Biology and control strategies for whitefly (*Bemisia tabaci* Gennadius) (Homoptera: Aleyrodidae) populations in Burkina Faso (West Africa)

By

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

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March 2003

Suggested short title

Control strategies for whitefly populations in Burkina Faso.

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Abstract

Ph.D **Lenli Claude Otoidobiga** **Natural Resource Sciences**

Field and laboratory investigations were conducted in Burkina Faso to diagnose the causes of severe outbreaks of *Bemisia tabaci* populations and general failures of chemical control against this pest in cotton. The research efforts were oriented in the following three areas:

I) The susceptibility of *B. tabaci* populations and its parasitoids to cotton insecticides. Insecticides have been and will likely remain the primary tools employed to control *Bemisia tabaci*. However repeated applications have often resulted in *B. tabaci* developing resistance to numerous conventional insecticides throughout the world. The need for promoting judicious insecticide use in association with other management practices led me to investigate the status of *B. tabaci* and its parasitoids susceptibility to conventional and novel insecticides.

a) Conventional insecticides. Research was conducted in 14 cotton fields (3 to 10 ha) selected in 7 localities (two fields per locality), with the objectives to: 1) estimate *Bemisia tabaci* Gennadus (Homoptera: Aleyrodidae) population abundance, 2) assess the levels of parasitism by *Encarsia* spp. and *Eretmocerus* spp. (Hymenoptera: Aphelinidae), and 3) estimate the susceptibilities of the pest and those of the parasites *Eretmocerus* spp. (Aphelinidae) to the insecticides currently sprayed on cotton. Yellow sticky cards and a leaf turning technique were used to estimate adult *B. tabaci* population densities. Yellow sticky cards were also used to estimate the densities of adult *Eretmocerus* sp. and the

susceptibilities of *B. tabaci* to insecticides. Leaf disk techniques were used to estimate *B. tabaci* red eye nymph populations, and parasitism by *Encarsia* spp. and *Eretmoceus* spp. were evaluated using stereomicroscopy. A leaf cage technique was used to estimate the susceptibilities of *Eretmocerus* spp. to insecticides. A mean of 6.5 to 27.4 adult *B. tabaci* were trapped per yellow sticky card and 5.5 to >34.9 were counted per leaf using the leaf turning technique. There were 0.14 to 13 *Eretmocerus* sp. trapped per yellow sticky card. The levels of parasitism varied between 36 and 87% by the end of the season and parasitism by *Eretmocerus* sp. predominated in most of the fields. The susceptibilities of *B. tabaci* and *Eretmocerus* sp. varied from field to field and with the insecticide tested. We suggest: 1) that broad-spectrum insecticides be replaced by more selective insecticides, or 2) be applied at the end of the season (i.e. open boll period) and be used only twice to allow the parasitoids to act, 3) that different classes of insecticides be alternated to reduce the risk of resistance buildup.

b) Novel insecticides. Bioassays were conducted in 2001 and 2002 to estimate toxicities and dose-response relationships of 24 *Bemisia tabaci* Gennadius populations to pyriproxifen, acemitaprid, and diafenthiuron newly introduced to deal with the *B. tabaci* crisis. LC_{50s} ranging from 0.014 to 0.096 mgl^{-1}, 0.60 to 1.3 mgl^{-1}, and 3.5 to 6.7 mgl^{-1} were observed for pyriproxifen, acemitaprid, and diafenthiuron, respectively. These LC_{50s} were extremely low compared to the field doses recommended for each compound. A fast increase in rates of mortality within a narrow range of lethal concentrations was observed for each compound, indicating that all three compounds were highly effective at killing whiteflies.

In a separate experiment, pyriproxifen, acemitaprid, and diafenthiuron were tested in 2001 and 2002 to compare their effectiveness and assess their impact on parasitism in the field. In both years all three compounds significantly prevented *B. tabaci* populations from reaching economic injury levels in cotton and minimized adverse effects on parasitism. Our results provide, for the first time baseline toxicological and field efficacy data of pyriproxifen, acemitaprid, and diafenthiuron against *B. tabaci* in West Africa. These compounds should be included in a resistance management program of the cotton pest complex and their use should be restricted to prevent the buildup of resistance in *B. tabaci* populations.

II) The relative abundance of *B. tabaci* and its parasitoids in the field. Whitefly infestations and parasitism were monitored year round in overlapping cotton crops sown at three dates to record the relative abundance of *B. tabaci* (Gennadius) and its parasitoids, *Eretmocerus* spp. and *Encarsia* spp. in control and insecticide sprayed plots. Low *B. tabaci* populations developed during the first half of the five-six month rainy-season (May-October). Pest populations increased from September and picked in November. The levels reached were higher in insecticide treated (48 nymphs/leaf) than in control (25 nymphs/leaf) plots. Parasitism reached 88.7% in control plots, and 53.7% in insecticide treated plots. *Eretmocerus* spp. were more abundant than *Encarsia* spp. in both treated and control plots. A curvilinear relationship was observed where % parasitism on a linear scale rose to a pleateau with logarithmic increase in host density. This relationship later reversed to a linear inverse relationship between densities of *B. tabaci* and % of parasitism. In general % parasitism followed the abundance of pest populations except in March and April where parasitism increased while *B. tabaci*

populations decreased. In a separate experiment, adult *Eretmocerus* spp. were released into caged cotton plants to study the impact of augmentative releases of the parasites on the population dynamics of the pest. Pest densities increased from 1.47nymphs/leaf to 39.4 nymphs/leaf in the control, but were reduced to 0.8 and 0.6 nymphs/leaf in the cages where respectively 4 and 8 parasitoids have been released per plant. Parasitism is an important factor reducing *B. tabaci* populations during and after the growing season and, *Eretmocerus* spp. are promising biological control candidates against the pest in cotton.

III) The biological activities of some botanical extracts as alternative insecticides against the pest. Botanical insecticides often have different modes of actions than synthetic insecticides. They can be sound alternatives to the management of *B. tabaci* because they do not persist in the environment, may have low mammalian toxicity, and resistance has not yet been reported against them. The ovicidal, adulticidal, larvicidal, repellent, and oviposition suppression activities of *Jatropha curcas* L, *Ricinus communis* L. and. *Datura innoxia* seed aqueous extracts were investigated against *Bemisia tabaci* Gennadius on cotton using foliar spray. No ovicidal activities were observed but the extracts caused moderate (33.5) to high mortality (54 to 100%) respectively of adults and nymphs. In addition *J. curcas* L, *R. communis* L. extracts significantly reduced adult abundance and oviposition on cotton foliage in the field and laboratory. The extracts represent promising tools for the control of *B. tabaci*.

Résumé.

Ph. D. Lenli Claude Otoidobiga **Natural Resource Sciences**

Des activités de recherches ont été effectuées en laboratoire et au champ au Burkina Faso pour étudier les causes des explosions des populations de *B. tabaci* en culture cotonnière et proposer des solutions. Ces activités ont été articulées autour des trois axes de recherches suivants :

I La sensibilité des populations de *B. tabaci* et de leurs parasitoïdes aux insecticides utilisés pour la protection cotonnière. Les insecticides ont été et resteront probablement encore pour longtemps le principal moyen de lutte contre *B. tabaci*. Cependant leur mauvaise utilisation a souvent provoqué le développement de la résistance aux insecticides par le ravageur. La nécessité de promouvoir une utilisation judicieuse des insecticides et des autres moyens de lutte m'a emmené à évaluer les niveaux de sensibilités de *B. tabaci* aux insecticides conventionnels qui étaient couramment utilisés depuis des années et à évaluer l'efficacité des insecticides qui ont été importés dans le pays pour juguler la crise.

a) Susceptibilité des populations de *B. tabaci* et des parasitoids aux insecticides conventionels. Des études ont été menées dans 14 champs de coton (de 3 à 10ha) sélectionnés dans 7 localités (2 champs par localité) et avaient pour objectifs : 1) d'estimer l'abondance des populations de *B. tabaci*, 2) d'étudier les niveaux de parasitisme par les parasitoïdes des genres *Encarsia* spp. et *Eretmocerus* spp. (Hymenoptera : Aphelinidae) et, 3) d'estimer les niveaux de susceptibilité des

populations du ravageur et ceux d'un de ses parasitoïdes les plus fréquents, *Eretmocerus* sp. aux insecticides couramment utilisés en protection cotonnière. Les techniques utilisant les cartes jaunes collantes et le retournement des feuilles ont été utilisées pour estimer les densités des populations de *B. tabaci* . La technique les cartes jaunes collantes a été aussi utilisée pour estimer l'efficacité biologique des insecticides utilisés contre *B. tabaci* et évaluer les densités du parasitoïde *Eretmocerus* sp. dans les champs. La technique du disque de feuille a été utilisée pour évaluer les densités des populations des nymphes du 4^{e} stade de *B. tabaci* et les parasitismes par *Encarsia* spp. et *Eretmocerus* spp. ont été estimés en utilisant un stéréo microscope. La technique des « leaf-clip » cages a été utilisée pour étudier la toxicité biologique des insecticides contre *Eretmocerus* sp. Des moyennes de 6.5 à 27.4 et 5.5 à plus de 34.9 adultes *B. tabaci* ont été comptées respectivement sur cartes jaunes et sur les feuilles par la méthode du comptage binomial et 60 à 100% de feuilles de coton étaient infestées par *B. tabaci*. On a dénombré 0.1 à 13 adultes d'*Eretmocerus* sp. par carte jaune collante. Les niveaux de parasitismes fluctuèrent entre 36 et 87% à la fin de la saison et le parasitisme par *Eretmocerus* sp. était le plus important dans la plupart des champs. Les efficacités biologiques des insecticides contre *B. tabaci* et *Eretmocerus* sp. varièrent d'un champ à l'autre et en fonction des insecticides testés. Nous recommandons: 1) que ces insecticides à large spectre d'action soient remplacés par des insecticides plus sélectifs, ou 2) soient utilisés vers la fin de la saison (pendant la période de l'ouverture des capsules) et soient épandus deux fois seulement pour permettre le développement des parasitoïdes, 3) La rotation entre insecticides de différentes classes pour réduire le développement de la résistance.

b) Susceptibilité des populations de *B. tabaci* aux nouveaux insecticides. Des bioessais ont été effectués en 2001 et 2002 pour estimer les niveaux de toxicité du pyriproxifen, de l'acétamiprid, et du diafenthiuron, insecticides qui ont récemment acquis une autorisation provisoire d'utilisation en Afrique de l'Ouest, et les types de relations entre les doses de ces produits et les réponses exprimées en taux de mortalité de 24 populations de *B. tabaci* . Des DL_{50s} comprises entre 0,014 à 0,096 mgl^{-1}, 0,60 à 1,30 mgl^{-1}, et 3,5 à 6,7mgl^{-1} ont été observées respectivement pour le pyriproxyfen, l'acétamiprid, et le diafenthiuron. Ces DL_{50s} étaient extrêmement bas comparées aux doses recommandées pour l'application au champ. Une augmentation rapide des taux de mortalité a été observée sur un échelle étroite de doses pour chacun des trois produits testés, indiquant que ces nouveaux produits étaient très efficaces contre les mouches blanches.

Dans une autre étude, les trois produits ont été expérimentés en 2001 et 2002 en utilisant des blocs complètement randomisés pour comparer leur efficacité et évaluer leur impact sur les parasitoïdes au champ. Pendant les deux années, tous les trois produits ont empêché *B. tabaci* d'atteindre le seuil économique des dégâts et ont eu un impact minimal sur le parasitisme. Nos résultats procurent pour la première fois une base de référence pour la toxicité du pyriproxyfen, l'acétamiprid, et le diafenthiuron en Afrique de l'Ouest, comme ces produits feront désormais partie de la gamme des produits utilisés contre *B. tabaci* en Afrique.

II Abondance relative de *B. tabaci* et de ses parasitoïdes.

Des parcelles de coton ont été semées à trois dates de façon à disposer de plants sans interruption. Le dispositif expérimental était constitué de bloc de parcelles complètement randomisées, dont la moitié était soumise au programme de traitements insecticides recommandés aux producteurs de coton, et les témoins étaient traitées avec de l'eau seulement. Les populations de *B. tabaci* et de ses parasitoïdes *Eretmocerus* spp. et *Encarsia* spp. ont été étudiées pendant toute l'année 2001 dans ces parcelles pour évaluer leur abondance relative. Les populations de *B. tabaci* étaient faibles pendant la période de forte pluviosité. Elles augmentèrent vers la fin de la saison des pluies, et étaient plus abondantes dans les parcelles traitées aux insecticides (48 nymphes/feuilles) que dans les parcelles témoins (25 nymphes/feuilles). Les taux de parasitisme atteignirent 88.7% dans les parcelles témoins, contre 53.7% dans les parcelles traitées aux insecticides. Les parasitoïdes du genre *Eretmocerus* étaient plus abondant que ceux du genre *Encarsia*. Une relation de type logarithmique qui devint plus tard une relation de type linéaire négative a été observée entre les densités de *B. tabaci* et celles de ces parasitoïdes. De manière générale, les % de parasitisme augmentèrent au fur et à mesure que les populations de *B. tabaci* augmentaient, excepté aux mois de Mars et Avril où les % de parasitisme augmentèrent pendant que les populations de *B. tabaci* diminuaient.

