Troisième réunion du Groupe de Travail de l'OILB-SROP "GMOs in Integrated Plant Production" : Ecological Impact of GMOs.

Varsovie, 23-25 mai 2007

Commentaires du participant :

Maurice. Vaissayre

Entomologiste, Département PerSysT, UPR Systèmes cotonniers paysans

Déplacement

Mardi 22 mai. Départ de Montpellier à 13h10, arrivée à Varsovie à 18h10

Mercredi 23 mai –Vendredi 25 mai : réunion du Groupe de Travail dans les locaux de l'Université Agronomique de Varsovie.

Vendredi 25 mai. Départ de Varsovie à 15h45, arrivée à Montpellier à 23h25 (pour cause d'orages sur Roissy CDG !)

Organisation

Keynote speakers:

Andrzej Anioł (Plant Breeding and Acclimatization Institute, Poland)	Research, politics and farmers' needs for GM crops in Poland.
Juan Ferre (Universitat de Valencia, Spain)	Exploring the potential of corn borers to develop resistance to Bt- corn in Europe.
Alison Haughton (Rothamsted Research, UK)	The impacts of novel management on ecosystem dynamics; tales from the UK Farm Scale Evaluations of GMHT crops.
Steve Naranjo (USDA-ARS, USA)	Integrating GM crops in IPM with emphasis on biological control systems.
Mark Sears (University of Guelph, Canada)	Risk assessment of non-target arthropods: the monarch butterfly and Bt maize pollen - a retrospective view.

Local organizer:

Zbigniew T. Dąbrowski

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Programme committee:

Jörg Romeis	Chairman, Agroscope Reckenholz-Tänikon ART, Switzerland
Ramon Albajes	Universitat de Lleida, Spain
Detlef Bartsch	Federal Office of Consumer Protection and Food Safety, Germany
Franz Bigler	Agroscope Reckenholz-Tänikon ART, Switzerland
Zbigniew T. Dąbrowski	Warsaw Agricultural University, Poland
Alan Raybould	Syngenta, UK

IOBC
OILB

International Organisation for Biological and Integrated Control of Noxious Animals and Plants
Organisation Internationale de Lutte Biologique et Integrée contre les Animaux et les Plantes Nuisibles
WPRS / SROP *West Palaearctic Regional Section / Section Régionale Ouest Paléarctique*

IOBC/wprs Working Group 'GMOs in Integrated Plant Production'

3rd EIGMO Meeting "Ecological Impact of Genetically Modified Organisms (EIGMO)" 23-25 May 2007, Warsaw, Poland

Under honorary patronage of Prof. dr hab. Tomasz Borecki
President of Warsaw Agricultural University

Program and abstracts

Warsaw, 2007

Quelques commentaires du participant

La réunion du Groupe de Travail sur l'impact écologique des organismes génétiquement modifiés est donc la troisième du genre, après celles de Prague (2003) et de Lérida (2005).

L'un des rôles que s'est assigné l'OILB au travers de ces ateliers concerne l'évaluation des risques, et la mise en place de recommandations (J. Romeis) lors de la mise en place de cultures transgéniques, dans la mseure où celles-ci peuvent avoir un impact sur la biocénose. Des personnes ressources ont été désignées pour collecter des données sur la formulation du problème et sur le cadre des activités d'évaluation du risque (A. Raybould, Syngenta), la sélection des espèces représentatives (F. Bigler, Agroscope) et sur les questions de méthodologie (J. Huesing, Monsanto). La question des modalités propres à l'évaluation de l'impact des PGM non insecticides (résistantes aux herbicides, ou d'intérêt plus spécifique) est également prise en compte. Noter qu'il existe une remarquable base de données accessible à l'adresse : http://delphi.nceas.ucsb.edu/btcrops/main/search

L'ouverture des travaux de l'atelier de Varsovie a été quelque peu perturbée par l'intervention imprévue du Ministre de l'Environnement polonais qui s'était déplacé pour faire part de l'opposition de son ministère à la libération de plantes génétiquement modifiées en Pologne, qualifiée de "GMO-free country", alors que dans sa communication d'ouverture le Prof. A. Aniol (IHAR Radzikòw) venait de faire part de l'impatience des cultivateurs polonais à pouvoir cultiver des PGM pour la résistance aux insectes !

Le Groupe de Travail poursuit, au travers d'échanges d'informations et d'expériences, la collecte d'informations sur les risques que représentent les plantes génétiquement modifiées (pour la résistance aux insectes) pour la faune utile, mais aussi sur les moyens d'en assurer une exploitation durable. La session du vendredi matin, où le Cirad est intervenu, était entièrement consacrée à ce sujet.

L'information obtenue jusqu'à ce jour est que, à l'exception remarquable des effets négatifs de la lectine (agglutinine) de *Galanthus nivale* (GNA) sur la chrysope *C. carnea* (Hogervorst *et al.* 2006), aucun impact négatif sur la biologie de la faune non cible n'a été observé après introduction des plantes transformées génétiquement pour la résistance aux insectes, et en particulier pour celles qui expriment des toxines de *B. thuringiensis*. On pourra consulter à ce sujet la mise au point de M.K. Sears (Univ. Guelft) à propos du Monarque (*Danaus plexippus*).

A noter que dans ce type d'atelier, un certain nombre de communications sont l'occasion pour des étudiants de se familiariser avec l'outil PowerPoint, sans que les informations présentées soient d'un grand intérêt. Il faut donc rester attentif à la personnalité des intervenants, les scientifiques reconnus étant généralement moins enclins aux présentations de résultats trop attendus ou trop peu intéressants. On a remarqué en fin de programme la prestation (quelque peu théâtrale) de K. Amman (Université de Delft), qui s'interroge sur le refus des tenants de l'*organic farming* d'y intégrer des cultivars génétiquement modifiés pour la résistance aux insectes.

En ce qui concerne la culture cotonnière, la présentation de S. Naranjo, qui traitait de l'intégration des cotons Bt dans la gestion intégrée des ravageurs aux Etats-Unis, a été suivie avec attention. Il a relevé en particulier que si les agriculteurs américains intervenaient sur seuil pour limiter les populations d'*H. zea* encore présentes sur cotonniers Bt, ils avaient fait le choix, faute de mieux (et de méthode fiable), d'intervenir systématiquement (en général à trois reprises lors de la campagne) pour contrôler les populations de Mirides et punaises vraies.

Pour ce qui est de la prévention de la résistance aux toxines de *B. thuringiensis* le sujet est aujourd'hui devenu d'un intérêt tel qu'il nous avait été demandé par les organisateurs de passer de notre proposition de poster à une intervention orale (vendredi matin, 10h15).

Programme

Warsaw Agricultural University (WAU), Nowoursynowska str., no. 166
(Old SGGW Capus – Ursynów), THE CRISTAL HALL (BUILDING NO. 9)
Szkoła Główna Gospodarstwa Wiejskiego (SGGW), Nowoursynowska 166
(Stary Ursynów –Kampus SGGW), AULA KRYSZTAŁOWA (BUDYNEK 9)

Wednesday, 23RD May, 2007

8.00-9.00	**Registration & poster mounting**
9:00-9:20	**Welcome address and introduction**
	Zbigniew T.Dąbrowski – Local organizer,
	Jörg Romeis – WG Convenor,
	prof. dr. Tomasz Borecki, Rector, Warsaw Agricultural University
9:20-9:30	**Short presentation on IOBC/WPRS**
	Franz Bigler
9:30-10:05	**Keynote: Research, politics and farmers' needs for GM crops in Poland**
	prof. dr Andrzej Anioł.

10:05-10:50 COFFEE

REPORT FROM A SPECIAL ACTIVITY ON „NON-TARGET RISK ASSESSMENT AND REGULATION"

10:50-11:00	**Introduction/background** (Jörg Romeis)
11:00-11:45	**Presentation of the outcome of this activity:**
	Problem formulation, risk assessment framework (Alan Raybould)
	Species selection (Franz Bigler)
	Study design (Joe Huesing)
11:45-12:00	**Implementation** (Jörg Romeis)
12:00-12:45	*Discussion*
	Moderator: Sabine Eber
	Protocol: Elisabeth Schulte

12:45-14:15 LUNCH (building No 6)

Proceedings of the 9th International Symposium on the Biosafety of Genetically Modified Organisms, September 24-29, 2006, Jeju Island, Korea, pp. 62-67 (2006)

Moving Through the Tiered and Methodological Framework for Non-Target Arthropod Risk Assessment of Transgenic Insecticidal Crops

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Abstract

Transgenic insecticidal crops have the potential to pose risks to non-target organisms. These risks need to be addressed as part of the environmental risk assessment that precedes the commercialization of any novel transgenic crop. An international initiative has been launched to develop a scientifically-sound, generic, and pragmatic approach to assess the risks to terrestrial non-target arthropods. The basis for this work is the widely-established and effective tiered testing approach from regulatory toxicology. The basic principles of this approach are described. These may provide guidance to countries that are currently developing their own non-target risk assessment guidelines and help to harmonize regulatory requirements in different regions.

Keywords

Non-target arthropods, risk assessment, study design, species selection, tiered approach, transgenic insecticidal crops

Introduction

Transgenic insecticidal crops that express Cry proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) have been grown on a steadily increasing area worldwide since their first introduction in 1996. A number of crops expressing novel insecticidal proteins are also under development and expected to reach the market stage in the near future. Like conventional agricultural pest control products, one of the risks associated with the growing of transgenic insecticidal crops is their potential impact on non-target organisms including a range of arthropod

species that fulfill important ecological functions such as biological control, pollination and decomposition. Potential non-target risks need to be assessed as part of the environmental risk assessment (ERA), prior to the cultivation of any transgenic crop.

Regulations and guidelines exist in the USA (Rose 2006), the European Union (EC 2002; EFSA 2004) and internationally (SCBD 2000). These provide general guidance on conducting an ERA of transgenic plants. However, there is still a need for detailed descriptions of non-target risk assessment procedures, for development of rigorous criteria for the selection of non-target species that need to be tested, and for establishment of test methods that apply to different regions.

