromones used in conjunction with an insecticide in a formulated product require to be registered, and although the pheromone may be exempt from tolerance for food and feed crop use, the insecticide and inerts must either have a tolerance or exemption from tolerance.

Types of semiochemicals

Arthropod pheromones: when used in traps for monitoring or for control are exempt from registration provided they are the sole active ingredient.
Arthropod pheromones: are exempt from registration when acting as a kairomone for a beneficial arthropod.
Arthropod pheromones: when used in retrievably sized polymeric matrix dispensers, applied to growing crops at a rate not exceeding 150 grams active ingredient/acre/year are exempt from registration but may require a tolerance expression.
Arthropod pheromones: when used in products for area-wide pest control strategies require registration, and require a tolerance expression unless used on a non-food crop.
Lepidopteran pheromones: when used in area-wide control strategies require registration however certain lepidopteran pheromones (SCLP) are exempt from the requirement of a tolerance when used at an active ingredient rate of up to 150 grams/acre/year.
Arthropod semiochemicals: when used only to attract pests for monitoring and survey and labeled accordingly are deemed not to be used for pesticidal purposes and are exempt from regulation. This also applies attractants and other semiochemicals.
Arthropod semiochemicals: which behaviorally manipulate beneficial arthropods are exempt from regulation.

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Commercialization of biopesticides experiences from a European process

Berndt Gerhardson[1]

Introduction

A spreading environmental concern and the problems encountered in using many conventional pesticides, e. g. resistance in target populations, residues in products, and health risks, have resulted in a worldwide strive for alternatives. This is often most clearly outspoken in research funding and political organizations, but an increasing number of researchers also have become involved. One of the important, potential alternatives identified, and often emphasized, is the use of biopesticides: preparations of pest, or disease suppressing organisms and/or their metabolites (Cook 1993.). Many research groups also have envisaged potential active agents/organisms resulting in a vast literature and several reviews addressing this topic (e.g. Butt et al. 2001, Charudattan 2001, Whipps 1997).

However, in looking worldwide it is paradoxical that despite great research efforts, political pressure in several countries, and an increasing organic farming, urgently needing new biopesticides, comparatively few products are on the marked, and even fewer have a large scale practical usage. Many factors, not least psychological, are behind this, and in drawing mainly from our own, European experiences I will shortly discuss some of these.

Commercialization is a costly process

From a tentative start in the middle of the last century, there have been a steady increase in research activities devoted to an even more intensive mining of nature for natural, plant disease, or plant pest suppressing organisms and/or metabolites, e. g. a number of Mistra programmes (www.mistra.org). Many of these organisms/ metabolites probably would function excellently as active ingredients in biopesticides, but they then normally have to be proliferated, and suitably formulated into products that can be packed, stored and distributed.

The development of such products, including large scale production/proliferation, formulation, product control, packing, distribution and marketing usually is a very expensive process (Hall and Menn 1999). This developmental cost is in itself a drawback, but it often, in addition and more importantly, lead to requirements for patents, or other forms of protection. However, since the active ingredients, especially where they are living organisms, usually are difficult, or in many cases impossible to patent, this requirement can seldom be met. Normal industrial funding for developmental work thereby is not available, and, as a consequence, the potential good products have to stay in the drawer as "promising possibilities".

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Agro-industrial complexes need adaptation – and time

Also in cases where you succeed in developing a biopesticides there might be a long way to go for marketing it. New products need acceptance and where there is no alternatives, this often is not a problem. However, where biopesticides interfere with a tradition of using cheap, efficient and easy to apply chemical pesticides, the end users, the dealers and the industry supplying application equipment etc. naturally are more skeptical and may be reluctant to change.

Moreover, even if these actors are convinced that biopesticides have economic and/or health and environmental advantages, the often complex backing up systems of tests applied, treatment equipment, extension services etc., normally are built and adapted for handling chemicals and nothing else.

Efficacy testing methods worked out for chemicals are, for example, in many cases misleading when applied for testing biological products with totally different modes of action (Gerhardson 2002). Many of the devices traditionally used for spraying, or for seed treatment are, likwise, often not suitable for applying biopesticides. This in turn, where changes of equipment, or specific adaptations have to be made, constitute an economical burden in marketing the biopesticides.

Other adaptations concern the strategies set up for pest and disease control management. Traditionally the control measures taken presently are often built on availability of chemical pesticides with broad actions on many pest, or diseases, and on efficient curative trestment. However, most biopesticides are not just other types of chemical pesticides that should, or can, be used in the same way. They rather constitute a totally new concept for plant protection, and thereby in most cases require different pest management regimes.