Dans une seconde étude, des adultes d'*Eretmocerus* spp. ont été lâchés dans des cages renfermant des plants de coton dans un champ pour étudier l'impact de l'augmentation de ces parasitoïdes sur la dynamique de population de *B. tabaci*. Les populations de *B. tabaci* augmentèrent de 1.47 à 39.4 nymphes/feuille dans les témoins, contre une réduction à 0,8 et 0,6 nymphe/feuille dans les cages où 4 ou 8 parasitoïdes ont été lâchés par plant. Ces résultats indiquent que le parasitisme est un important facteur

de réduction des populations de *B. tabaci* pendant et après la saison des cultures, et que les parasitoïdes du genre *Eretmocerus* constituent des agents de contrôle biologique prometteurs.

III Activités biologiques de quelques substances botaniques contre *B. tabaci*. Les insecticides botaniques possèdent des modes d'actions typiquement différents de ceux des insecticides synthétiques. Il pourraient constituer une alternative raisonnable aux insecticides synthétiques contre *B. tabaci* parce qu'ils ne persistent pas dans l'environnement, sont peu toxiques pour les animaux à sang chaud, et aucune résistance n'a encore été rapportée contre eux. La toxicité biologique des graines de *Jatropha curcas* L., *Ricinus communis* L., et *Datura innoxia* L. a été étudiée contre les œufs, les adultes, et les nymphes de *B. tabaci*. Les effets répulsifs et la suppression de la ponte des œufs chez les femelles mouches blanches par ces plantes ont aussi été étudiées en utilisant des extraits aqueux. Aucune toxicité n'a été observée sur les œufs, mais les extraits ont causé des mortalités modérées (33.5%) à élevées (54 à 100%) respectivement contre les adultes et les nymphes. En plus *J. curcas* L. et *R. communis* L. ont significativement réduit l'abondance des adultes et la ponte des œufs sur les feuilles de coton traitées au laboratoire et au champ. Ces extraits de plantes constituent des insecticides botaniques prometteurs contre *B. tabaci*.

Suggested short title

Control strategies for whitefly populations in Burkina Faso.

Dedication.

To my family, for which I stoically endured the hardships of this study.

Aknowledgements

This research was supported by scholarchip granted to me by the Institut de l'Environnement et des Recherches Agricoles (INERA) of Burkina Faso, my home country.

Colleagues and Friends in Burkina Faso.

I am grateful to Paco Sereme, former Director of INERA, and to Konaté Gnissa chair of the Department of Crop Production of this Institute for awarding me this postgraduate scholarship for this achievement. My gratitude is due to Abdoussalam Sawadogo, former chair of the "Programme Cultures Maraîchères, Fruitières et Plantes à Tubercules" for administrative support. I am indebted to Dakuo Déhou, adjunct Director of the "Direction de la Production Cotonnière" of Sofitex for his solicitude in providing transport facilities and fertilizer for my field experiments. I am grateful to Georges Yaméogo Director of the "Direction de la Production Cotonnière" of Sofitex for his encouragements. Thanks are due to Zagré Celestin former chef de la région cotonière de Dédougou, and acting Marketing Diretor of Sofitex for hosting me all the time I spent in Dédougou and Solenzo. I thank SAPHYTO, the local representative of the agrochemical company CALLIOPE for supplying the insecticides used in the study reported in chapter 2, and Sofitex for those used in the study reported in chapter 3. I am grateful to Bambara Issa, Dakuo Patrice, Sinon El Adj Adama, and Traoré Thomas for technical assistance.

Supervision.

I express my greatest admiration and gratitude to Dr. Charles Vincent for his invaluable patience, coaching, understanding, and diligence in reviewing the many versions of the papers and the thesis. I thank Dr. R.K. Stewart whose help and support has made my endeavor fruitful. Many thanks are due to Dr. D.J. Lewis for his administrative support.

Staff Members.

My gratitude is extended to Marie J. Kubecki for her kindness, Joanne Ten Eyck for managing my scholarship, and to all the professors and staff members in the Department of Natural Resource Sciences for diverse help and contributions in seminars.

External Scientists.

I am particularly indebted to Dr. Steve Naranjo for hosting me during two weeks in his laboratory at the Western Cotton Research Laboratory, Phoenix, Arizona. His help has been instrumental for my learning about the challenge raised by *B. tabaci* in the entire world. I am gratefull to Drs. Steve Castle and Nilima Prabhaker of the Western Cotton Research Laboratory, Phoenix, Arizona for teaching me bioassays techniques and giving me many materials I used in this study. I thank all the staff in the Western Cotton Research Laboratory in Phoenix, Arizona for contributing to my learning through the guiding tour they organized for me when I visited their laboratory. I thank Mr. Benoit Rencourt for technical assistance at Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu. Many thanks to Dr. Gregory Zolnerowich and Dr. Judy K. Brown respectively

of Kansas State University and the University of Arizona for respectively identifying *Eretmocerus* species and *Bemisia tabaci*. I am grateful to Dr. David Burns, Associate Dean of the Department of Chemistry at McGill University for managing to teach me analytical chemistry on his limited time.

Friends and Fellows Students in Canada.

I am grateful to Micheline Fauvel of the Public Health Laboratory of Québec in Montreal for her hospitality. I thank Nsitu Mbungu, Ph. D. student with whom I shared the same laboratory in the Department of Natural Resource Sciences.

Claims to originality.

The following findings from the present study, in the author's opinion, provide original knowledge on *Bemisia tabaci* control.

A) Information new at world level.

1. First development of a method for collecting adult *Eretmocerus* spp. in the field for bioassays.
2. First demonstration of tolerance of *Eretmocerus* spp. to insecticides in the field.
3. First demonstration of a curvilinear density-dependent, and an inverse linear density-dependent relationship between *B. tabaci* and its parasitoids in the field.
4. First demonstration of the insecticidal activity of *Datura innoxia* Linn. against *B. tabaci.*
5. First demonstration of the insecticidal activity of *Jatropha curcas* against *B. tabaci.*
6. First demonstration of the insecticidal activity of *Ricinus communis* against *B. tabaci.*
7. First demonstration of the repellant activity of *J. curcas* against *B. tabaci.*
8. First demonstration of the repellant activity of *R. communis* against *B. tabaci.*

9. First documentation of the population dynamics of *B. tabaci* and its parasitoids year round in continuous cultivation of the same crop.

10. This is the first assessment of the year round impact of ecological factors such as rainfall and temperature on field populations of *B. tabaci* and its parasitoids.

B) Information new to Africa.

11. First assessment of the impact of pyriproxifen, acetamiprid, and diafenthiuron on the parasitism of *B. tabaci.*

12. First documentation of *B. tabaci* resistance to cypermethrin, methamidophos, and omethoate.

13. First documentation of the baseline toxicities of pyriproxyfen, acetamiprid, and diafenthiuron to *B. tabaci.*

14. First assessment of the population dynamics of *Eretmocerus* spp. in cotton.

15. First assessment of the population dynamics of *Encarsia* spp. in cotton.

16. First assessment of the potential of *Eretmocerus* spp. against *B. tabaci* in field cages.

17. First demonstration of the role of insecticides in *B. tabaci* outbreaks in West Africa.

Overall my findings contribute to solving the *B. tabaci* crisis in Burkina Faso, and Africa.

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Introduction

A) Foreword

Bemisia tabaci (Gennadius) (Homoptera, Aleyrodidae) was described over 100 years ago as a tobacco pest in Greece, and has since become one of the most important pests of world agriculture. In addition to direct feeding damage, the insect vectors a number of devastating plant viruses, causes debilitating plant disorders of unknown etiology and, by excreting honeydew, reduces the quality of harvested products. There are several challenges to the development of economically efficient and environmentally sound management system for *B. tabaci*. The insect has a reported host range in excess of 600 plant species, a high reproductive rate, and the ability to readily disperse among hosts and breed year-round. The propensity of that pest to develop resistance to every class of insecticides makes it one of the most difficult pests to manage in the world. Previous distributions of this insect were limited to regions between the 30th parallels. However, in the past two decades, *B. tabaci* has invaded every continent in the world except Antarctica, and commercial trade has facilitated the regular occurrence of populations in temperate greenhouse production systems throughout areas like Europe and North America.

The challenges include not only management, biological and ecological issues, but even more basic problems with pest nomenclature. The recent geographic expansion of this pest has been closely associated with a new and more virulent biotype, known widely as biotype B, that may represent a new species (*B. argentifolii* Bellows and Perring). The taxonomy and systematic of this pest remains confused and controversial. Recent analyses, using a wide array of morphological and molecular tools, suggest that *B. tabaci* may represent a species complex. Because of the uncertainty of the pest's identity throughout the world and through time, the binomial *B. tabaci* have been recommended for use

in the broadest sense to represent all members of the species complex unless there is a compelling reason for a more specific designation. My Ph.D. thesis was undertaken in the contest of unprecedented *B. tabaci* population outbreaks that severely threatened the cotton industry of Burkina Faso.

B) Arthropods species in the cotton agro-ecosystems.

The number of arthropod species found in cotton agro-ecosystems across the world varies from a few hundred to more than a thousand (Luttrel *et al.* 1994). The vast majority of these species are predators and parasites of phytophagous species. Estimates of the number of pest species range from 20 to 60, but significant crop damage is caused by 5 to 10 key pests among which *Helicoverpa armigera* Hübner and *B. tabaci* are the most important in Africa (Eveleens, 1983). Chemical controls targeting specific pests in this multi-pest ecosystem often provoke the rise in importance of species from minor to major pest status, giving rise to recurring pest problems (Eveleens, 1983).

C) Climatic characteristics of the research area.

Situated in the sub-humid tropical zone between ten and thirteen degrees north latitude, the climate of the Western and North-Western Burkina Faso region in which most of my studies have been conducted, is characterized by distinct rainy and dry seasons. The movement of air masses that is NE-SW regulates the rhythm of wet and dry seasons. Rainfall lasts five to six months (May –October), and averages 800- to 1,100 mm annually. Rainfall is relatively intense and concentrated in the months of July-August (see Fig. 3, chapter 4). The dry season begins in October and ends in early May. "Harmattan", a dry and dusty wind blows NE-SW from the Sahara during that season. Together, these patterns give the region's climate a harsh character.

D) Rationale

D.1) *B. tabaci* population outbreaks in Burkina Faso.

In September 1998, Burkina Faso experienced severe outbreaks of whitefly populations on cotton. This was the first time that whiteflies, *Bemisia* spp., had so negatively impacted on cotton production in Burkina Faso. In the past *Bemisia* spp. was regarded as a secondary pest. Cotton is the main commercial crop of Burkina Faso, contributing to about 30% of its agricultural exports. Given this situation, the primary objectives of this study were to determine the causes of these outbreaks and to devise appropriate management strategies for the pest.

Cotton production in Burkina Faso is organised as follows: prior to the growing season, the company SOFITEX contracts to buy the cotton crop from farmers. Sofitex supplies the farmers with all the resources necessary to grow the crop (fertilizers, pesticides, and equipment) and deducts these costs from the payment for the crop.

Before 1991, six insecticide treatments per season were recommended to protect cotton against insect pests. Growers often applied only some of these treatments, because damage caused by insects was low. During 1991-1992, cotton growers experienced an outbreak of *Helicoverpa armigera* (Hübner)(Lepidoptera: Noctuidae), the cotton bollworm moth. This caused severe losses to SOFITEX, because most of the cotton growers could not repay their pesticide loans (Georges Yaméogo, personal communication). Because *H. armigera* (Hübner) seriously threatened cotton production in 1991-1992, and growers and Sofitex incurred severe losses, growers increased their annual number of insecticide treatments. Subsequently in 1996, a second *H. armigera* (Hübner) outbreak that was more severe than the first occurred. Most cotton fields were sprayed up to 12 times during the season. One treatment per week was frequently required to control *H. armigera*. As farmers could no longer support additional

insecticide costs, more toxic insecticides were searched for. Nevertheless, levels of pest control remained inadequate. As a consequence, peasants no longer trusted the insecticides offered by Sofitex, and many of them were buying their insecticides from independent retailers. This has not solved the problem since cotton crop losses have been higher. *Helicoverpa armigera* may have become resistant to most of the insecticides used against it in the country but this has not yet been documented.