The "West Palaearctic Regional Section" (WPRS) of the "International Organisation for Biological and Integrated Control of Noxious Animals and Plants" (IOBC) (http://www.iobc-wprs.org/) has a long history of assessing side-effects of plant protection products. A special initiative was launched in 2005, under the umbrella of the IOBC/WPRS working group "GMOs in Integrated Plant Production", with the aim of establishing generic ERA guidelines for transgenic insecticidal crops with particular emphasis on terrestrial non-target arthropods (NTAs) (Romeis 2006). This initiative involves scientists from diverse institutions including public research institutes, the agricultural biotech industry, representatives from regulatory agencies, and a commercial testing laboratory. The group has experience in the application of tiered risk assessment from a research and regulatory perspective. The final aim of this initiative is to propose a scientifically-sound, generic, and pragmatic NTA risk assessment method that can be adopted by different countries after adaptation to their specific regulatory needs and local circumstances.

A framework for assessing risk

A conceptual framework is critical in risk assessment and risk management. It can provide common understanding for regulators, registrants and scientists. It can also provide a predictable pathway for requesting, acquiring, organizing and evaluating data. Such a framework consists of four steps: (1) evaluation of need, (2) problem formulation, (3) information gathering, and (4) overall assessment. The initial evaluation of need determines whether a risk assessment is required for a specific case. Clearly defining the need as it meets the expectations of the final audience will help to design the overall risk assessment and determine how the information will be used and communicated. Common reasons for conducting an ERA include regulatory requirements, scientific inquiry, and scientific responses to public concerns. The main focus here is the ERA that is triggered by regulatory requirements. Once the need for the ERA has been clearly defined, the risk assessment moves forward to the problem formulation phase.

Problem formulation

The ERA is initiated with problem formulation (USEPA 1998; EFSA 2004). Problem formulation is used to define the scope of the risk assessment through generation of relevant risk hypotheses. For the ERA to go forward, a body of precursor information must determine that, other than for the expression of the trait of interest, the transgenic plant is equivalent to non-transformed comparators (see for example EuropaBio 2003). Once equivalence has been established on the basis of the transgenic plant characterization, the ERA can proceed with emphasis on stressor-mediated effects, where the potential stressor is the expressed trait, e.g., a Bt protein. The problem formulation considers the specifics of the stressor mode of action, the spectrum of activity and susceptibility, mode of expression, and relevant exposure profiles.

Additionally, it must also take into account ecological considerations that might affect the nature and extent of possible environmental impacts. One of the most significant factors in this regard is the intended scale of cultivation since ecological consequences of NTA impacts are likely to be positively correlated with scale. On this basis, the problem formulation then identifies assessment endpoints reflecting management goals and the scale and nature of the receiving ecosystem that is being considered. It should culminate in a conceptual model and analysis plan that is consistent with the risk hypotheses and establishes the relationship of the stressor to ecological impacts of concern. It also identifies possible surrogate test species and outlines an exposure analysis that accounts for the intended use and nature of the deployment of the transgenic plant.

Regardless of where in the world the ERA is conducted, the problem formulation approach should be very similar, using similar information that is modified by local cropping system information. The ERA process underlies the locally relevant tiered testing scheme, which should also reflect the basic design principles outlined below. The overall process may reflect additional national and regional regulatory needs and it must be achievable within the specific capacities and capabilities of the agency conducting the ERA.

The framework and progressing through it

A tiered risk assessment is recognized as being the most appropriate and rigorous approach to assess non-target affects from both scientific and regulatory standpoints. Both hazard and exposure can be evaluated within different levels or "tiers" that progress from worst-case hazard and exposure to more realistic scenarios. Lower tier tests serve to identify potential hazards, and they are conducted in the laboratory to provide high levels of replication and study control which increase the statistical power to test hypotheses. Where potential hazards are detected in these early tier tests, additional information is required. In these cases, higher tier tests can serve to confirm whether an effect might still be detected at more realistic rates and routes of exposure. Higher tier studies including semi-field or field-based tests offer greater environmental realism, but they may have lower statistical power. These tests are only triggered when early tier studies in the laboratory indicate potential hazards at environmentally relevant levels of exposure. In exceptional cases, higher tier studies may be conducted at the initial stage when early tier tests are not possible, for example plant tissue might be used because purified toxin is not available. Higher levels of replication or repetition may be needed to enhance statistical power in these circumstances.

In cases where a potential hazard is detected in a lower tier test, the tiered approach provides the flexibility to undertake further lower tier tests in the laboratory to increase the taxonomic breadth or local relevance of test species, thus avoiding the costs and uncertainties of high tier testing. Depending on the nature of the effect, one may also progress to higher tier testing, particularly in cases where there is no previous experience with the crop or toxin under investigation. The various tiered approaches that have been described for non-target risk assessment (e.g. Dutton et al. 2003; EuropaBio, 2004; Rose 2006) differ in their specific definitions of individual tiers, but they all follow the same underlying principles.

Movement between tiers during information gathering is based on the sufficiency of information that is available. If sufficient data and experience from toxicological testing and exposure analyses are available to characterize the potential risk as being acceptable, then there is no need to untertake additional testing. The process is designed to optimize the use of resources and to identify and define sources of potential risk. Where no reasonable hazard is detected, effective tiered processes prevent costly and unnecessary testing from taking place.

Species selection

For practical reasons, only a small fraction of all possible terrestrial arthropods can be considered for regulatory testing. It is therefore necessary to select appropriate species to serve as surrogates for ecologically and economically important NTAs that can be tested under worst-case conditions in the laboratory (Barrett et al. 1994). Species should be chosen to represent different ecological functions such as herbivory, pollination of cultivated and wild plants, predation and parasitism of pest organisms and decomposition in the soil. In order to reflect biogeographical variation, it is crucial to determine what relevant species are likely to occur in the cropping systems where the transgenic plant is expected to be grown. Another important source of information that serves as a basis for selecting relevant species is the information on the stressor (specificity, mode of expression and exposure profile) that is accumulated during problem formulation. The information collected in these previous steps will direct the selection of representative NTAs from a proposed set of species that capture key ecological functions. Criteria such as amenability to testing, availability of test methods that respect the standards of Good Laboratory Practices (GLP) and unambiguous taxonomic recognition are crucial for non-target testing. Based on these criteria, a list of NTA species that represent those living in the crop and in adjacent non-crop habitats is proposed. As a result of this process, test protocols for species that are of high relevance in particular regions may need to be developed.

The application of the surrogate species concept enhances the transferability of data from lower tier tests to a wide range of regions and to both annual and perennial crops. If higher tier studies are required, tests should be done using appropriate surrogates for the species potentially at risk. Appropriate surrogates may be the species used in the lower tier studies (Candolfi et al. 2000a); however it is not essential to use those species if the risk can be refined more effectively using others.

Study design

Once the surrogate test species are selected, they are evaluated in properly designed tests that fulfill established quality control standards, e.g., GLP. Experience has shown that early tier tests conducted under worst-case conditions in the laboratory (generally referred to as Tier 1 tests) can be well standardized. This is important to assure study repeatability, interpretability and quality, and thus to ensure a high level of confidence in the reported data. This process also facilitates the transportability of the test results among laboratories, countries and across crops, where this is appropriate. Protocols developed to assess the impact of pesticides (e.g., Candolfi et al. 2000b; OPPTS Series 885.4340, see http://www.epa.gov/opptsfrs/home/guidelin.htm) have historically formed the basis for the standard protocols used for the assessment of the potential effects of transgenic insecticidal crops on NTAs. Many of these protocols have been modified to consider the oral exposure pathway of plant-expressed insecticidal proteins and a number of new protocols have been developed.

Before entering into testing, the objectives of the individual studies need to be defined, and specific measurement endpoints described. Appropriate endpoints for risk assessment studies include life-table parameters such as mortality or fecundity, because they can easily be evaluated and the data can be related to measurable effects in the field. Other endpoints (e.g., weight, development and behavior) are possible, however, risk assessors should agree beforehand how to interpret these data. Because possible effects of insecticidal compounds expressed by transgenic crops may be delayed, multiple life-stage testing is recommended when possible. The life stages

that are selected should be chosen based on exposure, sensitivity and the amenability of the test system available for the selected arthropod.

Early tier tests usually entail a simple, well-defined test system designed to measure a specific endpoint (or set of endpoints) at concentrations that are several times higher than those that will be seen in the field. Elevated doses are applied since these tests use a small number of surrogate arthropods and because higher dose limit tests can add additional certainty to the safety assessment. All tests should adopt quality control parameters that help validate the test system which may include: (i) low negative control mortality, (ii) use of a positive control, (iii) homogeneity of test material, (iv) stability of the insecticidal compound, and (v) sufficient statistical power. It is recognized that there is a trade-off between the duration of the test, the number of life-stages that can be monitored, control mortality and thus the power of the test system. Flexibility to expand the range or number of lower tier tests may compensate for some of these constraints

Higher tier tests usually involve semi-field or field tests and sometimes are conducted when life-cycle (especially reproduction parameters) or tri-trophic evaluations are warranted. In general, these tests are problematic because of their complexity and high intrinsic uncertainty. Higher tier tests place high demands on skills in design, execution and data analysis and as a consequence they are subject to problems of low statistical power. These tests should therefore only be conducted when they can further reduce uncertainty in the risk assessment, and only when justified by detection of unacceptable risk at the lower tiers of testing.

Overall risk assessment

An ERA is a necessary step in the deregulation or regulatory approval of transgenic crops. It is comprised of the risk hypothesis, conceptual model, the characterization of hazard and exposure, and the results obtained from testing. The study quality, dosing levels, and the certainty levels associated with hazard tests should also be described. The test results should be placed in context and the following questions should be considered. Were any effects detected that were direct or indirect in nature? Were they restricted to one species or were they broad in taxonomic spectrum? Critical uncertainties should be identified and the temporal and spatial variability understood and explained at appropriate levels of detail. Once this information has been summarized, the predicted hazard is compared with the predicted exposure. Simple and powerful risk characterizations are based on the ratio between hazard and exposure values. Higher tiered, but more realistic, risk assessments involve the use of population and community responses which may include sources of geographic and temporal variability in exposure.

Two key factors should be kept in mind when completing a risk assessment. First, the risk assessment should be science-driven. Social and political concerns are important, but they are taken into account in risk management or in decision making that lies outside the risk assessment framework. Second, the risk assessment does not constitute a decision in itself, but represents a source of information for decision makers to use.