Many of the biopesticides have limited shelf-lives when stored, may be suitable or efficient only under certain weather or climate conditions, need specific timing for application, and few show good efficacy against several pests, or diseases. It might take much time - and efforts - to get a broad understanding and appreciation of such differences.

European registration procedures are serious bottlenecks

In most countries there is a requirement for some kind of registration of biopesticides. The European biopesticide registration processes have turned out to be especially complicated and time consuming, and are, thereby, for many potential biopesticides prohibiting expensive.

A general and remaining observation concerning the present European procedures is that many of the guidelines to be followed for registering organisms have been directly copied from guidelines for registration of chemical compounds. Since totally different concepts in identity, in modes of action and in use and handling are at hand for most biological agents, and since these are already naturally occurring, several of these requirements - mostly well motivated for chemical compounds - simply does not apply for untransformed microorganisms from our own ecosystems. The motivation for researchers to follow, or accept, certain of the present requirements thus is low.

The EU registration follow certain routines, long used for registering other plant protection products, and the counterparts of registration authorities have traditionally been large agrochemical companies. Some costly routines, like extra test requirements late in the process, or requirement to perform tests or documentations just in case, may for these companies be marginal economically. For those, often small companies, or organizations, introducing biopesticides, such extra registration costs are, on the other hand, often a main obstacle.

My conclusion is that to retain confidence in registration rules, and authorities, and to find the registration process for untransformed, naturally occurring organisms sound, reasonable and logical, the present (partly tentative) guidelines have to be in all the details/requirements well motivated and, thus, on a number of points radically changed. Clear and scientifically sound motivations in all details would also help to avoid the risk of unintentionally create barriers for registration of biocontrol agents.

GMM, or not GMM - an important crossroad

The constrains for commercializing biopesticides related above assumingly will continue to hamper these kinds of products -like the multinational agrochemical companies from fully going into this area. However, since the biopesticides can be seen as constituting a usable and economically and environmentally interesting potential, which we hardly can overlook, a number of new products will probably be marketed in the future anyway.

To what extent this will happen is, in my view, much a matter of political decisions, not least concerning registration costs and patent protection. but also concerning the possibilities of using genetically modified microorganisms, GMM. In case certain GMM would be allowed to be let out in nature, a lot of possibilities for designing new, and for enhancing the efficacy and activity spectrum of active agents in available biopesticides might be very much broadened.

Among possibilities so far little tested are also the combination of various pesticidal regimes, and combinations with other chemical or physical pest control methods (Vurro et al. 2001). Also the designing of biopesticides to suit specific plant cultivars, or a parallel development of plant resistance and biopesticides to enhance each others efficacy are possibilities that still have to be tested.

Conclusions

- Although, in many cases efficient and favorable to use, biopesticides as a group have so far not become a commercial success, mainly because of constrains in the commercialization process and in market acceptance.
- Main constrains for commercialization of new biopesticides are problems related to patenting this kinds of products, and the uncertainties around, and cost for product registration.
- In the case it will be possible to use and apply genetically modified micro-organisms many possibilities for designing and producing new and enhanced biopesticides will become available.

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Prospecting biodiversity for pest control

Ana Lorena Guevara[1]

Introduction

The indiscriminate use of agrochemicals during the last decades -and their negative consequences on human, animal and plant health- offers a challenge to researchers and entrepreneurs to find viable alternatives for agricultural production while diminishing to the minimum any potential impact on the environment and human health.

Protection of crops has taken place in three ways: through chemical control, through biological control and through genetic improvement to obtain resistant cultivars -three alternatives based on the knowledge and use of biodiversity (ten Kate y Laird 1999).

In the case of chemical control, some products called "natural" have been developed from compounds isolated from biodiversity, which are used without altering their chemical composition, except in processes of semi-synthesis. There are also other synthetic compounds that were not isolated from nature, but were modelled based on chemical structures of natural sources.

In biological control, protection takes place through direct use of organisms that naturally attack other beings; biological resources are used directly in the development of biopesticides.

The use of biodiversity for crop improvement -specifically resistance to pests- is widely known. Traditional improvement techniques applied to crops of economic importance have allowed the development of plant varieties resistant to pests and diseases.

Biodiversity provides resources for natural products and biopesticides; it is also a source for genetic alteration to obtain resistant varieties through both traditional improvement and biotechnology.

For crop protection, specifically the development and use of eco-friendly products, biological diversity is an important source of genetic and biochemical resources, which should be used to benefit human nutrition and health.

Bioprospecting: natural products and biopesticides

Through Bioprospecting defined us the "the systematic search for genes, natural compounds, designs and whole organisms in wildlife with a potential for product development by biological observation and biophysical, biochemical and genetic methods, without disruption to nature" (Mateo et al. 2000); the National Institute of Biodiversity (INBio, its acronym in Spanish) of Costa Rica has developed some projects with the national and international industries in the pest control area.