Meanwhile, from 1996 on, the whitefly *Bemisia* spp. (Homoptera: Aleyrodidae) started to seriously damage vegetables (mainly tomato). Cotton is grown from May to October while tomato is grown from November to April. As cotton matures, tomato is sown. From 1995 to 1998, many growers in the cotton area abandoned tomato production because of *Bemisia* attacks. *Bemisia* was not noted as a problem on cotton until the 1998 season. Scouts for cotton plant protection sometimes mentioned the presence of occasional individual whiteflies by the end of the cotton season, but identification may have been inadequate. Several scouts later said they confused *Bemisia* nymphs with the aphid *Aphis gossypii* (Homoptera: Aphidae) that was noted earlier in the season. Cotton yield losses to *Bemisia* amounted up to 40% in many fields (Georges Yaméogo, personnal communication).

B. tabaci also transmits Tomato Yellow Leaf Curl Virus (TYLCV), Cucumber Vein Yellowing Virus (CVYV) and Lettuce infectious yellows virus in Burkina Faso (Konaté 1995). On cotton, a leaf-reddening symptom is seen. The symptom does not persist on young leaves that develop after whiteflies emigrate out of the field at the end of the cotton vegetative period (personal observation).

D. 2). Hypotheses.

When dealing with an upsurge of an insect from minor to major pest status in a multi-pest crop subjected to intense insecticide pressure such as cotton, the most immediate requirement is to make a diagnosis of the problem. Questions such as the following should be answered to propose trustworthy

solutions: 1) has the pest developed resistance to the pesticides used? 2) Are the insecticides used disrupting the pest natural enemy activities? 3) What is the potential of natural enemies against it? 4) Are there reliable alternative control means for *B. tabaci* or for the others pest? These were the questions I aimed to answer about the *B. tabaci* crisis in Burkina Faso in this dissertation. I provide an answer to all these questions throughout the different chapters of the thesis, all of them drawn from scientific papers published or submitted to journals.

E). Guidelines for this thesis format.

The present thesis format, accepted by the Faculty of Graduate Studies and Research, and the department of Natural Resource Sciences, Macdonald Campus of McGill University, requires the full citation of the following indented section (see Manuscript and Authorship: Guidelines Concerning Thesis Preparation):

"I Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.)

II The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceding and following each manuscript are mandatory.

III The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts.

The thesis must include the following:

a table of contents;

a brief abstract in both English and French;

an introduction which clearly states the rationale and objectives of the research;

a comprehensive review of the literature (in addition to that covered in the introduction to each paper);

a final conclusion and summary;

a thorough bibliography;

IV. As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

V. In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers.

VI When previously published copyright material is presented in a thesis, the candidate must include signed waivers from the publishers and submit these to the Graduate and Postdoctoral Studies Office with the final deposition, if not submitted previously. The candidate must also include signed waivers from any co-authors of unpublished manuscripts".

I have followed the above recommendations. Chapter 1 presents an up to date literature review relevant to the topic. In chapter 2, I: a) estimate *B. tabaci* population abundance; b) assess the levels of parasitism by *Encarsia* spp. and *Eretmocerus* spp. (Hymenoptera: Aphelinidae); c) estimate the susceptibilities of the pest and those of its parasitoids to the older insecticides that were sprayed on cotton. In chapter 3, I estimate the toxicities and dose-response relationships of *B. tabaci* to novel insecticides. In chapter 4, I record the relative abundance of *B. tabaci* (Gennadius) and its parasitoids, *Eretmocerus* spp. and *Encarsia* spp. in control and insecticides sprayed plots and, assess the impact of augmentative releases of the parasites on the population dynamics of the pest. In chapter 5, botanical insecticides have been investigated, because they typically have different modes of actions and hence might be sound alternative to the management of *B. tabaci* because they do not persist in the environment, have low mammalian toxicity, and resistance has not yet been reported against them. Finally all the references cited in the dissertation are listed by alphabetical order.

Contribution of authors.

I have planned and conducted the research, with technical assistance as noted in the Acknowledgements, and have written the manuscripts with the guidance of my supervisors who also reviewed and coauthored all the papers.

Chapter 1. Literature review.

1. 1. Systematics

1.1.1. Historical records and synonimization.

Bemisia tabaci was originally described as *Aleyrodes tabaci* Gennadius from whiteflies collected on tobacco in Greece (Gennadius, 1889). Eleven years after this description, another whitefly was collected on *Physalis alkekengi* L. in southeastern USA and described as *Aleyrodes inconspicua* Quintance (Quintance, 1900). This species was moved into a new genus, *Bemisia*, in 1914, giving raise to *Bemisia inconspicua* (Quintance and Baker, 1914). However Quintance and Backer (1914) were unable to place *A. tabaci* in the new genus "on account of inadequate description". Over the following 50 years (through 1964) 19 additional species of whiteflies (that later were synonimized with *B. tabaci*) were described from 14 other countries on a variety of host plants (Perring, 2001). Significant in this list of species was the placement of *tabaci* in the genus *Bemisia* by Takahshy (1936), resulting in *B. tabaci* Gennadius, a denomination that remains today.

Bemisia tabaci belongs to the sub-family Aleyrodinae, family Aleyrodidae, superfamily Aleyrodoidea, which is placed either in the sub-order Homoptera or in the order Hemiptera (Richards and Davies, 1977; Woodward *et al.* 1970). The species shares many characteristics with other homopterans i.e. all are plant phloem sap feeders with piercing, sucking mouthparts; they are opisthognathus; adults of both sexes have four membranous wings and undergo incomplete metamorphosis.

Whitefly systematists noticed the difficulty of making good adult slide mounting resulting in few distinguishing characteristics (Martins, 1987), so the fourth instar nymph became the preferred

stage for diagnosis (Gill, 1990). However the key nymphal morphological characters i.e. crenulations on the margin, the structure and size of the thoracic tracheal folds, the shape of the lingua and opercula in the vasiform orifice, various pores and porettes on the dorsum of the nymph, and the shape, size, and presence of dorsal setae overlap substantially (Mound, 1963; Gill, 1990).

Mound (1963) raised offspring of a single virgin female of *B. tabaci* on different species of plants, and observed that two of these forms, from tobacco and cassava, differ significantly with respect to setal appearance as well as size. Host-correlated variation in the morphology of pupal integument of *B. tabaci* was also observed by Azab *et al.* (1969), on cabbage, cotton, *Euphorbia pulcherrima* L., and *Lantana camara* L. Mohanty and Basu (1986) and Basu (1995) demonstrated the combined effect of host plant and seasonal factors on pupal morphology. This host-correlated variation provides more evidence for the synonymization of various species with *B. tabaci* (Mound and Hasley, 1978). Variations in host plant preferences, life cycles, and disease transmission capacities have also been reported (Coudriet *et al.* 1985, Bedford *et al.* 1994). Collectively these variations undermine accurate identification of *B. tabaci* (Perring *et al.* 2001).

To facilitate communication with peers, scientists have used host association (Wool, *et al.* 1994, Brown *et al.* 1995), and biotypes to name *B. tabaci* populations. The term biotype designates populations that lack morphological distinction, but possess other characteristics that serve to separate them from other populations (Claridge *et al.* 1997). To date, 41 distinct populations of *B. tabaci* have been studied, of which 24 have been given a specific biotype designation. The remaining seventeen populations could not be labelled. Perring (2001) suggested that the best information might be derived from grouping biotypes according to their geographical provenance.

A number of studies have questioned whether all populations of *B. tabaci* can be grouped within a single taxon. Wool *et al.* (1991, 1994) presented data that supported a heterogeneous set of

sub-units under the species name *B. tabaci* and conclude that *B. tabaci* is genetically not the same in different regions of the world. Gill (1992), reviewing the status of *Bemisia* whiteflies in the south western US, suggested that *B. tabaci* biotype B was a sibling species to *B. tabaci* biotype A. Incompatible mating resulting in restricted gene flow has been shown between biotypes A, B, AN, D, K, L, and M. (Brown *et al.*, 2000; De Barro and Hart, 2000). Reviewing the literature through the mid-1990s, Brown *et al.* (1995) suggested that *B. tabaci* is a suite of highly cryptic sibling species, which Lane (1977) defines as "populations that are reproductively isolated and have separate genetic flow without much associated morphological change". This species complex hypothesis has been supported by more recent molecular and allozyme data (Frohlich *et al.* 1999, Brown *et al.* 2000). Due to this confusion, it has been recommended that the binomial *B. tabaci* be used in the broad sense to include all members of the species complex unless a specific designation is more convenient (Gerling and Mayer, 1996, Naranjo and Allsworth, 2001). This is the position I adopted in my Ph.D. thesis. Voucher specimens of insects have been deposited at the laboratoire de recherches de la protection des végétaux, ÌNERA Bobo-Dioulasso, Burkina Faso, the Laboratory of Mollecular Biology of Dr. Judy K. Brown, University of Arizona, Tucson, the Laboratory of systematics of Dr. Gregory Zolnerowich, Kansas State University, USA, and the Liman Museum of Macdonald Campus of McGill University, Canada.

1. 2. Geographical distribution

1. 2.1. Origin and dispersion.

The evolutionary affiliation of *Bemisia* taxa within the family Aleyrodidae suggests that they may have originated in tropical Africa and were introduced quite recently into the Neotropics and southern North America (Campbell *et al.*, 1996). Some evidence also suggests that *B. tabaci* may be

native to India or Pakistan, where the greatest diversity of the species' parasitoids have been found, a criteria that has been considered a good indication of a genus epicenter (Brown *et al.*, 1995).

The first *B. tabaci* in the New World were collected in 1897 in the United States on sweetpotato (Quaintance, 1900). In 1928, it was found in Brazil on *Euphorbia hirtella* (Mound and Halsey, 1978). In 1933, the species was collected in Taiwan (Mound and Halsey, 1978). Further extension of its geographical range from subtropical and tropical agriculture systems has occurred to include temperate climate areas such as Japan, Canada and the Netherlands, where greenhouse crops are affected (Martin, 1999). The species is now globally distributed and found on all continents except Antarctica (Martin *et al.*, 2000).

1. 2. 2. Host range

Bemisia tabaci has been recorded from more than 600 different plant species (Mound and Halsey, 1978; Secker *et al.*, 1998) and its polyphagous nature has been documented worldwide (Costa *et al.*, 1991; Burban et al., 1992). A large number of cultivated and non-cultivated annual and perennial plant species are recognized as acceptable feeding and/or reproductive hosts (Butler and Henneberry, 1986; Bedford *et al.* 1992). Of the plant species listed by Mound and Halsey (1978), 50% belong to five families: Fabaceae, Asteraceae, Malvaceae, Solanaceae and Euphorbiaceae. Among the plant families, the number of host plants per family ranges from 99 species in the Fabaceae (Basu, 1995) to one species each in the Begoniaceae, Lythraceae and Zygophyllaceae (Mound and Halsey, 1978). The association of *B. tabaci* with its hosts is complex, as symbolized in Puerto Rico where the polyphagous *Sida* biotype and the monophagous *Jatropha* biotype occur in the same geographical area but occupy separate ecological niches (Brown *et al.*, 1995).

1. 3. Biology and morphology.

Bemisia tabaci undergoes six life stages: the egg, the crawler (1st nymphal instar), two sessile nymphal instars (2nd and 3rd instar nymphs), the "pupa" (4th instar) and the adult or imago. The egg is 0.2 mm long and is whitish-yellow in colour turning to light brown before hatching. It is usually elongate-oval but may occasionally be reniform. Its basis is broader and has a pedicel or stalk by which the female attaches the egg to the host. Eggs are usually laid on the lower surface of the leaves, sometimes in small circular or semicircular groups produced by the female rotating about her feeding stylets whilst laying eggs (Mound, 1983). The duration of the egg stage varies depending on temperature, and the host plant. On cotton it lasts 22.5 days at 16.7°C, 7.7 days at 25°C, 5.0 days at 32.5°C, but eggs failed to hatch at 36°C (Butler *et al.* 1983). At 25°C, RH 75% and 16:8h L/D, the egg stage lasts 16.14 days on bean plants, 6.37 days on tobacco, 6.9 days on lantana, 7.30 days on tomato and 7.7 days on cotton (Cock, 1986). Fertilized eggs give males, unfertilized give females.