References

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SESSION:
NON-TARGET EFFECTS OF INSECTICIDAL GM CROPS I

Chair: **Louise A. Malone**

14:15-14:55 Naranjo S.E., Hellmich R.L *(KEYNOTE)*

Integrating GM crops in IPM with emphasis on biological control systems

14:55-15:15 Smith S., Raybould A., Poppy G.M.

Quality or toxicity – Comparative toxicity of the prey mediated and direct effects of Cry1Ab toxin in the parasitoid wasp *Cotesia marginiventris*.

15:15-15:35 Li Y., Romeis J.

Assessing the impact of Bt maize pollen on adult green lacewings

15:35-16:20 COFFEE

SESSION:
IMPACT OF BT CROPS ON SOIL ORGANISMS

Chair: **Silvia Fernandez**

16:20-16:40 Zurbrügg C., Nentwig W.

Cry1 and Cry3 toxins show different degradation patterns and thus exposure risk for soil organisms is higher for Cry1

16:40-17:00 Hönemann L., Nentwig W.

Effects of Bt-corn on the soil macro- and mesofauna – a litter bag field study

17:00-17:20 Büchs W., Prescher S.

Effects of Bt-maize with *Diabrotica*-resistance and other maize cultivars on saprophagous Diptera larvae

17:20-18:00 POSTER AND PRESENTATIONS *(THE CRISTAL HALL – building. no 9)*

&

18:00-20:00 COCKTAIL PARTY! (THE CRISTAL HALL ANNEX)

KEYNOTE

Integrating GM crops in IPM with emphasis on biological control systems

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Modern pest control is guided by the principles of integrated pest management (IPM) that has been articulated for over 50 years. Kogan defines IPM as "a decision support system for 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." The use of genetically modified (GM) crops, which have activity against insect pests, qualifies as one of the many tactics that can be integrated into IPM strategies for pest insects. Here we will focus primarily on the role of *Bacillus thuringiensis* (*Bt*) maize and cotton for managing pests of these crops over the past decade. Worldwide, 32.1 million hectares of *Bt* and *Bt* plus herbicide-tolerant crops were cultivated in 2006 and adoption and use of GM crops continues to grow rapidly. For most maize and cotton pests susceptible to *Bt* toxins, these GM crops are an extremely successful form of host plant resistance. As a result, they substantially alter target pest populations and can potentially influence non-target pests and beneficial arthropods, mainly through indirect paths. Given the prevalence of *Bt* crops, such changes have, and are, altering the implementation, utility and impact of other IPM tactics like insecticides, biological control, and decision aids. Numerous studies have examined the non-target impacts of *Bt* maize and cotton and have generally demonstrated, with the exception of specialist natural enemies, that negative effects are minimal. These general patterns have been confirmed recently through meta-analyses utilizing a large number of studies on maize and cotton. Some non-target pest problems, however, have arisen in GM crops, for example, whiteflies and plant bugs on *Bt* cotton, and aphids and mites in *Bt* maize. This rise of such pests usually is attributed to reductions in broad-spectrum pesticides that controlled these pests prior to *Bt* technology. Traditional IPM tactics like scouting and use of thresholds also can be altered by *Bt* crops whereby different strategies are applied to *Bt* and non-*Bt* crops. Given the apparent selectivity of *Bt* crops towards targeted pests, perhaps one of their greatest assets is the facilitation of biological control, particularly on non-target pests unaffected by *Bt* toxins. Many studies have clearly demonstrated enhanced natural enemy abundance in *Bt* crops compared with conventional crops subject to broad-spectrum chemical insecticides. A much smaller number of studies have focused on understanding the functional contribution of this conservation or considered the more subtle indirect effects which may alter natural enemy biology and behavior. For example, studies in the USA revealed that biological control function is unchanged in *Bt* cotton even though populations of some generalist natural enemies may decline slightly due to target prey reductions. Other studies in Europe have shown that parasitoid/host interactions are not altered in *Bt* rape and that caterpillar herbivory on *Bt* and non-*Bt* maize alters the amount of volatiles produced by the maize but does not change the behavior of parasitoids attracted to the odors. Overall, existing data generally support the beneficial role of *Bt* crops in providing selective control of key pests and generally reducing the need for disruptive broad-spectrum pesticides enabling more sustainable IPM tactics such as biological control to operate more effectively.

THURSDAY, 24RD May, 2007

LECTURE HALL 112 (BUILDING NO. 8)

SESSION:
NON-TARGET RISK ASSESSMENT

Chair: **Marco M.C. Gielkens**

9:00-9:40 Sears M.K. *(KEYNOTE)*

Risk assessment of non-target arthropods: the monarch butterfly and Bt maize pollen – a retrospective view

9:40-10:00 Todd J.H., Ramankutty P., Malone L.A.

A method for selecting non-target organisms for testing the biosafety of GM plants

10:00-10:20 Raybould A., Stacey D., Vlachos D., Joseph R., Graser G., Mead-Briggs M.

Environmental risk assessment of maize expressing mCry3A for control of corn rootworm

10:20-11:10 COFFEE

SESSION:
NON-TARGET EFFECTS OF INSECTICIDAL GM CROPS II

Chair: **Salvatore Arpaia**

11:10-11:30 Sweet J.

Application of ERA to different environments

11:30-11:50 Büchs W., Presher S., Schlein O.

Effects of transgenic maize with *Diabrotica v. virgifera* – resistance on the feeding rates and development of predatory beetles after consumption of Bt-contaminated prey larvae

11:50-12:10 Priesnitz K.U., Benker U., Roß-Nickoll M.

Impact of Coleopteran-specific Bt maize on Carabid beetles: results after two years of field and laboratory research

12:10-12:30 Konrad R., Babendreier D.

Effects of insect-resistant transgenic plants on solitary bees

12:30-14:00 LUNCH (building no 6)

KEYNOTE

Risk assessment of non-target arthropods: the monarch butterfly and Bt maize pollen – a retrospective view

Sears, M.K.

University of Guelph, Guelph, Ontario Canada N1G2W1

The purported impact of Bt maize pollen to larvae of monarch butterflies as reported in 1999 has been evaluated by extensive research from 1999-2004 in Canada and the United States. This collaborative effort resulted in a risk analysis that indicated the impact of Bt maize pollen on populations of monarch butterflies in North America was negligible. Since these data were published in 2001 and 2004, little concern regarding Bt pollen and monarch populations has been reported in popular media, but the study has become a touchstone for risk analysis of transgenic insecticidal crops and their risk to non-target organisms. Regulatory agencies around the world are keenly interested in developing a practical, tiered system of risk analysis of genome transformations to non-targets and this example serves as a model for such an approach. Details of the risk analysis approach used in the monarch butterfly issue and developments towards a unified process of non-target impact from such pesticide-incorporated plants will be presented.

SESSION:
IMPACT OF GMHT CROPS ON BIODIVERSITY

Chair: **Jeremy Sweet**

14:00-14:40 Haughton A.J., Bohan D.A. *(KEYNOTE)*

The impacts of novel management on ecosystem dynamics; tales from the UK Farm Scale Evaluations of GMHT crops

14:40-15:00 Szekere D., Kádár F., Dorner Z.

Ground beetles (Coleoptera: Carabidae) in herbicide tolerant (HT) maize hybrid test fields: Impact of HT crop or of weed control practice?

15:00-15:20 Albajes R., Eizaguirre M., Casado D., Pérez M., López C., Lumbierres B., Pons X.

Impact of glyphosate use on arthropods related to the cultivation of transgenic herbicide-tolerant maize

15:20-16:10 COFFEE

SESSION:
NON-TARGET EFFECTS OF INSECTICIDAL GM CROPS III

Chair: **Jozsef Kiss**

16:10-16:30 Thu Nguyen H., Jehle J.

Monitoring the Cry3Bb1 expression of corn line Mon88017 at the field trial in Germany

16:30-16:50 Rauschen S., Schuphan I., Eber S.

Assessment of possible non-target effects of the novel Bt-corn variety MON88017 resistant to the Western corn rootworm *Diabrotica virgifera virgifera* (LeConte)

16:50-17:10 Habuštová O., Doležal P., Hussein H.M., Spitzer L., Turanli F., Růžička V., Sehnal F.

Lack of effect of maize expressing bacterial toxin Cry1Ab on the composition of insect communities

17:10-17:30 Husáková J., Svobodová Z., Doležal P., Habuštová O., Sehnal F.

Effect of Bt toxin Cry3Aa on *Spodoptera littoralis* (Boisd.)

18:30 CONFERENCE DINNER (BUILDING 38)
RESTAURANT LIMBA HOTEL – NEW URSYNOW CAMPUS

KEYNOTE

The impacts of novel management on ecosystem dynamics; tales from the UK Farm Scale Evaluations of GMHT crops

Haughton, A.J., Bohan, D.A.

Ecosystem Dynamics & Biodiversity Group, Division of Plant & Invertebrate Ecology, Rothamsted Research, West Common, Harpenden, Herts. AL5 2JQ. UK (alison.haughton@bbsrc.ac.uk)

Concerns about the possible negative impact of the novel herbicide management associated with GM herbicide-tolerant (GMHT) crops on British farm wildlife led to the establishment of the Farm Scale Evaluations (FSEs). This series of field trials evaluated wildlife changes by comparing the wildlife of a GM crop, with its associated herbicide management, against a conventional variety and current 'best practice' herbicide management. Using a half-field design, in some 65 fields per GMHT crop distributed across the arable growing areas of Great Britain, the abundance, biomass and diversity of weed plant and invertebrate species or taxa was assessed.

The results showed that there were marked changes in some groups of weed plants and invertebrates with GMHT management. A basic assumption for the trials was that there were no direct effects of GMHT herbicide management on the invertebrates, and that any effects on invertebrates were caused by changes in the weed plants due to differences in herbicide management. Although the experimental approach taken was scientifically rigorous, the repeated testing of the null hypothesis for each species or taxa was bound to generate significant effects just by chance. What was not clear was whether all the observed changes indicated a broad risk to wildlife in farmland. Were the changes important?

The aim of the Ecosystem Dynamics and Biodiversity group is to understand whether there are better approaches to understanding changes in ecosystems with management, and the risk posed. Using analyses of data from the FSEs, I outline some hypotheses for agro-ecosystem structuring, the impact of management on this, and the wider risks to wildlife of these changes. Specifically, I ask: can we detect changes in the agro-ecosystem with novel management; how are invertebrates linked to plants, and can we observe novel management effects on these links; and can we extrapolate to biodiversity groups of social importance, such as birds, but which are difficult to measure in the field?