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INBio was founded in 1989 as a non-profit, public good, *non-governmental* organization. INBio's main activities at the time focused on creating an inventory and hence, on cataloguing a number of life forms such as plants and insects. Its mission is to promote a new awareness on the value of biodiversity, and thereby achieve its conservation and to improve the quality of life of human beings.

INBio is organized into strategic action units that develop activities in five main thematic areas: the National Biodiversity Inventory, responsible for the generation of information on species, their taxonomy, and distribution in protected areas of the country, as well as some elements of natural history; bioinformatics which seeks to arrange the information stemming from the inventory and other sources; social outreach that promotes bioliteracy by disseminating and using information about biodiversity for intellectual and spiritual purposes; conservation management which promotes the development of planning processes both at the national and institutional levels in matters of conservation and biodiversity management and bioprospecting which promotes the development of sustainable, novel, and profitable uses of biodiversity resources.

INBio has a number of strategic alliances both with industry and academia. In the case of Bioprospecting, INBio has established over 30 bioprospecting agreements with industry and academia. The Institute carries out a systematic analysis, documentation, and inventory of the species found in Costa Rica's conservation areas and processes the biodiversity components in its labs with financial support by private contracting partners.

Agreements are structured so that private partners in turn have rights to utilize the data and the active substances identified for their own pharmaceutical, industrial, agricultural or cosmetics research. Proceeds of commercial uses that may arise out of the research will be shared between the Companies, INBio and the Costa Rican Government. INBio and the Government will share these benefits in a 50:50 ratio.

INBio's portfolio of research collaborative agreements includes some in the agricultural area, which involve antagonist microorganisms and compounds from plants:

INBio-BTG-Ecos La Pacífica Agreement

This agreement was signed between INBio, ECOS (through Hacienda La Pacífica) and the British Technology Group (BTG) with the purpose of generating knowledge on the nematocidal activity of a compound called DMDP (2,5-Dihidroxymethyl-3,4-dihidroxypryrrolidine), isolated from a tree which is part of the biodiversity of Costa Rica.

Based on its activity as insecticide, Fellows et al. (1987) reported the potential use of DMDP in agriculture. Later, Birch et al. (1993) reported the activity of this compound as a control agent of plant attacking nematodes.

The research on DMDP in Costa Rica has required the participation of other governmental and private entities and made possible, for over ten years now, the development of several research activities including: study and identification of Costa Rican species belonging to the genus *Lonchocarpus* sp., which lead to the discovery of a new species, *Lonchocarpus felipei*; evaluation of several methods to propagate the species; development of domestication trials; laboratory essays to determine the compound's mode of action; development and optimization of a protocol for the compound isolation; development of toxicity trials and establishment of trials of efficacy for DMDP in greenhouses and field, in both tropical and temperate climates.

INBio- La Gavilana S.A. Agreement

Thanks to a partial non-refundable support from the Inter American Development Bank (IADB) and Multilateral Investment Fund, INBio initiated in 2000 an exploration of different micro- fungi species from the forest of the gender *Hypocrea/Trichoderma,* in order to know their potential us antagonist of the main fungal and bacterial diseases that attack vanilla crop. The project which goal was to produce *Vanilla* sp. in an eco-friendly fashion was conducted through a RCA with a national small company named La Gavilana S.A.

Costa Rica has an extremely rich microbial diversity. It is estimated that in its territory there are more than 25 000 species of algae and micro-algae and close to 65.000 species of fungi, of which only 3% have been described (Obando 2000).

Considering the natural wealth of the country and the pathological problems of vanilla, this research involved antagonism trials with different micro-organisms from Costa Rica's diversity, specifically the genus *Trichoderma*. The goal was to identify those that killed pathogens from the soil or reduced the damage they provoked.

INBio's research strengthened the findings of other scientists in relation to the antagonistic capacity of this micro-organism, and also lead to the discovery of a new, much more aggressive species. Greenhouse and field trials demonstrated the efficacy of this new species against soil pathogens the affect vanilla production.

Final considerations

The use of biodiversity as a source of genetic and biochemical resources for crop protection is irrefutable. Natural compounds, bio-controllers and genes are waiting to be discovered and utilized to the benefit of agriculture and the environment. Still there are few products along this line in the market, while the need of innocuous foods and clean production increases.

Organizations such as INBio work in the generation of knowledge that allows future generations to enjoy the natural wealth in their territory and use it in intelligent ways to contribute to the country's sustainable development.