The crawler (1st instar nymph) is 0.3 mm long, transparent to opaque, with color ranging from light green (due to transparency) to yellow, light brown through dark brown and black. It is mobile although in most species it settles within a few millimeters of the eggshell. Crawlers have four, possibly five leg segments and 2-3 antennal segments (Azab *et al.* 1970). Segmentation is unclear and most species may appear to have only three leg segments and two antennal segments. On each side of the abdominal cavity there is a yellow spot, the mycetome, which is quite apparent through the integument (El-Helaly, El-Shazli and El-Gayer (1971). Small amounts of powdery white wax usually are produced after the crawler settles and begins feeding.

The second and third nymphal instars are respectively 0.4 and 0.5 mm long, oval to elongated-oval. Some are circular or nearly heart-shaped. The body is greenish-yellow, the mycetome visible

through the integument is orange-yellow while the legs and the antennae are atrophied (Azab, *et al.* 1970).

The early fourth nymphal instar is 0.6mm long, greenish-yellow and elliptical in shape; the eyes are still small in size as in the previous instars. There is no moult between the early fourth instar and the late fourth instar, but some morphological characters are quite different. The late fourth instar nymph is 0.7mm long and 0.36mm wide at the broadest part, the mesothorax. The eyes look like two red spots constricted in the middle and are quite visible while the mycetomes are less apparent. The number and distribution of dorsal setae in the pupal stage is subject to the degree of pubescence of the host plant (Harakly, 1974). The "pupal" transformation to adult takes place without a "pupal" moult.

The newly emerged adult is soft, 0.8-0.9mm long, and whitish-yellow. After a few hours its colour changes to completely white due to the deposition of wax on the body and wings. The compound eyes are red and comprise one optical mass, which is crossed on the surface by a triangular strip-like cuticular projection, which is covered with white wax (Azab, *et al.* 1970).

The life cycle of *B. tabaci* varies considerably according to seasonal temperatures and host plants. It is shorter in summer (14-20 days), than in winter (74-75 days) in Egypt (Azab *et al.* 1971). At 26.7°C developments were completed in 30% less time on lettuce, cucumber, egg plant, and squash than on broccoli or carrot (Coudriet *et al.* 1985). *Bemisia tabaci* reproduce throughout the year. Adults and eggs are found during the winter on newly sprouted and germinated plants (Gerling, 1984). Overwintered whiteflies initiate infestation of large acreages of crops that are available during spring and early summer.

1. 4. Population ecology

The basis of any population ecology study is a sampling plan used to assess the population and its natural enemies. Sampling methods for whiteflies include sticky traps, suction samplers and, counts per plant, leaf or sub-leaf unit (Natwick, *et al.* 1994, 1995, 1996). At fixed-precision levels of sequential sampling recently developed, enumerative and binomial sampling plans correlate well at moderate to high densities (Naranjo *et al.* 1997). *B. tabaci* aggregates on and around a main stem leaf whose location varies according to plant growth and cotton variety. A method for absolute estimates of red eye nymphs (REN) was developed by von Arx *et al.* (1984). Action thresholds are estimated at between 5 ands 10 adult whiteflies per leaf (Ellsworth *et al.* 1994). Based on a threshold of 3 adults per leaf (i. e. a leaf is considered infested when it has 3 or more adults), a binomial sampling scheme ranks percentages of infestations and levels of populations in the field. For example, if 14% of leaves are infested, the population in the field is 1 adult per leaf. Between 28 to 49% infestation correspond to a range of population of 2 to 4 adults per leaf in the field, while 57% of leaves infested correspond to a population of 5 adults per leaf in the field. Sixty four to 82% of infestations indicate a range of 6 to10 adults per leaf in the field.

Sampling strategies for defined levels of accuracy have been developed for cotton (Naranjo and Flint, 1994; von Arx and Delucchi, 1984). For monitoring purposes to implement IPM decision rules, such strategies considerably reduce sampling costs, while for population studies they can be used to generate data of determined accuracy. Adults and eggs *B. tabaci* mostly occur on young leaves, and the sessile and early stages mature with leaves so that the pupae tend to be on older leaves. Thus to accurately estimate populations, adults and nymphs should be sampled separately.

1. 5. Economic impact

Reliable estimates of the economic impact of *B. tabaci* on worldwide agriculture have been difficult to obtain because of the extensive areas affected, the numbers of crops and ornamentals involved, and different monetary systems. Over the last three decades, *B. tabaci* has caused excessive annual crop losses. Direct feeding and honeydew excreta favor sooty mould production and affect crop yield in both quantitative and qualitative terms. Increased control costs and reduced product marketability and profitability are also important factors (Ellsworth, 1999).

B. tabaci was first reported to be a serious pest of cotton in the late 1920s and early 1930s in northern India (now part of Pakistan) (Misra and Lamba, 1929; Husain and Trehan, 1933). Subsequently, severe infestations on cotton were recorded in the Sudan and Iran (1950), El Salvador (1961), Mexico (1962), Brazil (1968), Turkey (1974), Israel (1976), Thailand (1978), Arizona and California, USA (1981), Ethiopia (1984) (Basu, 1995) and Burkina Faso (Otoidobiga *et al.* 2003). Historically, heavy infestations in cotton fields often resulted in significant dispersal to other field crops and vegetables following termination of the cotton crop. Insecticide use in many cases resulted in the development of resistance and a general failure of control efforts.

In Africa the epizootic outbreaks of the pest populations are best documented in cotton in the Sudan Gezira agricultural complex. This complex of approximately one million ha is situated in the fork of the Blue and White Nile. The British have irrigated it during colonisation. More than 100 000 tenant farmers exploit it but the Sudan Gezira Board centrally controls pests by aerial sprays. *Bemisia tabaci* population outbreaks started in the seventies following decades of intensive usage of DDT in cotton primarily against *H. armigera*. Eveleens (1983) considered suppression of aphelenid parasitoids of *B. tabaci* by broad-spectrum insecticides as the major cause of the *B. tabaci* outbreaks. Dittrich *et al.* (1985) considered that intensive use of insecticides resulted in the development of resistance in *B.*

tabaci and general failure of control efforts against this pest. Dittrich *et al.* (1990) found that the pest population outbreaks were compounded by the ability of insecticide-resistant *B. tabaci* individuals to increase their oviposition rates when under insecticidal stress (hormoligosis). Castle (1999) reappraised the Sudan *B. tabaci* situation and offered a more complex explanation for the outbreaks. His counter argument to the hypothesis that *B. tabaci* outbreaks were solely induced by pesticides involved the influence of agricultural intensification of cotton acreage, increased fertilizer use and other production technology, later planting dates, and managerial overuse of insecticides. I concur with Castle (1999) it is likely that all these factors affect *B. tabaci* population outbreaks. *Bemisia tabaci* also vectors African cassava mosaic virus (Swanson and Harrison, 1994), and Tomato Yellow Mottle Virus (Konaté *et al.*1995) that deprive most African farmers of their most important food and commercial crops.

In the USA, *B. tabaci* had been a long time resident in various agricultural regions throughout the southern and western states but rarely was an economic pest until 1981 when serious outbreaks occurred in California and Arizona. In 1986 in Florida, poinsettias were heavily infested with whiteflies, and silverleaf symptoms were reported in squash (Price *et al.*, 1986; Maynard and Cantliffe, 1989). California's Imperial Valley felt the full destructive potential of the insect in 1991 (Toscano *et al.*, 1998). The losses caused in Arizona, California, Texas, and Florida, in 1991 and 1992 were estimated at about 200 and 500 million US $, respectively. Between 1994 and 1998, Arizona, California and Texas cotton growers spent 153.9 million US$ to control *B. tabaci* and prevent cotton lint stickiness (Ellsworth *et al.*, 1999). In a socio-economic impact study in California, Gonzalez *et al.* (1992) concluded that for every 1 million US$ of primary-induced crop loss, there was an estimated 1.2 million US$ in loss of personal income as well as the elimination of 42 jobs.

The years 1991 and 1992 were critical to Mexico, when *B. tabaci* caused losses exceeding 33 million US$ by damaging fall melon and watermelon, sesame and cotton crops in the Mexicali Valley. Also in Mexicali Valley, cotton production was reduced from 39,415 ha in 1991 to only 653 ha in 1992 (Medina Esparza and Leon Paul, 1994). The area planted with cotton between 1995 and 1996 in Sonora, Mexico was reduced by 65% as a result of *B. tabaci* infestations (Silva Sanchez, 1997).

Soybean production in Sonora, Mexico has also been greatly reduced by *B. tabaci* infestations. Areas planted ranged from 89,000 to 124,000 ha from 1992 to 1994 (Olivera *et al.* 2001) and profitability was marginal. Increasing *B. tabaci* infestations began to occur in 1991. Chemical control costs were about $120/ha and not considered particularly effective. Yields declined and profitability was reduced further (American Soybean Association, 2000). Since 1998 areas planted have been reduced to about 500 ha annually. (American Soybean Association, 2000).

In Central America and the Caribbean, extensive losses on tomato, okra, cotton, tobacco and melon have occurred in Cuba, Barbados, Costa Rica, Dominican Republic, El Salvador, Haiti, Honduras, Guatemala, Jamaica, Monserrat, Nicaragua and Santa Lucia (Hilje, 1996, Vazquez, 1999). In Guatemala, costs of whitefly control increased from 30 to 50% on melon, tomato and pepper. In 1998 and 1999, melon losses exceeded 40% as a result of sooty mold and geminiviruses (Dávila, 1999).

Since 1995, Brazil has been seriously affected by *B. tabaci*. Accumulated losses have exceeded 5 billion US$ (Lima *et al.*, 2000). The main crops attacked are beans, tomatoes, cotton, melons, watermelons, okra, and cabbage, but numerous other crops are also infested. *Bemisia tabaci* has spread from the Southeast to almost all areas of the country (Lima *et al.*, 2000). Other South American countries such as Argentina (Viscarret *et al.*, 2000), Colombia (Quintero *et al.*, 1998), and Bolivia (Morales and Anderson, 2001) have reported increasing problems with *B. tabaci*.

Most countries surrounding the Mediterranean Basin encountered severe *B. tabaci* infestations beginning in 1974. In Italy and southern France, severe damage occurred on poinsettias and tomatoes. *Bemisia tabaci* was recorded from Azerbaijan and Georgia in the former Soviet Union, and has been observed on citrus growing in glasshouses along the southern coast of the Crimea and the Black Sea Coast of the Caucasus (Traboulsi, 1994).

In the Near East, *B. tabaci* has been reported as a major pest in Algeria, Bahrain, Cyprus, Egypt, the Islamic Republic of Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Malta, Morocco, Saudi Arabia, Somalia, Tunisia, Turkey, the United Arab Emirates and Yemen. It attacks vegetables and ornamentals outdoors and under protected cultivation (Traboulsi, 1994). It also occurred on citrus and cotton in Pakistan and Israel and on olives and pears in Morocco (Traboulsi, 1994). Watermelon crops in Yemen have been decimated since 1989 (Bedford *et al.*, 1994b).

In China severe local outbreaks of *B. tabaci* were recorded in 1953 in Taiwan and in 1972 in Yunnan, and at present, *B. tabaci* has spread from the south to northern parts of the country, expanding from six to 12 provinces between 1953 and 1995 (Rumei, 1996).

In 1994 *B. tabaci* was reported in cotton in Australia. It was first described in the country in 1959 but it had not been an economic problem (Gunning *et al.* 1995; 1996; Fransmann *et al.*, 1998). In the mid-1990s, in the Pacific Region, *B. tabaci* was found on 18 islands including Cook Island, Guam, New Caledonia, American Samoa, Fiji, Marshall Islands, Niue, Federated States of Micronesia, Kiribati, Papua New Guinea, and Solomon Islands (De Barro *et al.*, 1998).

The magnitude of *B. tabaci* infestations and the nature and extent of injury vary with plant species, season and localities. Some plant species that are severely infested in one area may be relatively free of infestation in other areas. For example, *B. tabaci* is a serious threat to cotton in Sudan

but an insignificant problem on cotton in Egypt. Cassava is affected in Africa, but not in South America (Costa and Russell, 1975).

1. 5. 1. *Bemisia tabaci*-transmitted viruses

Bemisia tabaci transmits seven distinct plant virus groups including: geminiviruses, closteroviruses, carlaviruses, potyviruses, nepoviruses, luteoviruses and a DNA-containing rod-shaped virus (Duffus,1996). The most economically significant of these are the geminiviruses (Family *Geminiviridae*: Genus *Begomovirus*) and the closteroviruses (Family *Closteroviridae*: Genus *Crinivirus*). One or more whitefly transmitted viral diseases have affected cassava, beans, cowpea, soybean, cotton, tobacco, tomatoes, peppers, okra, squash, melon, watermelon, lettuce and papaya. However, geminiviruses infecting tomatoes, beans, and cassava have been cited as the most widespread and economically important.