FRIDAY, 25TH MAY, 2007

LECTURE HALL 112 (BUILDING NO. 8)

SESSION: RESISTANCE MANAGEMENT

Chair: **Sabine Eber**

9:00-9:35 Ferré J., González-Cabrera, J., Bel Y., Escriche B. *(KEYNOTE)*
Exploring the potential of corn borers to develop resistance to Bt-corn in Europe

9:35-9:55 Moeser J., Vidal S.
Possible resistance development of *Diabrotica virgifera virgifera* against transgenic MON 88017 Bt-maize

9:55-10:15 Engels H., Schuphan I., Eber S.
F2-Screen and field sampling with light trap cages, two methods for a resistance monitoring in transgenic crops

10:15-10:35 Brevault T., Prudent P., Vaissayre M.
Baseline susceptibility of *Helicoverpa armigera* (Hübner) to Bt toxins in West and Central Africa

10:35-11:15 COFFEE

SESSION: FREE TOPICS

Chair: **Zbigniew T. Dabrowski**

11:15-11:35 Zaritsky A., Ben-Dov E.
Transgenic bacteria expressing combinations of genes from *Bacillus thuringiensis*

11:35-11:55 Hussein H.M., Procházková M., Habuštová O., Sehnal F.
Effect of GNA potatoes on the Egyptian armyworm, *Spodoptera littoralis*

11:55-12:15 Sanvido O., Stark M., Romeis J., Bigler F.
Ecological impacts of genetically modified crops: experiences from ten years of experimental field research and commercial cultivation

12:15-12:35 Ammann K.
Farming organically and with transgenic plants: a comparison of environmental impact

12.35-12.55 Judziński B.
Activities and objectives of the Grain and Feed Chamber: actions on objective evaluation and decisions related to GMO

13.00-14:00 LUNCH (BUILDING NO. 6)

14:00-14:45 Discussion on future activities of the working group; working-group business

14:45 CLOSURE OF THE MEETING

KEYNOTE

Exploring the potential of corn borers to develop resistance to Bt-corn in Europe

Ferré, J., González-Cabrera, J., Bel, Y., Escriche, B.

Universitat de València, Department of Genetics, Faculty of Biology, Burjassot, Spain (Juan.Ferre@uv.es)

After 10 years of Bt-corn planting in the US, no development of resistance in corn borer populations to *Bacillus thuringiensis* (Bt) insecticidal proteins has been found in the field. One reason is for sure the strict resistance management imposed by the EPA to the seed companies: a high expression of the Bt protein in the plant and the use of refuges. Europe has started with the adoption of Bt-corn much more cautiously and most of the countries have been reluctant to approve Bt-corn planting. Spain has been the exception, and it has been always the European country with the largest area planted to Bt-corn (over 60,000 ha in 2006).

One of the main threats of adopting Bt-corn is the high selection pressure imposed to the corn borer populations which can lead to the development of resistance. It was for this reason that the EU funded a project with the title "Protecting the benefits of Bt-toxins from insect resistance development by monitoring and management (ProBenBt)" (contract number QLK3-CT-2002-01969), which covered the period from November 2002 till April 2006. The project was coordinated by Ingolf Shuphan from Aachen University (Aachen, Germany) and involved 11 research groups. The objectives of this project were, among others, to characterise the corn borer populations in Europe to determine the genetic diversity and the frequency of resistance alleles. Our group was involved in the mode of action of Bt insecticidal proteins in the corn borers *Ostrinia nubilalis* and *Sesamia nonagrioides*, the biochemical characterisation of the resistance mechanism in laboratory-selected resistant strains of *O. nubilalis*, and the characterisation of major candidate genes for the future application to molecular monitoring of resistance alleles.

In collaboration with the group of Blair D. Siegfried, (University of Nebraska, Lincoln, Nebraska, US), we determined that the mechanism of resistance in a laboratory-selected resistant strain of *O. nubilalis*, derived from insects collected in Italy, was most likely due to a reduction in the cadherin protein, one of the membrane receptors for the Bt proteins. We have determined the genomic structure of the *cadherin* gene in this species and have found that it has 34 introns, one of them in the 5'-UTR region, one feature that we also found in the *cadherin* ortologous genes in other lepidopteran species. The sequence variability in some regions of this gene will be used to design molecular probes for monitoring of resistance alleles in this species.

Ecological impacts of genetically modified crops: experiences from ten years of experimental field research and commercial cultivation

Sanvido, O., Stark, M., Romeis, J., Bigler, F.

Agroscope Reckenholz Tänikon Research Station ART, CH-8046 Zürich, Switzerland

The worldwide commercial cultivation of genetically modified (GM) crops has raised concerns about potential adverse effects on the environment, which could result from the use of these crops. Consequently, the risks of GM crops for the environment, and especially for biodiversity, have been extensively assessed before and during their commercial cultivation. Substantial scientific data on environmental effects of the currently commercialized GM crops is available today. We have been commissioned by the Swiss Expert Committee for Biosafety to review this scientific knowledge deriving from the past ten years of worldwide experimental field research and commercial cultivation. The sources of information included peer-reviewed scientific journals, scientific books, reports from countries with extensive GM crop cultivation, as well as reports from international organizations. Although there is currently no general consensus on the notion of environmental damage, the data available so far provides no scientific evidence that the commercial cultivation of GM crops has caused significant environmental impacts that would be judged as drastic changes in environmental quality. Nevertheless, a number of issues related to the interpretation of scientific data on effects of GM crops on the environment are debated controversially. The ongoing debate is not primarily due to a lack of scientific data, but more to a lack of clear definitions on how to put a value on effects of GM crops on biodiversity in the context of current agricultural systems. The study highlights these scientific debates and discusses the effects of GM crop cultivation on the environment considering the impacts caused by cultivation practices of modern agricultural systems.

F2-Screen and field sampling with light trap cages, two methods for a resistence monitoring in transgenic crops

Engels H., Schuphan I., Eber S.

Institute for Environmental Research (Biologie 5), Aachen University, Aachen, Germany

Large-scale cultivation of *Bt* crops will exert high selection pressure on the target pest, which may consequently evolve resistance. So far, no resistance of the European corn borer *Ostrinia nubilalis* to *Bt* crops has been reported. As yet, no anticipatory resistance monitoring plan has been established for Europe. Routine target pest susceptibility screens or the routine use of F2-screens for different Lepidopteran species are in discussion.

When resistance alleles are assumed to be rare, the most efficient method is the F2-screen. This method preserves the genetic variation among isofemale lines and concentrates potential resistance alleles into homozygous genotypes of the F2-generation. This way it is possible to test whether they are recessive or dominant. In our study 450 isofemale lines, started from 650 females, captured in several maize fields in four German regions, were screened over three cultivation periods.

As the F2-screen is time consuming and labour intensive, a simpler long-term monitoring method has been developed and tested. Target pest insects are thereby attracted to light-trap cages containing insect-resistant crop plants. Trapped target insects lay their eggs onto these plants during the cultivation period. Sensitive neonates feeding on the transgenic plants, will die, resistant neonates will survive and develop on the plants. At the end of the cultivation period these larvae can be counted and used for further analyses on the mechanisms of resistance. As a basis for modelling egg masses can be quantified in the light-trap cages throughout the season, so that the number of females can be estimated. So far about 1670 egg masses and thus 50,000 larvae were screened in test trials during one cultivation period in one cage. Neither of the methods revealed any resistant corn borer larvae.

Farming organically and with transgenic plants: a comparison of environmental impact

Ammann, K.

Technical University of Delft, Faculty of Applied Sciences, Department of Biotechnology, Julianalaan 67, 2628 BC Delft, The Netherlands

In the years since the Convention on Biological Diversity was adopted, issues of traditional knowledge have come to affect the legitimacy of the multilateral trading system, in general, and its IP (intellectual property) aspects, in particular. In order to engage indigenous knowledge in furthering socio-economic development, policy-makers will need to reconsider the prevailing notion of a fundamental dichotomy between indigenous and scientific knowledge and begin to challenge both types of knowledge. This contribution concentrates on traditional knowledge – and how it relates to the ecology of agriculture, in all of its variants – and compares it to recent advances in scientific knowledge and the resulting applications of biotechnology and organic farming in global agriculture.

Deeper examination of the genetic integrity of plants used within organic and biotechnology-based agricultural systems shows that the respective crop varieties being used under each system are more similar than they are different. Increasingly, organic farming is building on scientific knowledge, and agricultural biotechnology is seeking to draw on traditional knowledge.

This contribution challenges policy-makers and scientists to examine and, ultimately, to move beyond those conceptual worldviews, or constructs, that maintain the current divide between traditional knowledge/organic agriculture and scientific knowledge/agricultural biotechnology.

By building the bridge between traditional knowledge and science and becoming free to draw upon the best existing ideas and practices from both, a larger palate is available to draw from. But, more importantly, by integrating the innovation systems of both traditional and scientific communities, a much larger range of new ideas and practices could be generated. The contribution calls such dynamic integration the "participatory approach" to agricultural innovation, building upon the "unifying power of sustainable development" and leading to balanced choices in agricultural production chains and rural land use.