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Problems related to production and commercialization of an alternative insecticide: 5 years of experience with neem in Nicaragua

Anne Kathrina Gruber[1]

Introduction

Neem (*Azadirachta indica* A. Juss, Meliaceae) is a rapid growing, medium high tree from southeast Asia (India, Sri Lanka, Burma), well adapted to tropical dry climates and introduced to Central America from 1975 on. In India it was long known as a medicinal plant, but also as a source of repellents against pests of stored grains.

In the 1970's real insecticidal properties were observed, too. And in the 1980's the main responsible substance, azadirachtin, was isolated from the seeds and its molecular structure elucidated. In the following 15 years the rest of the different active substances were isolated from seeds, leafs, bark and roots of the tree, all belonging to the biochemical group of the triterpenoids (limonoids).

Since then more than 340 species of insects and nematodes have been tested as positively responding to these substances. There exist more than 24 patents registered in the USA about different ways to obtain stable neem products and formulations. The worldwide interest on neem as a natural source of alternative products for pest control results from the non-toxicity, the target specificity and very reduced effect on natural enemies, the complete biodegradability and the relatively cheap and easy way of production.

The neem tree grows well in all tropical and subtropical dry regions up to 800 m above sea level and the seed is a renewable source. In Nicaragua it is estimated to grow now more than 1 million of trees with a yield of at least 1 kg of decorticated seed, that means a potential of 1,000 t of finished neem products. Neem insecticides are commercialized in several countries of the world including Sweden, Germany, USA, Canada, Dominican Republic, Nicaragua and most of all India with more than 20 registered products.

Neem production technology and its problems in Nicaragua

The development of an adequate neem production technology in a small, poor and insufficiently developed country, like Nicaragua, needed a cautiously selected level between primitive handicraft and highly technical processes.

During the Project "Natural Insecticides from the Neem Tree" (1987 to 1994), a semi-industrial method was developed. It operates with motor driven machines mainly of national construction and a good part of handwork. Harvesting and fruit selection, washing and drying of the seeds and the final selection of the decorticated seeds on a conveyor, highly important for good quality products, were done by hand. For depulping the fresh fruit, decorticating the dried seeds, grinding, milling and pressing the oil, machines were proved and adapted to the neem form and consistence.

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The main problems were:

a. How to harvest at the right time, at the lowest cost and without recollecting unripe fruits.
b. How to avoid loss of raw material by fungi contamination.
c. How to avoid degradation of the thermo labile and UV-sensible azadirachtins during all steps of the process and storage of the finished products.
d. How to achieve alcoholic extractions to obtain a stable and sufficiently high azadirachtin content in the finished product using a simple technology.
e. How to formulate the pressed neem oil without using synthetic emulsifiers .

In 1992 production in Nicaragua initiated with 2 tones and comes now to 14 tones of finished products annually, that means 1.4% of the Nicaraguan potential. Actually the set of seven products consists of ground neem seeds, pure pressed oil, formulated oil, ground neem cake, paste, ethanol extract of the ground seeds or ground cake, ground dried leaves. Each product is developed for a specific group of insect pests or nematodes.

Commercialization of neem in Nicaragua as a fight to overcome competition and bureaucracy

The existing Nicaraguan neem industry sold a bit more than the value of US$ 70,000.00 in 2003 with an average growth rate of 14.3% since 1999. This production comes now near to self sustainability but still with the risk of losses. The main market within the small country consists mostly of NGOs propagating and subsidizing the organic production of legumes, fruits, fibers, meat and other agricultural production. The percentage of small or medium farmers buying by their own interest is slowly growing. The main export is done to Mexico.

The difficulties for better commercialization in the Nicaraguan market and more exportation consists in:

a. The restricted financial capacity of the small industry to propagate the new products.
b. The relatively high producing costs including registration fees of a still very small neem production.
c. The average low prices of synthetic pesticides.
d. The believing of the agricultural producers in the "good toxics" of the transnational companies.
e. The division of the Central America market, which obliges to register all the products in all these countries to be allowed to sell and the bureaucratic regulations to achieve these registrations.
f. The absolute lack of political and/or economical support and assistance from the public hand to shift from toxic synthetic to natural, non toxic and environmental friendly pesticides.
g. The competition of cheap neem oil coming from India to USA and Europe.

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A bitterwood (*Quassia amara*) shrub in Talamanca, Costa Rica (Róger Villalobos, CATIE); masses of an unidentified bacteria acting as an antagonist of late blight (*Phytophthora infestans*) (Vera Sánchez, CATIE); CIPRONA (University of Costa Rica) laboratory (Guillermo Flores, MAG); a pheromone dispenser for the tobacco budworm, *Heliothis virescens* (Luko Hilje); and field testing of pheromone blends against the mahogany shootborer (*Hypsipyla grandella*) at CATIE (Isis Pinto).

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