In almost all countries where tomatoes are grown commercially, they are infected with geminiviruses. In the Middle East, Tomato yellow leaf curl virus (TYLCV) outbreaks were sporadic in the 1960s (Cohen and Harpaz, 1964), but have become a serious economic problem since the early 1970s, when total crop failures occurred (Czosnek and Laterrot, 1997). The virus affected all tomato-producing regions in the Middle East by the end of the 1970s (Abak *et al.*, 1991; Czosnek and Laterrot, 1997). In southern Europe, extensive tomato losses have been caused by TYLCV (Benuzzi *et al.*, 1990; Traboulsi, 1994). The virus is now distributed in Asia, Africa, Europe, the Caribbean, Mexico and North America.

A review by Polston and Anderson (1997) on the tomato-infecting geminiviruses in Latin America indicated that the number of new geminiviruses infecting tomatoes increased from three in the

1970s to 17 in the 1990s. Some of these geminiviruses also infect peppers and other horticultural crops of economic importance (Morales and Anderson, 2001).

According to Polston and Anderson (1997), no formal crop loss assessments have been made for tomato diseases caused by geminiviruses, but empirical data suggest the potential for extensive reductions in crop production. In the Dominican Republic, crop damage reported from 1988 to 1995 ranged from 5 to 95% with economic losses of 10 million US$ in 1988, and 50 million US$ between 1989 and 1995. In Florida, USA, disease caused by Tomato mottle virus was found in all tomato production areas reducing the value of the 1990–1991 production by 20% or 140 million US$. Puerto Rico suffered an estimated 40 million US$ loss, and in Honduras, tomato production in the Comayagua Valley losses were estimated at 4.6 million US$ in 1992. In Brazil, economic loss estimates have not been made, but in the last 4 years, more than 11,000 jobs have been lost in the tomato industry because of whitefly-transmitted geminiviruses (Olivera *et al.* 2001).

Numbers of whitefly transmitted geminiviruses, numbers of crops affected, yield losses, and agricultural areas devastated have been exceptionally high in Latin America (Morales and Anderson, 2001). Common bean is grown on more than 9 million ha with Brazil producing over 50% of the total crop harvested (Morales and Anderson, 2001). Bean golden mosaic virus (BGMV) and Bean golden yellow mosaic virus (BGYMV) are considered the most limiting pathogens to common bean production in the Americas. Over one million hectares traditionally planted to beans have been abandoned because of bean golden mosaic disease (Morales and Anderson, 2001).

The most serious whitefly virus-vectored problem in Africa is cassava mosaic disease (CMD) caused by a complex of cassava mosaic geminiviruses (Harrison *et al.*, 1997; Zhou *et al.*, 1997). Cassava is the most important food crop grown on the African continent, and yield losses due to CMD

are excessive. Yield losses range from insignificant to 95% depending upon the cultivars used, environmental conditions, and geminiviruses involved (Legg, 1999).

Although Cotton leaf curl virus (CLCV) has been known for many years in Pakistan (Mahmood, 1999), rapid spread of the disease started in 1988 (24 ha) and increased to about 121,458 ha in 1992 reducing cotton production by 30–40% in 1993 and 1994. Estimates of losses from 1994 to 1999 were about 7.4 million cotton bales valued at 4.98 billion US$ (Mansoor *et al.*, 1999). In India CLCV was first detected from Sri Gangenagar in 1993 and Punjab in 1994 (Singh *et al.*, 1999). At present it is widespread over the entire northern cotton-producing zone of India. In Punjab (India), cotton production in 1998 decreased 75% compared to 1990 (Singh *et al.*, 1999). Cotton leaf curl is suggested as a major factor in the decline.

Mung bean yellow mosaic virus (MYMV) is a major constraint to the cultivation of grain legumes in India, particularly mung bean (*Phaseolus aureus*) and black gram (*P. mungo*). According to Varma *et al.* (1992), in epidemic years, losses due to MYMV have exceeded 300 million US$ in three major crops: black gram, mung bean and soybean (Basu, 1995). Okra yellow vein mosaic virus in India, Sri Lanka and Bangladesh occurs in cultivated vegetable crops, with losses reported to exceed 80% if the plants are infected within 4–5 weeks after germination (Basu, 1995).

The whitefly-transmitted closteroviruses (Family *Closteroviridae*: Genus *Crinivirus*) have recently been recognized as causing extensive losses in North America, the Mediterranean Basin and the Middle East (Wisler *et al.*, 1998). The *B. tabaci*-transmitted lettuce infectious yellows virus (LIYV) caused losses estimated at 20 million US$ in lettuce, sugar beets and melons during the 1981 growing season (Duffus *et al.*, 1996; Wisler *et al.*, 1998). However, in the late 1980s and early 1990s with increasing populations of *B. tabaci* biotype B, the occurrence of LIYV began to decline. At present LIYV infections of lettuce and melons in Southern California and Arizona are rare. This has

resulted from the fact that the B biotype transmit 100 fold less efficiently LIYV than the A biotype it has replaced (Duffus *et al.*, 1996). Other *B. tabaci*-transmitted criniviruses include Cucurbit Yellow Stunting Disorder Virus (CYSDV), Lettuce Chlorosis Virus (LCV), Tomato Chlorosis Virus (ToCV), Sweetpotato Chlorotic Stunt Virus (SPCSV), and a group of related closterovirus isolates infecting sweetpotato (Winter et al., 1992; Duffus *et al.*, 1996; Livierateos *et al.*, 1999).

1. 5. 2. *Bemisia tabaci*-induced plant physiological disorders and other aspects of plant damage

Since 1987, increases in field populations of *B. tabaci* in Florida, Arizona, California, Puerto Rico, Texas, and Hawaii have been associated with plant disorders of unknown etiology (Costa *et al.*, 1993). Squash silverleaf (SSL) (Yokomi *et al.*, 1990; Demichelis *et al.*, 2000), uneven ripening of tomato (Maynard and Cantliffe, 1989), pumpkin white stem (Costa and Brown, 1991), white streaking in cole crops (Brown *et al.*, 1992), and reduced growth, yellowing and stem blanching in lettuce (Costa *et al.*, 1993; Bedford *et al.*, 1994b) have been attributed to biotype B. In Brazil, high-level infestations in okra plantings resulted in inedible woody fruit and blanching of *Solanum gilo* fruit (M.R.V. Oliveira, pers comm.)

B. tabaci nymphs and adults feed in phloem and obtain sap containing various sugars (Hendrix *et al.*, 1992). Their excretions, called honeydew, may contain these and other metabolized sugars. Honeydew accumulates on the upper surfaces of plant parts where it serves as a substrate for sooty molds. Quality may be reduced or the product rendered unmarketable. Honeydew contamination of cotton lint makes the cotton sticky and also is a substrate for sooty molds that discolor the lint. Sticky cotton adheres to machinery in textile mills and interferes with processing. Sticky cotton reduces harvesting and ginning efficiency (Johnson *et al.*, 1982; Hector and Hodkinson, 1989). Sticky cotton

may also contain leaf trash and dirt causing health problems for textile mill workers (Ayars *et al.*, 1986). Sticky cotton is a serious problem in many cotton production areas in the world (Strolz, 1992). Losses of 10% of the lint value may occur (Hector and Hodkinson, 1989). On vegetables, melons, and ornamentals, honeydew and sooty mold reduce quality and marketability (Riley and Palumbo, 1995).

1. 6. Control of *B. tabaci.*

1. 6.1. Insecticidal control

In many cropping systems the use of insecticides is the primary strategy used to control *Bemisia tabaci.* Conventional insecticidal control of *B. tabaci* in field and vegetable crops consists predominately of foliar-applied sprays of active ingredients that are dependent on spray coverage and deposition (Sharaf, 1986). In many cropping systems, repeated spray applications have been necessary and often resulted in overuse of these chemicals. Consequently, *B. tabaci* has developed resistance to numerous conventional insecticides throughout the world (Denholm *et al.*, 1996, 1998). Several new classes of insecticide chemistry have been developed recently that effectively control *B. tabaci*, and perhaps more importantly, provide producers with a diversity of chemicals with which to battle resistance (Horowitz and Ishaaya, 1996). Consequently, the implementation of integrated pest management (IPM) programs that promote judicious insecticide use in association with other management practices has allowed economic management of *B. tabaci.* I will primarily address the development and implementation of insecticide use patterns for conventional and novel insecticides and secondly, report on the status of insecticide resistance, and recent developments in insecticide resistance management (IRM) programs.

1. 6. 1. 1. Conventional insecticides

Single neurotoxic conventional insecticides have been less effective at controlling *B. tabaci* than mixtures (Horowitz and Ishaaya, 1996, Otoidobiga *et al.* 2003). Of the mixtures tested and used for *B. tabaci* control, synergized pyrethroids have been the most effictive (Horowitz and Ishaaya, 1996; Prabhaker *et al.*, 1998). These spray mixtures involve combining high rates of pyrethroid insecticides with moderate rates of compounds from a different chemical class such as organophosphates, carbamates, formamidines and cyclodienes. The increased efficacy of these mixtures can, to a lesser extent, be attributed to the additive toxic effects of both compounds, but in most cases, greater toxicity results from the inhibition of insecticide resistance mechanisms (Dittrich *et al.* 1990; Denholm *et al.* 1998). Since resistance mechanisms in *B. tabaci* have been shown to involve increased esterasic activity and insensitive acetylcholinesterase (AchE) towards inhibitors (Ishaaya *et al.*, 1987; Byrne and Devonshire, 1993), pyrethroid toxicity to *B. tabaci* populations can be restored when combined with an esterase inhibitor, such as methamidophos, acephate or endosulfan (Ishaaya *et al.*, 1987).

Synergized pyrethroid sprays are primarily effective against adult whiteflies through contact action (Horowitz and Ishaaya, 1996). Although nymphs are susceptible to these active ingredients (Prabhaker *et al.* 1989), control of immature populations on plants with conventional treatments is inherently difficult to achieve because the sessile nymphs reside on the abaxial surface of leaves and are difficult to contact with sprays (Palumbo and Coates, 1996). Thus, in practice, pyrethroid mixtures control *B. tabaci* populations by reducing oviposition via adult mortality, and subsequently, the establishment of immature populations on leaves (Fig. 1). Since synergized pyrethroids are efficacious primarily through contact activity, proper spray coverage and deposition on leaf surfaces is important. Unfortunately, there have been very few advances in application technology that have improved the efficacy of foliar spray treatments.

The lack of significant improvements in foliar application technology places even greater emphasis on the importance of timing. Past experiences in whitefly control suggested that economic management of *B. tabaci* populations with conventional foliar insecticides can only be sustained when accurate sampling techniques and economic thresholds are available (Sharaf, 1986; Horowitz and Ishaaya, 1996). Furthermore, socio-economic demands have been placed on producers to reduce the total number of insecticide sprays applied to crops. The recent development of novel insecticide chemistries with improved routes and modes of action have made the diversification of insecticide exposure possible and reduced selection pressure for resistance.

1. 6. 1. 2. Novel insecticide chemistries

1. 6. 1. 2. 1. Nicotinoids

The nicotinoids are a new class of neurotoxins with a mode of action unique from any insecticide currently available for use in field, vegetable or protected cropping systems (Ware, 2000). Compounds within the nicotinoid class are also referred to as nitroquanidines, nitromethylenes, chloronicotinyls, or neonicotinoids (Yamamoto *et al.* 1995). They were modeled after naturally occurring nicotine compounds and similarly act on the central nervous system of insects, causing irreversible blockage of postsynaptic nicotinergic acetylcholine receptors (Bai *et al.* 1991; Liu and Casida, 1993). In general, nicotinoid insecticides possess low mammalian toxicity relative to other neurotoxins, are relatively non-toxic to non-target species, and are highly efficacious against a broad range of insect pests (Wollweber and Tietjen, 1999).

These compounds have excellent systemic properties and long residual activity that make them particularly effective against sucking insects such as *B. tabaci* (Kagabu, 1999; Yamada *et al.* 1999; Maienfisch *et al.* 2001). These properties also allow them to be applied in diverse ways such as foliar

sprays, soil drenches, in-furrow and subsurface granule and liquid applications, seed treatments, and with paint-on plant containers. Overall, the novel biochemical attributes, biological activity, and versatility in application of the nicotinoids make this class of chemistry particularly effective against *B. tabaci*.

1. 6. 1. 2. 2. Insect growth regulators (IGRs)

The need for a greater diversity of insecticides for *B. tabaci* control was met by the development of the non-neurotoxic IGRs (Horowitz and Ishaaya, 1996). These potent control agents include buprofezin and pyriproxyfen.