For the full account on the topic see:

Ammann, K. (2007) Reconciling Traditional Knowledge with Modern Agriculture: A Guide for Building Bridges. In: Intellectual Property Management in Health and Agricultural Innovation a Handbook of Best Practices (eds A. Krattiger, R.T.L. Mahoney, L. Nelsen, G.A. Thompson, A.B. Bennett, K. Satyanarayana, G.D. Graff, C. Fernandez & S.P. Kowalsky), pp. 1539-1559. MIHR, PIPRA, Oxford, U.K. and Davis, USA

www.ipHandbook.org. (as of September 2007),

Flyer: http://www.botanischergarten.ch/TraditionalKnowledge/ipHandbook-Flyer.pdf

and the definite chapter free of copyrights:

http://www.botanischergarten.ch/TraditionalKnowledge/Ammann-Traditional-Biotech-2007.pdf

Poster Presentations

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   Interaction of rhizospheric nitrogenic bacteria with roots of transgenic alfalfa plants containing different transgenes
2. ***Ferré, J.*****, MacIntosh, S.C.** (page 39)
   Insect species of importance to currently deployed Bt-crops that have developed resistance to *B. thuringiensis* toxins in the laboratory
3. ***Gaspers, C.*****, Engels, H., Schuphan, I., Eber, S.** (page 40)
   Geographic and host plant distribution of pheromone races of the European Corn Borer (*Ostrinia nubilalis*)
4. ***Meissle, M.*****, Romeis, J.** (page 41)
   Assessing the risk of *Diabrotica*-resistant Bt maize for the web building spider *Theridion impressum* (Ara.: Theridiidae)
5. **Górecka, J., Dąbrowski, Z.T.** (page 42)
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6. ***Twardowski, J.P.*****, Hurej, M.** (page 43)
   European corn borer (*Ostrinia nubilalis* Hübner) on transgenic Bt corn in Lower Silesia, Poland. Preliminary results
7. ***Garcia-Alonso, M.*****, Raybould, A.** (page 44)
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8. **Lawo, N.C.,** ***Romeis, J.*** (page 45)
   Performance of *Aphis gossypii* on Indian *Bt* cotton varieties
9. **Dhillon, M.K., Lawo, N.C., Sharma, H.C.,** ***Romeis, J.*** (page 46)
   Assessing the direct effects of *Galanthus nivalis* agglutinin (GNA) and avidin on the ladybird beetle *Coccinella septempunctata*
10. ***Farinós, G.P.*****, De la Poza, M., Hernández-Crespo, P., Ortego, F., Castañera, P.** (page 47)
    Diversity and temporal phenology of above-ground fauna in transgenic and conventional maize fields of Central Spain
11. ***Kiełkiewicz, M.*****, Gajc-Wolska, J., Szwacka, M., Maleszy, S.** (page 48)
    Unintended changes in biochemistry of cucumber carrying the thaumatin II gene that can affect insect/mite pests
12. ***Zemek, R.*****, Vávrová, Z.** (page 49)
    Assessing the effects of Bt-maize pollen on *Typhlodromus pyri* (Acari: Phytoseiidae)
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    Effects of transgenic DK626 Bt (MON810) corn variety to corn stalk borer, *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae) on second corn crop under natural infestation conditions in Turkey
14. ***Li, Y.*****, Romeis, J.** (page 51)
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16. ***Żurawska, M.*****, Linkiewicz, A.M.** (page 53)
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17. ***Arpaia, S.*****, Di Leo, G.M.** (page 54)
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18. ***Bereś, P.K.*****, Gabarkiewicz, R.** (page 55)
    Preventing expansion of *Ostrinia nubilalis* Hbn. by cultivation of Bt transgenic maize – First field experiments in southeastern Poland
19. ***Tekiela, A.*****, Gabarkiewicz, R.** (page 56)
    Reduction of a threat caused by mycotoxins to mammals and birds by growing Bt maize cultivars in Poland

Poster 2

Insect species of importance to currently deployed Bt-crops that have developed resistance to *B. thuringiensis* toxins in the laboratory

Ferré, J.[1], MacIntosh, S.C.[2]

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[2]MacIntosh & Assoc. Inc., Saint Paul, MN, USA (Juan.Ferre@uv.es)

Bt-crops have been adopted by many countries and so far, after 11 years of their adoption, no case of insect resistance has been reported in the field. However, laboratory selection has long shown that insects have the potential to develop resistance against *Bacillus thuringiensis* (Bt) insecticidal proteins (Cry proteins). Here we list and give details of the strains that have been successfully selected for resistance, in the laboratory, of species of importance to current Bt-crops: *Heliothis virescens* (4 strains), *Helicoverpa armigera* (9 strains), *Pectinophora gossypiella* (2 strains), and *Ostrinia nubilalis* (8 strains).

Resistance levels against Cry1Ab or Cry1Ac have been particularly high in two strains of *H. virescens* (400 and >10,000-fold), both from North Carolina (USA); 5 strains of *H. armigera* (over 200-fold) from India, Australia and China; two strains of *P. gossypiella* (over 100-fold) from USA; and 3 strains of *O. nubilalis* (>500-fold) from Kansas (USA), Italy, and a mixed population of insects collected in Italy and Nebraska (USA). Selection for resistance to Cry2A protoxins has also been successful, reaching very high levels in *H. armigera* from New South Wales (Australia) (6800-fold to Cry2Ab and 9600-fold to Cry2Aa) and with *O. nubilalis* from Kansas (USA)(>640-fold).

Resistance is, in all cases, autosomically inherited. However, depending on the strain considered, resistance can be monogenic or due to more than one gene, and its inheritance pattern can be from completely recessive to partially dominant.

Insect species of importance to currently deployed Bt-crops that have developed resistance to *B. thuringiensis* toxins in the laboratory

Juan Ferré[1] and Susan C. MacIntosh[2].

[1] University of Valencia, Burjassot, Spain, and [2] MacIntosh & Assoc., Inc., Saint Paul, MN, USA

Abstract

Bt crops have been adopted by many countries and so far, after 11 years of their adoption, no case of insect resistance has been reported in the field. However, laboratory selection has long shown that insects have the potential to develop resistance against *Bacillus thuringiensis* (Bt) insecticidal proteins (Cry proteins). Here we list and give details of the strains that have been successfully selected for resistance, in the laboratory, of species of importance to current Bt-crops: *Heliothis virescens* (4 strains), *Helicoverpa armigera* (9 strains), *Pectinophora gossypiella* (2 strains), and *Ostrinia nubilalis* (8 strains).

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Resistance is, in all cases, autosomically inherited. However, depending on the strain considered, resistance can be monogenic or due to more than one gene, and its inheritance pattern can be from completely recessive to partially dominant.

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Species	*Origin*	*Selecting agent[a] (form[b])*	*Strain name*	*Resistance and cross-resistance levels*			*Genetics of resistance (dominance levels estimated as D_{LC})[c]*	*Reference*
				ES[c]	*Toxin/Formulation[d]*	*RR[e]*		
H. virescens	North Carolina (USA)	Cry1Ab (CC) / Dipel (FSC)	SEL	14	Cry1Ab protoxin	20	–	Stone et al, 1989
				22	Cry1Ab protoxin	69	Polygenic, PD (0.71)	Sims and Stone, 1991
H. virescens	North Carolina (USA)	Cry1Ac (T)	CP73-3	17	Cry1Ab	13	–	Gould et al, 1992
				17	Cry1Ac	50	PR (0.31)	
H. virescens	North Carolina (USA)	Cry1Ac (T/CC)	YHD2	19	Cry1Ac	>10000	–	Gould *et al.*, 1995
				–	Cry1Ab	>2300	Monogenic, PR (0.24)	
				–	Cry2Aa	25	PD (0.80)	
H. virescens	North Carolina (USA)	Cry1Ac (T)	KCB	–	Cry1Ac	400	–	Forcada *et al.*, 1999
H. armigera	Various locations (India)	Cry1Ac (IB)	–	10	Cry1Ac protoxin	76	–	Kranthi *et al.*, 2000
H. armigera	Gujarat (India)	Cry1Ac (Bt-cotton/CC)	Res-Bt	15	Cry1Ac protoxin	93	Monogenic, SD (0.42)	Kranthi *et al.*, 2006
H. armigera	Maharastra (India)	Cry1Ac (CC)	Res-AC	14	Cry1Ac protoxin	205	Monogenic, SD (0.56)	Kranthi *et al.*, 2006
H. armigera	Various locations (Australia)	Cry1Ac (SC)	BX	21	Cry1Ac protoxin	321	PR	Akhurst *et al.*, 2003
H. armigera	New South Wales (Australia)	Cry2Ab (SC)	SP15	–	Cry2Ab protoxin	6830	Monogenic, CR	Mahon *et al.*, 2007
H. armigera	China	Cry1Ac (Bt-cotton)	–	16	Cry1Ac	43*	Monogenic, PD (0.64)[f]	Liang *et al.*, 2000
H. armigera	Henan (China)	Cry1Ac (Bt-cotton)	–	42	Cry1Ac protoxin	1680	–	Meng *et al.*, 2003
H. armigera	Hebei (China)	Cry1Ac (T)	GYBT	28	Cry1Ac	564	PR (0.24)	Xu *et al.*, 2005
H. armigera	Henan (China)	Cry1Ac (CC)	–	52	Cry1Ac	425*	–	Luo *et al.*, 2006
H. zea	Mississippi and Texas (USA)	Cry1Ac (PT)	FZ	7	Cry1Ac	119	–	Luttrell *et al.*, 1999
P. gossypiella	Various locations (USA)	Cry1Ac (Bt-cotton/CC)	APHIS-98R	–	Cry1Ac protoxin	>100	SD to CR depending on the toxin conc. CR on Bt-cotton	Liu *et al.*, 1999; Liu *et al.*, 2001
P. gossypiella	Arizona (USA)	Cry1Ac (CC)	AZP-R	28	Cry1Ac protoxin	3100	Monogenic, PR (0.20)	Tabashnik *et al.*, 2002
O. nubilalis	Kansas (USA)	Dipel (FSC)	KS-SC-R	7	Dipel	65	Monogenic, PD (0.86)	Huang *et al.*, 1999
O. nubilalis	Minnesota (USA)	Cry1Ac (CC)	S-I	17	Cry1Ac protoxin	162 (FR)[g]	–	Bolin *et al.*, 1999
O. nubilalis	Minnesota (USA)	Cry1Ac (CC)	S-II	14	Cry1Ac protoxin	58 (FR)	–	Bolin *et al.*, 1999
O. nubilalis	Minnesota (USA)	Cry1Ac (CC) / Cry1Ab (Bt-corn)[h]	S-IV	40	Cry1Ac protoxin	8.4	–	Bolin *et al.*, 1999
O. nubilalis	Nebraska (USA)	Cry1Ab (SC)	N	7	Cry1Ab protoxin	14* (FR)	–	Chaufaux *et al.*, 2001
O. nubilalis	France and Switzerland	Cry1Ab (C)	LAS	9	Cry1Ab protoxin	32 (FR)	–	Chaufaux *et al.*, 2001
O. nubilalis	Italy	Cry1Ab (SC)	Europe-R[i]	9	Cry1Ab protoxin	13* (FR)	–	Chaufaux *et al.*, 2001
				95	Cry1Ab	2000	Polygenic, SD (0.44)	Alves *et al.*, 2006
O. nubilalis	Nebraska (USA) and Italy	Cry1Ab (SC)	RSTT-R	41	Cry1Ab protoxin	9	–	Siqueira *et al.*, 2004
				56	Cry1Ab	1300	Polygenic, SD (0.60)	Alves *et al.*, 2006
D. saccharalis	Louisiana (USA)	Cry1Ab (PM)	Isoline 52	–	Cry1Ab	Completed larval devel. on *Bt*-corn	Monogenic, CR	Huang *et al.*, 2007

[a] Dipel is a tradename for a commercial formulation of *B. thuringiensis* var. *kurstaki*.