Buprofezin was the first selective IGR introduced for control of *B. tabaci* in cotton (Horowitz and Ishaaya, 1992). It is a thiadiazine chitin synthesis inhibitor which has selective activity on some homopteran pests by inhibiting the incorporation of N-acetyl-[D-H^3] glucosamine into chitin and interfering with cuticle formation (Kanno *et al.* 1981). This compound acts specifically on immature developmental stages resulting in nymphal mortality during ecdysis (Yasui *et al.* 1987; Ishaaya *et al.*, 1988). Buprofezin has no direct effect on *B. tabaci* adult longevity or oviposition, but reduces egg hatch and the fecundity of female whiteflies exposed to treated leaves (Ishaaya *et al.* 1988). Buprofezin also causes mortality to *B. tabaci* nymphs through its vapor phase activity (vapor pressure, 9.4×10^{-6} mm Hg), where it acts through inhalation by nymphs, and through direct contact adsorption by the integument (De Cock *et al.*, 1990).

Pyriproxyfen is a non-terpenoidal juvenile hormone analog (Horowitz *et al.* 1999b). It is a very potent growth regulator with persistent activity against several insects, including *B. tabaci* (Schaefer *et al.* 1988; Ishaaya and Horowitz, 1992). The compound exhibits juvenoidal activity by disrupting normal hormonal balance (Dhadialla *et al.*, 1998) resulting in the suppression of embryogenesis,

metamorphosis, and adult formation (Ascher and Eliyahu, 1988). Pyriproxyfen does not possess any direct toxic activity against whitefly adults; however, the compound has strong translaminar activity within foliage that enhances the suppression of embryogenesis by direct (ovicidal) contact to eggs deposited on lower leaf surfaces, or indirectly (transovarial) through adult contact on treated foliage (Ishaaya and Horowitz, 1995).

The vapor phase and translaminar activities of buprofezin and pyriproxyfen, respectively, have important practical implications for field application. Since these routes of activity do not rely heavily upon contact action, spray deposition on and under leaf surfaces is not as critical as with conventional sprays. Topical coverage of foliage is generally adequate for achieving field mortality with IGRs. Thus, both buprofezin and pyriproxyfen are applied to crops as foliar sprays and have been shown to be highly efficacious against *B. tabaci* (Ellsworth and Naranjo, 1999).

Since buprofezin and pyriproxyfen are not toxic to adults and stage-specific mortality involves complex physiological developmental processes, *B. tabaci* population regulation with these compounds is very different from neurotoxic compounds such as imidacloprid or synergized pyrethroids (Horowitz and Ishaaya, 1996; Naranjo and Ellsworth, 1999). Buprofezin, for example, is most toxic to whitefly crawlers and 2nd instar nymphs, and population regulation occurs primarily by rapidly preventing the developmental transition of individuals from the 2nd to 4th nymphal instars (Beevi and Balasubramanian, 1991) (Fig. 1).

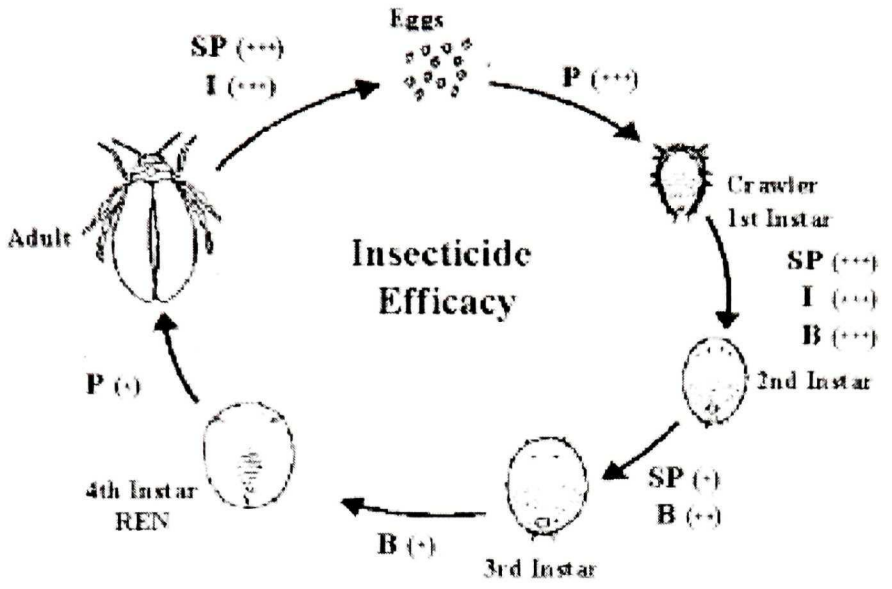

Fig. 1. Efficacy of synergized pyrethroids (SP), imidacloprid (I), buprofezin (B), and pyriproxyfen (P) against *B. tabaci*. Assuming the insecticide is applied properly, position of asterisks indicates the point during the *B. tabaci* life cycle that each compound is providing control. (***), primary lifestage controlled; (**) moderate control at this lifestage; (*), limited control at this life stage; REN = red eye nymphs (From Palumbo *et al.* 2001a).

Conversely, pyriproxyfen can affect all preimaginal life stages; by direct ovicidal activity, by indirect transovarial suppression of egg hatch, and to a lesser degree, by inhibition of the developmental transition from pupa to adult (Ishaaya and Horowitz, 1992) (Fig.1). Consequently, reductions in adult abundance and immature colonization following application in the field may not be readily apparent for 7–14 days. Therefore, the use of these compounds by growers and IPM practitioners requires a fundamental understanding of *B. tabaci* biology and ecology, as well as the adoption of different sampling procedures and thresholds for timing applications and targeted at appropriate life stages.

Another IGR, novaluron (Rimon®; Makhteshim-Agan, Tel Aviv) a novel benzoylphenyl urea chitin synthesis inhibitor is in the advanced stages of development and has been found to have activity against *B. tabaci* (Ishaaya *et al.* 1996). The compound works by contact with *B. tabaci* eggs and nymphs, and appears to have no significant effects on various parasitoids or phytoseiids, and a mild effect on other natural enemies (Ishaaya *et al.* 1997). Its efficacy against *B. tabaci* under field conditions has been shown to resemble or exceeds other IGRs (Ishaaya *et al.* 2001).

1. 6. 1. 2. 3. Other novel chemicals

Other novel compounds have recently been introduced or have potential uses for some cropping systems. Most notably, diafenthiuron, a thiourea derivative has both insecticidal and acaricidal activity against a narrow range of homopterans and phytophagous mites (Steinemann *et al.*, 1990; Horowitz and Ishaaya, 1996). Under field conditions, diafenthiuron is phytochemically converted to a carbodiimide derivative in the presence of sunlight, resulting in insecticidal activity greater than the parent compound (Steinemann *et al.*, 1990). It directly effects insect respiration through the inhibition of oxidative phosphorylation and disruption of mitochondrial ATP synthesis (Ruder and Kayser, 1993). The compound has very low mammalian toxicity with little toxicity to natural enemies and pollinators (De Clercq et al., 1995; DeCock *et al.* 1996). Diafenthiuron has been shown to suppress the formation of progeny, when adult females are exposed to treated plants (Ishaaya *et al.*, 1993), and is more potent against nymphs than pupae or eggs.

Another new compound is pymetrozine. It represents a new and novel class of chemistry, the pyridine–azomethines, which are selectively active against sucking insects within the Homoptera (Nicholson *et al.* 1996). This chemistry exhibits a unique mode of action that can be characterized as neural inhibition of feeding behavior (Fluckiger *et al.* 1992; Kayser *et al.*, 1994) that selectively

interferes with normal feeding activities by affecting the neural regulation of fluid intake. It has been postulated that pymetrozine affects the activity of the cibarial and salivary pumps (Harrewijn and Kayser, 1997). Pymetrozine is used as a foliar spray and has translaminar activity. Pymetrozine is active against a number of whitefly species, including *B. tabaci,* and is active primarily against adults (Koenig *et al.*, 1998; Ishaaya and Horowitz, 1998). However this compound has not performed as well against *B. tabaci* populations in cotton because of its marginal nymphal activity (Ishaaya and Horowitz, 1998; Koenig *et al.*, 1998). Nevertheless, control of adult and immature stages was enhanced in cotton when pymetrozine was combined with pyriproxyfen (Koenig et al., 1998).

Collectively, nicotinids, IGRs, thiourea derivatives, and the pyridine–azomethines represent the most recent classes of chemistry available or in advanced stages of development for managing *B. tabaci* in agricultural production systems. This diversity of effective modes of action potentially provides producers with options to concurrently limit chemical use and sustain long-term field efficacy. Although, the susceptibility of insect populations to new modes of action can be influenced by past exposures to insecticides (Georghiou and Taylor, 1986), these newer chemistries appear to be less affected by existing resistance mechanisms (Horowitz *et al.* 1999a; Denholm *et al.*, 1998; Cahill and Denholm, 1999). However, if not used judiciously in carefully devised IPM programs, the risk of resistance to all these compounds can be expected to increase.

1. 6. 2. Status of insecticidal resistance in *Bemisia tabaci*

1. 6. 2. 1. Synergized pyrethroid combinations

Bemisia tabaci has developed resistance to chloronatids insecticides, organophosphates, carbamates, pyrethroids, IGRs, and nicotinids insecticides used against it throughout the world (Horowitz and Ishaaya, 1996; Denholm *et al.*, 1996). Efforts were undertaken to understand resistance

mechanisms, determine cross-resistance and develop resistance management strategies for synergized pyrethroids (Prabhaker *et al.*, 1998; Sivasupramaniam and Watson, 2000).

In 1994 evidence of reduced susceptibility to synergized pyrethroids in *B. tabaci* populations collected from cotton fields in central Arizona was documented (Dennehy *et al.*, 1995). Monitoring of field-collected *B. tabaci* populations during 1995 confirmed significant reductions in susceptibility to these combinations in major cotton growing regions (Dennehy *et al.*, 1997, Dennehy and Williams, 1997). By the end of the 1995-growing season producers in some growing areas experienced unacceptable yield losses and sticky lint contamination following repeated use of synergized pyrethroids. In the Imperial Valley of California, a significant shift in reduced susceptibility to fenpropathrin mixed with acephate was detected in laboratory bioassays in 1997, and again in 1999 (Castle *et al.*, 2001).

1. 6. 2. 2. Nicotinoids

Evidence of the high propensity for nicotinoid resistance was recently demonstrated by the rapid rate at which resistance to imidacloprid was selected in a *B. tabaci* population placed under continuous laboratory exposure (9-fold resistance in the F_5 generation; >80-fold resistance after 24 generations) (Prabhaker *et al.*, 1997). Spatial and temporal monitoring of *B. tabaci* populations within cropping systems has allowed researchers to detect changes in susceptibility to imidacloprid and other nicotinoids. Cahill *et al.* (1996b) were the first to report reduced susceptibility to imidacloprid in *B. tabaci* populations collected from the Almeria region of southern Spain. Elbert and Nauen (2000) subsequently confirmed this reduced susceptibility. Comparisons among nicotinoids also confirmed the presence of a strong cross-resistance between imidacloprid, thiamethoxam and acetamiprid (Li *et al.*, 2001). Poor control of *B. tabaci* populations following foliar imidacloprid and thiamethoxam

applications in one of four greenhouse pepper trials in 1998 further corroborated the laboratory bioassays (Elbert and Nauen, 2000). However the compound still provides adequate control in most areas within the regional cropping system, but the need for strict adherence to recommended resistance management strategies is strongly emphasized (Elbert *et al.*, 1996).

Monitoring studies in other parts of the world have shown that *B. tabaci* have become only slightly less susceptible to imidacloprid and other nicotinoids. In Israel, three years of acetamiprid use in greenhouses resulted in a 5 to10-fold decrease in susceptibility of *B. tabaci* to the compound (Horowitz *et al.*, 1999a). In Arizona, where imidacloprid has been used since 1993, monitoring of *B. tabaci* populations showed declines in *B. tabaci* susceptibility to imidacloprid from 1995 to 1998 (Dennehy *et al.*, 1999). Subsequent monitoring showed that these populations had actually regained and sustained susceptibility to imidacloprid in 1999 and 2000, at levels similar to those reported in 1997 (Williams *et al.*, 1998; Li *et al.*, 2001) suggesting that IRM program in force was effective. While neither thiamethoxam nor acetamiprid was registered for use in the United States, susceptibility of *B. tabaci* populations to these compounds followed the same general trends as imidacloprid from 1997 to 2000.

1. 6. 2. 3. Insect growth regulators

Changes in susceptibility to buprofezin and pyriproxyfen have been well documented and, as for imidacloprid, were first detected in intensive, enclosed-production systems. Reduced susceptibility to buprofezin was first reported in *B. tabaci* populations collected from glasshouses in the Netherlands, and in greenhouses in Spain and Israel (Cahill *et al.*, 1996c; Horowitz and Ishaaya, 1994), where changes in susceptibility were associated with repeated applications of the compound. Recent

bioassays of *B. tabaci* collected from greenhouses in Almeria, Spain, showed that resistance to buprofezin has increased since 1994 (Elbert and Nauen, 2000).

Resistance to buprofezin under field conditions, where use is restricted to a single treatment per season (i.e., Israel and United States), has developed at a much slower rate. In Israel no appreciable change in susceptibility to buprofezin was found during the first two years after its use on cotton in 1989, but susceptibilities decreased slightly after three years of use (Horowitz and Ishaaya, 1996). Most recently, significant decreases in susceptibility to buprofezin were detected in *B. tabaci* populations collected from cotton fields in the Ayalon Valley of Israel from 1992 to 1995 (Horowitz *et al.*, 1999a).

In Arizona susceptibility to buprofezin in populations of field-collected and reared immatures during the 1998 growing season showed that *B. tabaci* susceptibility had increased when compared with populations assayed in 1993 and 1996, prior to any buprofezin use in the region (Yasui *et al.*, 1997, Ellsworth *et al.*, 1999b). In 1999 susceptibility to buprofezin decreased significantly, but returned to lower levels in 2000, where a 10-fold reduction was reported in several populations (Li *et al.*, 2001). The differences in susceptibility reported in these studies may be attributed to differences in the bioassay procedures employed, variability in the geographical populations assayed or the lack of significantly compromised susceptible populations in the region.

Pyriproxyfen was first used in Israel in 1991, and within one year, a high level of resistance (>500-fold the initial level) was detected in *B. tabaci* collected from a rose greenhouse, where populations had been exposed to three consecutive applications (Horowitz and Ishaaya, 1994). In contrast, pyriproxyfen was restricted to a single use per season in cotton (Horowitz *et al.*, 1999b), and changes in susceptibility were initially much lower. During the first few years of use, seasonal trends in susceptibility to pyriproxyfen in field populations revealed only slight increases in tolerance during

the cotton season but, due to its restricted use, *B. tabaci* susceptibility to pyriproxyfen was restored by the following season (Horowitz and Ishaaya, 1994). However by 1997, despite the limited use of pyriproxyfen, susceptibility of *B. tabaci* in the Ayalon Valley of Israel decreased dramatically failing to recover between seasons (Horowitz *et al.*, 1999b). An increase in susceptibility was observed in 1998 following the use of acetamiprid and diafenthiuron instead of pyriproxyfen in most cotton fields. It is not clear as to why *B. tabaci* developed resistance so rapidly in the Ayalon Valley after only five years of use. These changes may have been encouraged by the geographic isolation of the area that mimics an enclosed, greenhouse environment where there are fewer susceptible individuals in unsprayed refugia to dilute resistance gene pools in the cotton zones, and the almost universal adoption of pyriproxyfen use by cotton producers during a five-year period. In addition, the exposure of *B. tabaci* populations to adverse genetic, ecological, and agronomic factors likely contributed to losses in susceptibility (Horowitz *et al.*, 1999b).

Pyriproxyfen was first used as a rotational alternative with buprofezin in cotton resistance management programs in Arizona beginning in 1996 and in California in 1997. Initial monitoring of *B. tabaci* collected from cotton in Arizona from 1996 to 1998 showed no reductions in susceptibility to pyriproxyfen (Dennehy *et al.*, 1999). However, a significant decrease in susceptibility was observed in populations collected from some Arizona cotton growing regions in 1999 and 2000 (Li *et al.*, 2001). Similar to buprofezin, this trend in reduced susceptibility in *B. tabaci* populations has occurred despite a significant decline in pyriproxyfen use in Arizona cotton fields during this period (Ellsworth and Jones, 2001). No cross-resistance has been reported between Buprofezin and pyriproxyfen.

1. 6. 3. Insecticide Resistance Management (IRM)

Insecticide resistance management is a key component of IPM programs. IRM for *B. tabaci* has been best documented in Israeli and Arizona cotton. Thus I will use these two examples to illustrate recent development in this matter.

1. 6. 3. 1. The Israeli cotton IPM–IRM program

In Israel failure to control *B. tabaci* with conventional insecticides led to the introduction of the first IRM program implemented for managing *B. tabaci* in cotton in 1987. The program used strategies that had been adapted from a similar program being used in Australia against *Helicoverpa armigera* Hubner (Forrester, 1990). In essence, the Israeli strategy divided the cotton-growing season into four-week periods during which specific insecticide groups could be used. A rotational scheme was developed that allowed use of insecticides with different modes of action to be applied only once during each period (the approximate duration of one pest generation). The primary goals of the program were to restrict use of pyrethroids and endosulfan for short periods during the growing season to delay the onset of resistance and, upon introduction, sustain the long-term efficacy of new insecticides by optimizing their use. In addition, by limiting and optimizing chemical use, another goal was to conserve natural enemy populations that aided in suppression of *B. tabaci* and other important cotton pests. The program has been continually modified over the past decade, reflecting the introduction of novel chemistries and changes in *B. tabaci* susceptibility to insecticides (Table 1).

Table 1. Insecticide resistance management program for *B. tabaci* and other pests in Israeli cotton from 1987 to 2000 (From Palumbo *et al.* 2001).

Years	Period II* Mid-May to mid-July	Period III Mid-July to mid-Aug.	Period IV Mid-August to mid-Sept
1987-1988	Aldicarb, Endosulfan	Pyrethroids	Organophosphates Carbamates
1989-1990	Aldicarb[a], Endosulfan	Buprofezin	Pyrethroids
1991-1997	(Endosulfan)[b]	Pyriproxyfen Diafenthiuron (Pyrethroids)	Buprofezin (Ops and carbamates)
1998-2000	Endosulfan, pheromone (PBW) mating disruption	(Pyriproxifen)[c], acetamiprid, imidacloprid (soil) abamectin (Pyrethroids)[d]	Buprofezin, diafenthiuron, Benzoyphenylureas (Ops and carbamates)[d]
Pest	*Helicoverpa armigera* *Pectinophora gossypiella*	*Bemisia tabaci,* *Pectinophora gossypiella,* mites (Tetranichidae)	*Bemisia tabaci, Pectinophora gossypiella, Spodoptera litteralis*

* Period I last from April to mid-May. Aphids and cutworms are the predominant pests during that period.

[a] Aldicarb was removed from the system following the introduction of the IGRs.

[b] Applications of insecticides in parenthesis are recommended only if high adult populations develop.

[c] Not recommended for use in some areas where high levels of resistance have been detected; use of acetamiprid or diafenthiuron are recommended.

[d] Limited use against *P. gossypiella.*

During 1987–1988, control of whiteflies was based on the restricted use of conventional insecticides within specific four-week periods in the growing season, and pyrethroid use delayed to mid-July. During the 1989–1990 growing seasons buprofezin was introduced into the system and became the principle compound for controlling *B. tabaci,* resulting in a further delay in pyrethroid use.

In 1991, pyriproxyfen was incorporated into the system, and its use was targeted at *B. tabaci* infestations occurring in July–August, postponing buprofezin use to later in the season if required. In many fields a single pyriproxyfen application during this treatment window was sufficient to control *B. tabaci* populations for the remainder of the cotton season. The program had become focused on preserving the susceptibility to the IGRs by using a rotational scheme where each IGR was used once during the season, and the use of conventional insecticides was complementary. In 1998, the program was further modified to reflect recent changes in areawide IPM approaches to insect management in cotton and to compensate for moderate to high levels of resistance to pyriproxyfen developing in limited areas such as the Ayalon Valley (Horowitz *et al.*, 1999b). To overcome this problem, compounds such as acetamiprid and diafenthiuron were used instead of pyriproxyfen in most cotton fields in Israel (Table 1). Although susceptibility to pyriproxyfen has rebounded somewhat in certain areas, growers in regions with cropping systems similar to the Ayalon Valley have been advised to consider implementing "pyriproxyfen-free years" in order to promote more effective containment of resistance. Period-specific uses were also identified for the limited use of compounds for common pests such as *H. armigera* and *Pectinophora gossypiella* Saunders.

The Israeli IRM program has been very successful in managing *B. tabaci* populations in Israel. By dramatically reducing the number of insecticide applications needed even against other cotton pests, it increased the impact of natural enemies on *B. tabaci.* Hence the more impact natural enemies had on *B. tabaci*, the less growers are forced to use insecticides and, the less selection pressure there is for resistance. To sustaining the insecticides, particularly for the IGRs, ideally a restriction to one application per season (or year) appears to be essential. Depending on the cropping system it may require annual periods of non-use. Furthermore, successful compliance to the recommendations of the program were exceptional and reflect the importance of educational outreach programs in helping

growers meet the important goals of resistance management (Palumbo *et al.* 2001). The important implication this strategy has for managing resistance to novel compounds is that the "big picture" also affects the onset of resistance, not just *B. tabaci* treatments in isolation.

1. 6. 3. 2.The Arizona whitefly resistance management strategy.

As previously discussed, control of *B. tabaci* in central Arizona cotton during the 1995-growing season had become extremely difficult with conventional insecticides, resembling the situation that had occurred in Israel during the late 1980s. Anecdotal reports estimated that in some fields as many as 10–12 applications were made to control *B. tabaci* resulting in exceedingly high control costs. This management crisis prompted the development of a new whitefly management program for control of *B. tabaci* in cotton that was modeled after the successes of the Israeli IRM program and incorporated the use of IGRs (Ellsworth *et al.*, 1996b; Dennehy and Denholm, 1998).

Similar to the Israel program, the Arizona strategy is centered on the need to restrict and optimize insecticide use, while conserving natural enemies. Both approaches strongly emphasize the deployment of non-chemical control practices, and identify specified stages during the season when insecticide groups (i.e. IGRs) could be applied. However, the strategies differ somewhat operationally in that use and sequence of either IGR in Arizona cotton is based specifically on population densities, rather than time of year (Ellsworth *et al.*, 1996b).

The Arizona IRM strategy recommends insecticide use for *B. tabaci* control in cotton to follow three stages (Table 2).

Table 2. Three stage chemical use plan for Arizona cotton as part of an integrated resistance and integrated whitefly management plan that encourages first use of IGRs and delayed usage of pyrethroids (From Ellsworth *et al.*, 1996a).

Stage I: Insect growth regulators	Use IGR of choice when whitefly counts exceed threshold. Threshold of 1 large nymph per leaf disk and 3-5 adults per leaf. Buprofezin at 393g a.i./ha used once, no sooner than 21 days after Pyriproxifen sterilizes adults and young eggs, prevent adult emergence. Pyriproxifen at 60.3 g a.i./ha used once, no sooner than 14 days after Buprofezin juvenoid chitin synthesis inhibitor use against nymphs.
Stage II: non pyrethroids	When populations average more than five adults per leaf, use Stage II materials at least once before using Stage III materials, in order to delay the need for pyrethroids. Rotate among classes of insecticides and among different insecticides within classes. Use no active ingredient more than twice per season.
Stage III: Pyrethroid mixtures	Delay pyrethroid use until the end of the control season approaches (for example, September-October). Plan to use the pyrethroid class no more than twice per season. Rotate the classes of compounds mixed with the pyrethroid and rotate among pyrethroids.

Stage I involves the application of either IGR when *B. tabaci* infestations exceed specific adult- and nymph-based thresholds. Using this two-component threshold insures that IGRs are used during periods of rapid population increase, when the compounds are most effective and potentially provide up to six weeks of control (Ellsworth *et al.*, 1997a; Ellsworth and Martinez-Carrillo, 2001). If additional whitefly control is required, then a non-pyrethroid conventional insecticide is recommended (Stage II), thus postponing the use of synergized pyrethroid mixtures to later in the season (Stage III). Use of all other active ingredients and pyrethroids as a class is further limited to no more than two uses per season against all pests in an effort to delay the development of resistance to these chemistries and conserve natural enemy populations. Since the whitefly IRM strategy is part of an overall cotton IPM

program, standard whitefly management practices, such as host plant sanitation, host crop sequencing, optimal crop management, and scouting continue to remain the foundation for *B. tabaci* management (Ellsworth and Martinez-Carrillo, 2001). Another important aspect of the program was the extensive educational outreach component that was critical for program implementation. During the first two years of the program, cotton growers and pest control advisors were annually provided mandatory training on the fundamentals of whitefly biology and management, and proper IGR use (Ellsworth *et al.*, 1997b).