[b] Different forms of selecting agent have been used: formulated spore-crystal preparations (FSC), spore-crystal preparations (SC), microencapsulated recombinant *P. fluorescens* cells expressing a *cry* gene (CC), parasporal crystals (C), inclusion bodies from recombinant *E. coli* cells expressing a *cry* gene (IB), protoxin (PT), activated toxin (T), and transformed plant material expressing a *cry* gene (PM).

[c] When available, the number of episodes of selection after which the insects were tested, is given.

[d] RR= resistance ratio. For *Bt* formulation or (pro)toxins this is defined as the LC_{50} (or LD_{50}) of resistant strain divided by the LC_{50} (or LD_{50}) of susceptible control strain; when the value is followed by "*" it refers instead to EC_{50} values (the concentration responsible for 50% growth inhibition). All values for Cry proteins refer to activated toxins unless otherwise indicated.

[e] In all reported cases resistance was autosomal and its type of inheritance: completely recessive (CR), partially recessive (PR), semi-dominant (SD), or partially dominant (PD). Dominance levels have been calculated from LC_{50} values (D_{LC}, dominance of insecticide resistance) (Bourguet *et al.*, 2000) as $D_{LC} = (\log LC_{SS} - \log LC_{RS}) / (\log LC_{SS} - \log LC_{RR})$. The range for D_{LC} is 0 (complete recessivity) to 1 (complete dominance). When LC_{50} values were available from the two reciprocal crosses (RR females × SS males and SS females × RR males), the mean value was used to calculate D_{LC}.

[f] The authors reported partial recessive inheritance, but according to the LC_{50} values, the calculated D_{LC} is 0.64, which corresponds to partial dominant inheritance.

[g] FR indicates that resistance levels fluctuated considerably from generation to generation, and the value given is the maximum value obtained.

[h] *O. nubilalis* Europe-R strain was originally referred to as strain I.

Poster 3

Geographic and host plant distribution of pheromone races of the European Corn Borer (*Ostrinia nubilalis*)

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The European corn borer *Ostrinia nubilalis* (ECB) is one of the most damaging pests of maize in Europe and North America. Two pheromone races of ECB have been identified. Both use 11-tetradecenyl acetate (11-14:OAc) isomers (E and Z) as sex pheromones. Females of the Z-race produce and males respond to a 97:3 ratio of Z11-14:OAc to E11-14:OAc, whereas the opposite E-blend in E-races ranges from 97:3 to 99:1. Small numbers of hybrid females producing an intermediate E/Z pheromone blend of 65:35 were found in regions where the two races occur sympatrically. It is assumed that also in laboratory studies hybrid moths are produced only at a low rate.

Regarding the distribution of different pheromone races on various host plants the E-race is commonly associated with hop and mugwort, and in only a few European countries (Switzerland and Italy) and Eastern North America with maize. The Z-race is associated solely with maize plants. For a resistance management in transgenic crops it would therefore not be advisable to use hop and mugwort as refuges because susceptible E-individuals from these refuges would not mate with resistant individuals from Bt-maize fields.

The pheromone composition of several European ECB populations sampled in maize fields, and from one population each sampled in hop and mugwort was analysed by gaschromatography. Pheromones were extracted from ECB tips incubated in hexan. E-; EZ- and Z- females were identified by comparing retention times and masses of natural compounds with those of synthetic external standards of the isomers. GC-analyses were performed with the parental or the 2^{nd} / 3^{rd} generation, but mostly with advanced laboratory populations.

Populations from Germany, France, Spain and Serbia showed the pheromone composition of the Z-race, but with varying frequencies of hybrids (EZ). In Italy and Greece we also found E-individuals and a considerable number of hybrids. Contradictory to the literature we found Z-individuals in hop and all pheromone types (E, EZ, Z) in mugwort both in an advanced laboratory population and in a freshly sampled field population (both from the same area).

Poster 8

Performance of *Aphis gossypii* on Indian *Bt* cotton varieties

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Insect-resistant transgenic cotton plants expressing Cry proteins derived from *Bacillus thuringiensis* (*Bt*) are cultivated on an increasing area in India since 2002, reaching 3.8 million hectares in 2006. *Bt* cotton provides a very effective control of the main pest, the pod borer *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae).

In recent years, several studies examined the effect of *Bt* crops on non-target organisms. This includes aphids which are not targeted by the Cry proteins expressed by the *Bt*-transgenic varieties but occasionally reach pest status and play an important role in arthropod food-webs. Since little is known about the interaction of Indian *Bt* cotton varieties with aphids we have conducted laboratory experiments to investigated the performance of cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), on three Indian *Bt* cotton varieties (MECH 12, MECH 162, MECH 184) expressing Cry1Ac, and their non-transformed near isolines. *Bt* protein and cotton variety effects on different aphid life-table parameters were determined. Furthermore we have investigated whether the *Bt* toxin can be detected in the aphids. This would indicate that the protein is transported in the phloem sap on which the aphids feed on.

Our study revealed no significant differences between *Bt* and non-*Bt* cotton varieties for any of the different aphid life-table parameters assessed. However, some small differences were observed among the three cotton varieties. None of the aphid samples contained Cry1Ac. As a consequence, natural enemies that feed on aphids are not exposed to the toxin. This finding is consistent with precious studies from other *Bt*-transgenic plants (maize, oilseed rape, rice and cotton) which have reported only trace amounts of Cry proteins in sap-feeding insects.

Our study allows to conclude that *Bt* cotton poses a negligible risk for aphid specific antagonists.

Poster 9

Assessing the direct effects of *Galanthus nivalis* agglutinin (GNA) and avidin on the ladybird beetle *Coccinella septempunctata*

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Genes encoding *Galanthus nivalis* agglutinin (GNA) and avidin have been incorporated in several crops to enhance their resistance to a range of insect pests. The ladybird beetle, *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) is an important predator of aphids and other soft-bodied insects in different crops. Thus, *C. septempunctata* is likely to ingest insecticidal proteins expressed by transgenic plants either directly by feeding on pollen or through its prey organisms. The present study was conducted to test the direct effects of GNA and avidin on a range of life-table parameters of *C. septempunctata*. The insecticidal proteins were provided dissolved in a 2M sucrose solution at a concentration of 1% (weight per volume). Neonate *C. septempunctata* larvae were fed either a pure sucrose solution (control) or a sucrose solution containing GNA or avidin. Every alternate day, predator larvae were fed exclusively with aphid prey. Ingestion of avidin resulted in a significant reduction in larval survival, adult emergence, and adult weight as compared to *C. septempunctata* receiving pure sucrose solution. Larvae of *C. septempunctata* appeared to be even more sensitive to GNA since this insecticidal protein caused a 100 % mortality. The results indicate that both GNA and avidin pose a hazard for larvae of the predatory beetle, *C. septempunctata*.

Poster 11

Unintended changes in biochemistry of cucumber carrying the thaumatin II gene that can affect insect/mite pests

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Thaumatin II gene codes for sweet tasting protein, which shares a significant amino acid homology to PR-5 (pathogenesis-related) proteins, also known as TL (thaumatin-like) proteins. Like other PR proteins, TL proteins when expressed in plants may contribute to endogenous resistance to pathogen/pest attack. They can also be involved in resistance and stress response in plants, although their precise functions are still unknown. Our recent studies showed that transgenic cucumber plants expressing the thaumatin II gene affect the abundance of some piercing-sucking pests but not natural enemies. However, there was no clear relationship between pests density and leaf thaumatin level. Therefore, to better understand the consequence of the presence of thaumatin II protein for chemical composition of transgenic cucumber plants we investigated unintended changes in biochemistry of modified cucumber plants carrying the thaumatin II gene that can influence insect/mite pests.

Four lines of GM-cucumbers (T 224 09, T 225 03, T 212 01, T 210 06) and non-GM inbred line of *Cucumis sativus* L. cv. Borszczagowski (line B) were grown under field conditions. During the vegetative period, leaf samples were collected and chosen primary and secondary metabolites essentially in host plant – pest interactions were analysed. Additionally, fruit samples at the stage of full fruiting of plants were collected and evaluated for the content of the same compounds as assayed in the leaf samples.

Biochemical analyses revealed that among examined cucumber lines the constitutive level of leaf/fruit biochemical components differs. In some of GM-cucumber lines the concentration of leaf total soluble phenolics and lignin content as well as the value of the lignin:phenolics ratio were altered significantly as compared to the controls. The concentration of glucose, fructose and soluble proteins in GM-cucumber lines was similar to the concentration of leaf primary metabolites in non-transformed line. The changes in the quality of fruits of transformed cucumber lines could be a result of changes in biochemistry of leaves. Disturbance in chemical composition of both leaves and fruits of GM-cucumber lines strongly suggests the alternations of existing biosynthetic pathways in response to transformation. The importance of biochemical properties of cucumbers carrying the thaumatin II gene in herbivorous pests settling, feeding and development is discussed. Further studies are necessary to determine, whether unexpected metabolic changes in GM-cucumber organs are existed in each of the subsequent generation.

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Poster 14

Impact of snowdrop lectin (GNA) on adult *Chrysoperla carnea*

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The gene encoding for snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) has been engineered successfully into a variety of crops including wheat, rice, tobacco and potatoes to control homopteran pests such as aphids. Since GNA has a broad range of activity and only provides partial control of the target pest(s), the impact of GNA on biological control organisms needs to be assessed.

The common green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), is an important natural predator of aphids in different crops. A previous study by Hogervorst et al. (2006) showed that the longevity of *C. carnea* larvae was directly affected by GNA. In addition, it was shown that the GNA could not be digested. Since *C. carnea* larvae are unable to excrete faeces, the protein remained in their bodies.

We investigated whether GNA-feeding during the larval stages has consequences for the performance of adult lacewings. In addition, the direct impact of GNA on adult performance was assessed by providing adults with an artificial diet that contained different amounts of GNA. Subsequently a number of important life-table parameters (survival, fecundity and fertility) were recorded. In addition, we examined the fate of GNA after ingestion by lacewing larvae.

Adult *C. carnea* were found to be sensitive to GNA and also affected when larvae were sublethally damaged by consumption of this insecticidal protein. Western-blots revealed that GNA ingested by larvae of *C. carnea* is (partly) transferred to the adult stage and was subsequently excreted or digested within a few days.

The established bioassay was found to be suitable to assess the impact of orally active insecticidal proteins on adult *C. carnea*.

Reference:

Hogervorst PAM, Ferry N, Gatehouse AMR, Wäckers FL & Romeis J (2006) Direct effects of snowdrop lectin (GNA) on larvae of three aphid predators and fate of GNA after ingestion. *Journal of Insect Physiology* 52: 614-624.

Poster 15

The influence of somaclonal variation on agronomic traits in potato

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Somaclonal variation is a term used to describe a phenomenon in plants caused by genetic or epigenetic changes induced during callus formation on plant tissues cultured *in vitro*. Typical genetic alterations are changes in the number and structure of chromosomes, and in DNA sequence, typical epigenetic related events are gene amplifications and gene methylation. The regenerated plants can show genotypic and phenotypic variations. Current techniques used in genetic transformation experiments, involving callus culture and plant regeneration, can result in other traits varying in addition to the expected changes caused by gene transfer.

In order to improve one or more agronomic traits of potato cultivars or breeding clones for potato breeding programmes a study of the induction and analysis of somaclonal variation in plants from *in vitro* callus cultures of stem and leaf explants, on medium supplemented with different phytohormones, was initiated. About 13,000 somaclones of seventeen cultivars and clones of potato were studied. For this purposes a somaclone is defined as a clone that differs from the donor genotype in at least one or more agronomic traits, induced, by the disorganised cell growth associated with the *in vitro* procedure. The somaclones were planted in a greenhouse, followed by tuber generations grown in the field. These plants were subjected to the multistage selection procedure commonly used in potato breeding. Over a period of five years and three field generations the haulm growth, earliness, yield, tuber number, size, shape, eye depth, starch content, starch yield and tuber appearance of these somaclones were assessed and compared with that of the controls, the donor varieties. In addition to the frequencies of positive and negative variants the percentage of invariant and positively varying somaclones among the total number of potted somaclones (gain rate) was used to define the effectiveness of this method of potato breeding.

The results indicate that the somaclones of potato vary in haulm growth, earliness, yield and tuber traits. This variation persists for several generations in the field. The degree of variation depends on the genotype and specific characters of the donor. After selection there is a small proportion of desirable aberrants, with better performance in terms of foliage, earliness, yield and tuber characters. Depending on trait the average gain rate for all donor genotypes ranged between 0.2 and 2.3% for –deviants, between 12.2 and 15.5% for invariants and between 0.1–1.4% for +deviants. Therefore, this method could be exploited in potato breeding programmes for improving cvs. or breeding clones specifically for breeding targets such as increasing tuber number per plant, starch content or earliness of one maturity type. For most of the traits tested, 67-98% of the somaclones were indistinguishable from the donor genotype in the first to third field generations. Nevertheless, depending on genotype, up to 15%, 15% and 20% of the somaclones of the second generation in the field consisted of negative variants, in terms of haulm growth, length of the vegetation period and tuber yield per plant, respectively. In the third field generation 1.2–5.5% of the somaclones showed negative variants in tuber number per plant, size of tubers, starch content and yield per plant, tuber shape and eye depth for on average 5 donor cvs. and breeding clones. The unpredictable and uncontrollable nature of somaclonal variation has to be taken into consideration when using *in vitro* culture for the production of transgenic potato material.

THIEME, R.; GRIESS, H.: Somaclonal variation in tuber traits of potato. Potato Research 48, 2005, 153-165.

Baseline susceptibility of *Helicoverpa armigera* (Hübner) to Bt toxins in Western and Central Africa

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Introduction

Burkina Faso will be the first country in West Africa to grow Bt cotton at a commercial scale in 2007. Others cotton producing countries all-around are considering the technology and will observe the economical results as well as the crop management strategy adopted by local authorities in Burkina Faso.

Bt cotton (expressing Cry1Ac toxin) was introduced in the Old World (Australia, China, India) to manage insecticide-resistant bollworm (*Helicoverpa armigera* Hübner) populations. Many scientists consider the possibility of bollworms developing resistance to the Bt toxin as an important concern. In large farming (USA, Australia) resistance management relies on the HDR strategy (High level of toxin expression, associated with cultivated refuges). In small-scale farming (China, India), associated crops and wild host plants are currently claimed as satisfactory refuges (Wu et al., 2002; Green *et al.*, 2003) but elements of doubt hang over the matter. To obtain a sustainable efficacy of Bt cotton in the fragmented landscape associated with small farming, African scientists choose to introduce Bt cultivars expressing two toxins: Cry1Ac and Cry2Ab.

Up to now, the susceptibility of African populations of the bollworm to Bt toxins is not known. CIRAD is currently developing a model (Vaissayre *et al.* 2006; Nibouche *et al.* 2007) to point out key factors associated with Bt cotton sustainability and resistance development: among these factors are the efficacy of Bt toxins against the bollworm, and the frequency of resistant individuals in naïve bollworm populations. The purpose of the study reported here was to determine the susceptibility to Cry1Ac and Cry2Ab of bollworm strains collected in various cotton growing areas of western and central Africa, either in terms of mortality, or, according to the specificity of Bt toxins, in terms of inhibition of larval growth.

Material and Methods

Insect strains

Bioassays were performed in 2006, from mid September to the end of October in IRAD facilities in Garoua, Cameroon as well as in INRAB facilities in Bohicon, Benin. Bollworm larvae were collected from 7 localities in Cameroon, 2 localities in Chad and 1 cotton growing village in North Eastern Nigeria. Other strains were collected in various cotton growing regions of Benin. In each place, 100 to 200 late instars of *H. armigera* larvae were collected along a 10 km transect. Larvae were reared on a semi-artificial diet in the laboratory. Adults obtained were placed in jars for egg laying. The bioassays were performed on the first instars (24 h after hatching) from F1 and F2 progeny.

Toxins
Two toxins were tested. Cry 1Ac (200 mg/g) was obtained from MVPII bioinsecticide and Cry 2Ab (6 mg/g) from lyophilized corn leaf powder. Toxins used were provided by Monsanto Company (Chesterfield, USA). When the temperature of the artificial diet was reduced to 45-50°C, the liquid diet was mixed with the toxin suspension, to obtain a volume of 150 ml. Dilutions in distilled water led to seven doses and a control.One ml of diet with the toxin was dispensed into each of the 24 cells of a box, 6 boxes for each dose of toxin. The diet was allowed to get cold under UV for 1 hour.
Bioassays were conducted by exposing neonates (< 24 h after hatching) to treated artificial diet. One bollworm larva was transferred in each cell, the cell closed with Parafilm® and the boxes maintained at a temperature of 25 ± 2°C, RH 60 ± 20% and photoperiod 14:10h. A toxin mixture (Cry1Ac + Cry2Ab), 1/1 ratio, was also tested. Each test was repeated 4 to 6 fold.

Observations
Mortality was recorded, if any, after 1, 2, 5 and 7 days. For the determination of the LD_{50}, we considered not only dead larvae, but larvae not reaching the third instar after 7 days were also considered as dead ones.
According to Siegfried *et al.* (2000) the feeding disruption effect of Bt toxins was evaluated: the weight of the living larvae was recorded at 7 days, and compared with the control, to obtain a growth inhibition factor (GI_{50} = dose corresponding to 50% of larvae on treated diet reaching less than half the weight of larvae growing on the control diet).
Statistical analysis was performed using WinDL V2.0 software (CIRAD).

Results and discussion

Data were obtained in Benin on strains collected from cotton in November, and from tomato later on (January-March). Rough data show that mortality (fig 1) as well as growth inhibition (fig 2) obtained by increasing toxin concentration is higher on cotton than on tomatoes, and higher on populations collected at the end of the growing season than later on, during the dry season. Results could be affected either by the host plant or by the collection date.

Data obtained in Central Africa (fig 3) for LC_{50} as well as for GI_{50} appear when both toxins and their association are compared for their effect on mortality (fig 4) as well as on growth inhibition (fig 5).

Cry 1Ac confirms a high level of toxicity, expressed as direct mortality through LC_{50}, and a repellent effect, expressed as a decrease or an arrest of feeding, followed by a loss of weight (illustrated by GI_{50} data). The mean value for LC_{50} is 1.1 µg/ml and ranges from 0.2 to 2.28. Growth inhibition occurs for concentrations ten-fold lower: 0.15 µg/ml (0.05 to 0.38). Our results are in accordance with previous ones (Kranthi *et al,* 2001; Jalali *et al,* 2004) for Cry1Ac on *H. armigera*.

Cry 2Ab has a lower impact on both mortality and feeding. The mean value for LC_{50} is 2.14 µg/ml and ranges from 0.65 to 5.9, when growth inhibition occurs for concentrations ten-fold lower: 0.26 µg/ml (0.04 to 0.55). Associating both toxins in a 1/1 ratio leads to intermediate values for the LC_{50} : 1.63 µg/ml, but remains similar to Cry2Ab alone for the GI_{50}

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Fig 1. Mortality according to populations of *H. armigera* (Benin, 2007)

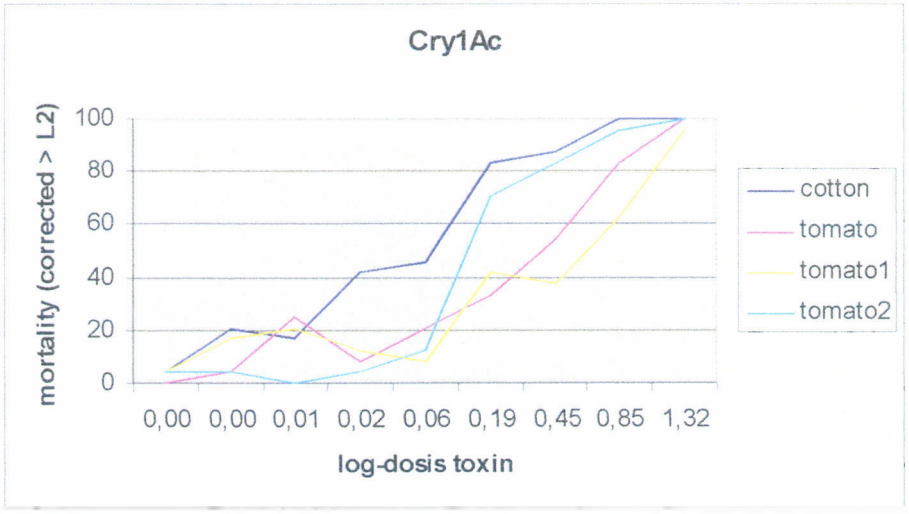


Fig 2. Weight decrease/Populations of *H. armigera* (Benin, 2007)

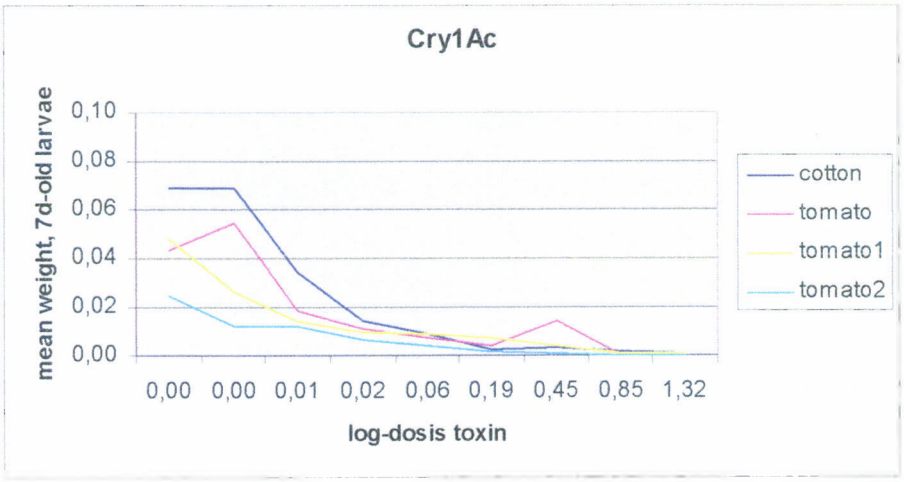


Fig 3: Insect collection sites in Central Africa

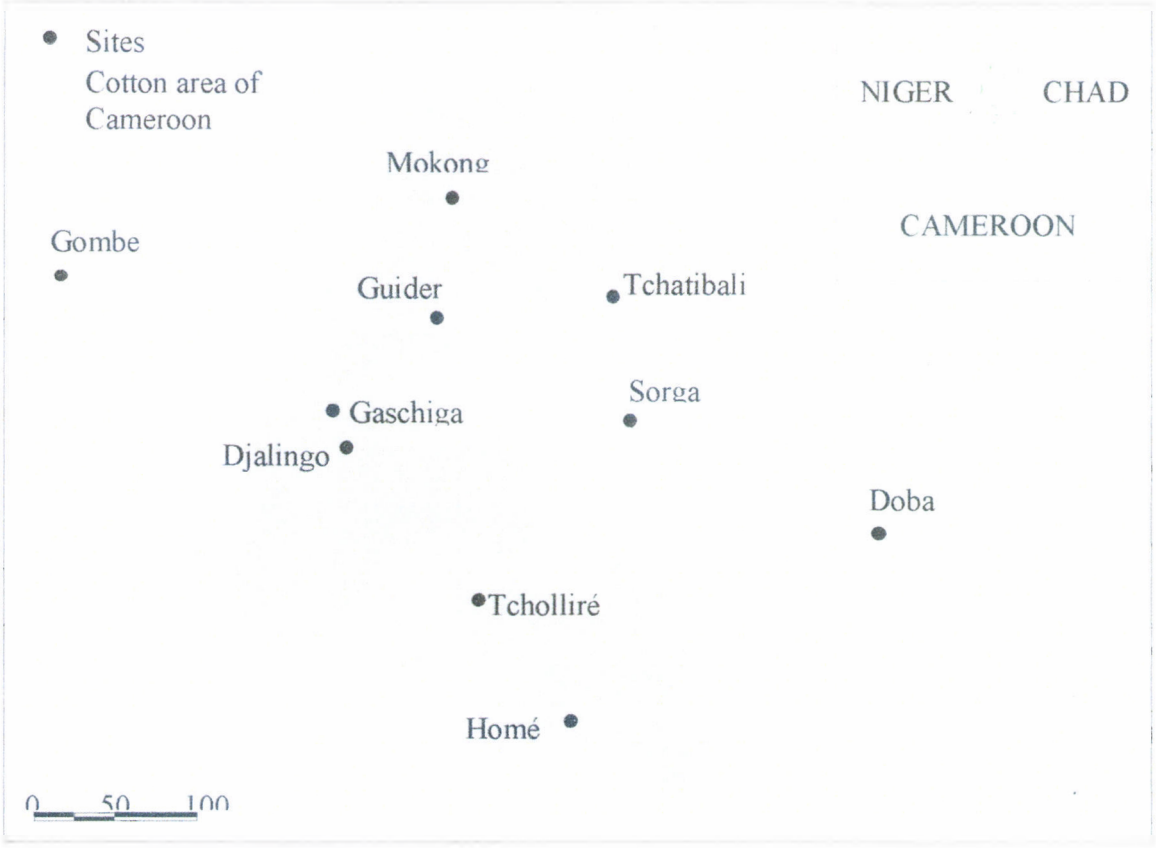


Fig 4

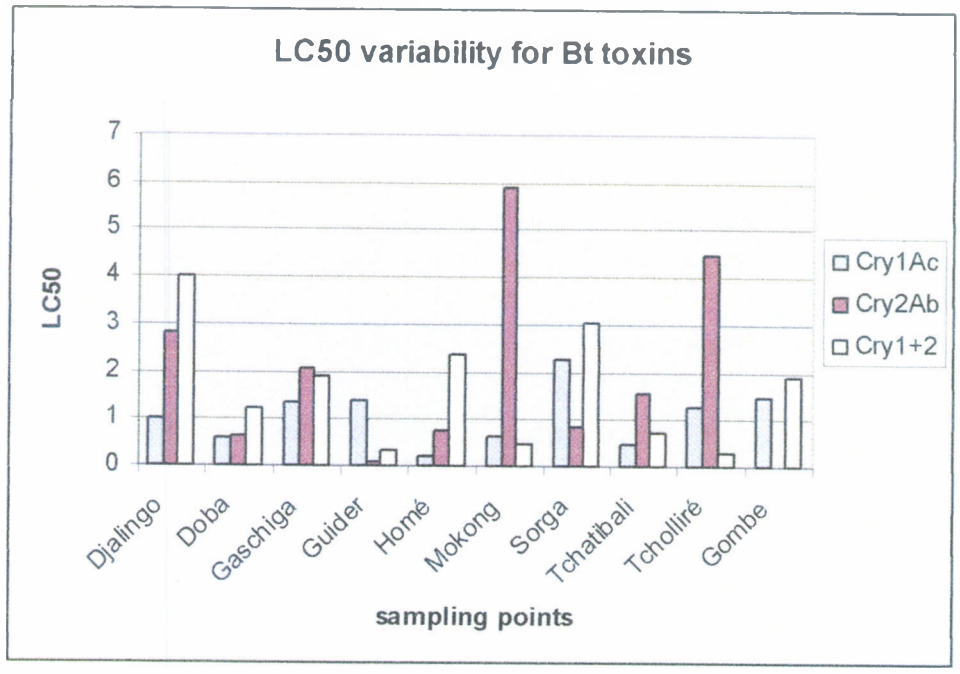
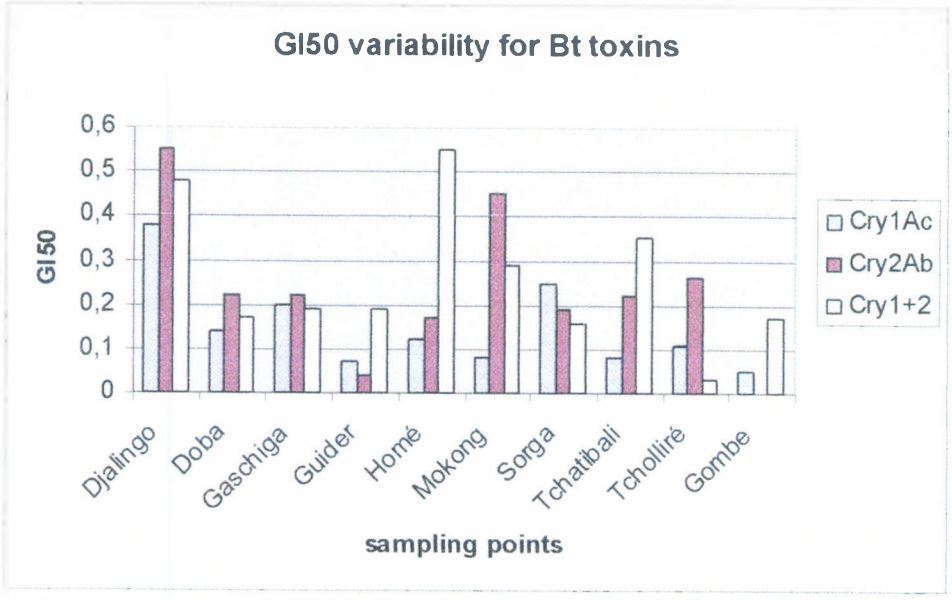

LC50 variability for Bt toxins
LC50
7
6
5
4
3
2
1
0
Djalingo
Doba
Gaschiga
Guider
Homé
Mokong
Sorga
Tchatibali
Tcholliré
Gombe
sampling points
Cry1Ac
Cry2Ab
Cry1+2

Fig 5


GI50 variability for Bt toxins
GI50
0,6
0,5
0,4
0,3
0,2
0,1
0
Djalingo
Doba
Gaschiga
Guider
Homé
Mokong
Sorga
Tchatibali
Tcholliré
Gombe
sampling points
Cry1Ac
Cry2Ab
Cry1+2

J'y étais !

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