The implementation of the IPM/IRM strategy was followed by an immediate and dramatic adoption of IGR use, with nearly two-thirds of the cotton acreage receiving an IGR application in 1996 (Ellsworth and Jones, 2001). IGR and conventional insecticide usage in cotton has since declined (Agnew *et al.*, 2000), largely due to the areawide suppression of *B. tabaci* populations following successive years of IGR use in cotton (Frisvold *et al.*, 2000). Since the implementation of the IPM/IRM strategy five years ago, Arizona cotton producers have not experienced an outbreak of *B. tabaci*. The chemical effectiveness of the IGRs in Arizona cotton, in combination with their relative specificity and subsequent conservation of natural enemies, have provided opportunities for predators and parasitoids to have a significant impact on *B. tabaci* population growth (Naranjo, 2001). Because these materials did not significantly disrupt the natural enemy complex, biological control was able to contribute to pest suppression. Thus, it was not the only chemical effectiveness of the insecticides themselves that permitted long-term control, but also the preservation of biological control activity. The increased activity of natural ennemies in IGR treated field has been nickmaned "bioresidual activity" and, it ultimately resulted in the longer control periods of *B. tabaci* populations achieved by IGRs compared to conventional insecticides (Naranjo and Ellsworth, 1999; Ellsworth and Martinez-Carrillo, 2001). The experiences in Israel and Arizona stress the importance of adhering to resistance

management strategies developed for sustaining long-term chemical efficacy as part of an IPM program that develops and integrates additional cultural, biological and novel chemical control approaches.

1. 6. 3. 3. Cross-commodity whitefly management

As expanded uses for IGRs and nicotinoids are introduced into more crops, additional selection pressures on *B. tabaci* populations may increase the resistance risks that already exist. This is particularly important, when registrations allow for multiple applications of novel insecticides in multiple crops. Given the tremendous value of effective compounds in *B. tabaci* control, all interested parties need to work together to develop and improve existing IRM strategies while still expanding non-chemical tactics of the IPM plan. For example in Arizona cross-commodity whitefly management strategy was initiated in 1998 in an attempt to manage whiteflies and to harmonize chemical uses in multi-crop communities. A coalition of research and extension scientists, cotton, melon and vegetables producers, industry and commodity group representatives, and regulatory officials came together cooperatively to establish a regional strategy for *B. tabaci* management across multiple commodities (Palumbo *et al.*, 1999). The program was prompted by the anticipation of multi-crop registrations that authorized up to three applications of buprofezin in a multi-crop community during a six-month period (i.e., once on spring melons, once on cotton during the summer, and once on fall melons). Furthermore, registration of buprofezin now allows two applications per crop in cotton, melons and lettuce. Since exposure to buprofezin would potentially overlap among these crops, it was apparent that cooperation would be needed among growers to harmonize insecticide use among commodities, to accommodate management needs of the respective groups, and to protect long-term chemical efficacy. Thus, the guiding principles behind the program were to maximize the efficacy and longevity of all

chemical modes of action for all commodities while providing suppression of *B. tabaci* below economic levels. In practical terms, the goal was to develop a chemical use strategy that optimized frequency of buprofezin use (e.g., limited number of applications per season or year), and avoided sequential exposure of multiple generations of whiteflies across commodities. An immediate concern was identification of regional differences in crop production, insecticide use and *B. tabaci* bionomics in Arizona so an assessment could be made of the potential for buprofezin exposure to successive whitefly generations as populations periodically move from crop to crop. A similar conflict of uses existed for imidacloprid; however foliar use in cotton remains very low, rendering this issue less important. However, the recent registration of thiamethoxam and expected registration of acetamiprid in the US will make harmonization of neonicotinoid use among Arizona's producers of multiple crops a priority issue.

General IPM practices were recommended for all crops that stressed *B. tabaci* avoidance through cultural management, scouting, and strategies for ensuring effective chemical use (Ellsworth and Martinez-Carrillo, 2001; Palumbo *et al.*, 2000a). Specific recommendations for buprofezin use included (i) limit use to one application per crop season, (ii) restrict use to no more than three applications per year in a multi-crop community, (iii) avoid exposing multiple, sequential generations of whitefly to buprofezin, (iv) coordinate treatments of adjacent fields such that, when thresholds indicate the need, buprofezin applications be made within one week (within the same whitefly generation), or the interval between buprofezin sprays is extended to four weeks, or two whitefly generations, (v) if a field requires treatment for *B. tabaci* and an adjacent field has been treated with buprofezin within 28 days (see (iv)), use an alternative chemistry with a different mode of action (i.e., pyriproxyfen in cotton), and (vi) assuming imidacloprid is used on lettuce, use alternatives to buprofezin for controlling *B. tabaci* in lettuce. In some regions where cotton and melons are produced

concurrently from April to November, the strategy recommends modifying *B. tabaci* control by eliminating buprofezin use during the F3 and F4 generations in cotton, and delaying use on fall melons. Recommendations were also made for limiting imidacloprid use by focusing mainly on the use of alternative modes of action in melons and vegetables for control of *B. tabaci* and aphids (Kerns and Palumbo, 1995) and avoiding all uses against *B. tabaci* in cotton.

1. 6. 4. Biological control of *Bemisia tabaci*

Natural enemies attacking *B. tabaci* include fungi (Osborne and Landa, 1992; Fransen *et al.* 1987; Meade and Byrne 1991), predators (Cohen and Byrne 1992) predators and parasitoids (Schuster *et al.* 1998; Goolsby *et al.* 1998; Gerling 2001). The potential for *Bemisia tabaci* populations to develop resistance against every class of synthetic insecticides has stimulated studies on integrated pest management strategies in which biological control may play a significant role.

1. 6. 4. 1. Fungi.

Most reports of natural fungal infections of *Bemisia* spp. refer to species of Hyphomycetes, especially *Paecilomyces*, *Verticillium* and *Aschersonia* spp. Most entomopathogenic fungi infect their hosts by direct penetration of the body wall. Surveys have revealed that they are among the most important natural enemies of whiteflies (Lacey *et al.*, 1996)

Recent advances in production, formulation, and application of insect pathogenic fungi have resulted in improvements in long-standing whitefly mycoinsecticide products based on *Verticillium lecanii*, and development and registration of several new products based on *Paecilomyces fumosoroseus* and *Beauveria bassiana* (Wraight and Carruthers, 1999). These products have the

capacity to suppress and, in some instances, provide good control of whiteflies in both greenhouse and field crops.

However, numerous factors continue to impede the commercial development of fungi as whitefly biological control agents. These include slow action, poor adulticidal activity, potentially negative interactions with commonly used fungicides, relatively high cost, limited shelf life, and dependence on favorable environmental conditions. Commercial markets for these products have been slow to develop and remain unstable in the face of strong competition from less costly, highly efficacious chemical insecticides (Vicentini *et al.*, 2001), Development of methods and strategies for overcoming these limitations has progressed, however, and various practices that enhance mycoinsecticide efficacy have been identified. These include: (1) initiating treatments against the early stages of the pest to prevent population build up, (2) targeting pest populations developing under moderate environmental conditions (e.g., during spring or fall growing seasons), (3) selecting crops amenable to multiple, highly efficient spray applications, and (4) applying fungi asynchronously with incompatible fungicides (Faria and Wraight, 2001).

Little is known regarding the potential for insect pest populations to develop resistance to fungal pathogens applied on a broad and intensive scale. It is encouraging that resistance is not reported to have developed as a consequence of the long-term use of *M. anisopliae* against sugarcane spittlebug in Brazil or of *B. bassiana* against pine caterpillar in China (St. Leger, 1995). Insect pathogenic fungi possess redundant enzyme systems that confer capacity to degrade the insect integument. This redundancy reduces the potential for development of resistance based on disruption of enzymes responsible for penetration of the host cuticle (St. Leger, 1995). On the other hand, the frequency and heritability of other resistance factors (e.g., chemical constituents of the host cuticle with antifungal properties) and the capacity of insect fungi to overcome these factors are essentially

unknown. Estimating the potential for development of mycoinsecticide resistance and establishing recommendations for resistance management will require extensive fundamental and applied research.

1. 6. 4. 2. Predators and parasitoids.

Bemisia tabaci predators include arthropods belonging to 9 orders and 31 families. Most are beetles, (Coccinelidae), true bugs (Miridae, Anthocoridae), lacewings (Chrysopidae, Coniopterrydae), mites (Phytoseiidae), and spiders (Araneae). Few natural enemies species have been studied in details, and are commercially available for *B. tabaci* (Gerling *et al.*, 2001). Primary parasitoids of *B. tabaci* are known from the genera *Encarsia, Eretmocerus* (Hymenoptera, Aphelinidae) (Gerling *et al.* 1980) and *Amitus* (Hymenoptera, Platygasteridae) (Joyce *et al.*, 1999). In addition, a *Metaphycus* Sp. (Hymenoptera, Encyrtidae) has been recorded (Polazsek *et al.* 1992) from *B. tabaci.* Hyperparasitic *Signiphora* spp. (Signiphoridae) has also been recorded.

Predators and parasitoids have been exploited by employing proper conservation and augmentation techniques (Naranjo, 2001). In addition to use of extant fauna, importation of parasitoids belonging to the genera *Encarsia* and/or *Eretmocerus* and of various predators has been successfully employed in greenhouses and in the field. Successes in greenhouses and in the field lead the way to a better understanding of the types of studies necessary for implementing future programs. Others natural enemy species have proven effective components in *B. tabaci* control, and there are still unexplored, potentially valuable species in many areas of the world (Gerling *et. al.* 2001).

The most significant factor disrupting biological control of arthropod pests in most cropping systems is the use of insecticides (Croft, 1990). Results from both laboratory toxicology studies and field application studies of conventional, broad-spectrum insecticides have shown that use of such

materials typically leads to direct natural enemy mortality in the laboratory and reduced population densities and activity in the field (Schuster and Stansly, 2000; Simmons and Jackson, 2000).

Because insecticides are likely to remain a major component of pest suppression for *B. tabaci*, minimizing the effects of insecticides on natural enemies in cropping systems will require more selective approaches for use of broad-spectrum insecticide and/or more selective materials. Strategies that focus on conventional or broad-spectrum insecticides, termed ecological selectivity, have been successful in other pest systems (Newsom *et al.*, 1976; Johnson and Tabashnik, 1999). These approaches include reduced rates of application, use of less persistent materials, temporal and spatial changes in application methods, and changes in formulation and delivery (Croft, 1990). Recently the use of systemic formulations of imidacloprid and other related nicotinid compounds appears to achieve selectivity by avoiding contamination of the plant surface (Palumbo *et al.*, 2001a). In addition, much research has been conducted to examine the effects of putatively selective and biorational materials such as IGRs which have both become more readily available worldwide in the last decade. IGRs cause little disruption of the natural enemy complex, allowing biological control to contribute to pest suppression (Naranjo, 2001).

1. 6. 5. Cultural control.

Cultural controls are preventative in nature (Gerling and Mayer, 1996). They consist of the manipulation of current or new components of the agroecosystem to prevent pest damage from reaching economic levels (Hilje, 2000a). In contrast with the first three tactics there are a heterogeneous group of practices, without well-defined boundaries or a coherent conceptual framework (Gerling, 1990). One approach to classifying this diverse group of practices might be a scheme based on underlying biological and ecological mechanisms. Hilje (2001) proposed avoidance in time or

space, behavioral manipulation of the insect, host suitability, and insect removal. This approach can be complemented with criteria related to the scale on which the practice is expected to operate, i.e. *regional*, *local* or *individual*. In such a scheme, crop sequencing and crop-free periods intended to remove or decrease inoculum sources over an entire area would be categorized as regional. Living barriers, trap crops and mulches intended to manage whiteflies in a single field would be classified as local. Fertilization regimes, although applied over an entire field, are intended to alter the suitability or susceptibility of individual plants and so would be characterized as individual (Hilje, 2001). Practices such as crop-free periods, altering planting dates, crop rotation, and weed and crop residue disposal, can play a significant role in integrated pest management (IPM) systems targeting whiteflies (Ellsworth, 2001).

The history of managing whitefly borne diseases with cultural practices can be traced back to the 1920s when cotton leaf curl disease was controlled in the Gezira region of Sudan by a two-month "dead season" during which cotton was not planted and ratoon growth removed (Bailey, 1930). During the same general timeframe, tobacco leaf curl was controlled in south central Africa through a legally mandated "closed season" during which tobacco was not grown and ratoon growth destroyed (Cock, 1986b). Recent example of application of crop free period occurred in the Dominican Republic tomato industry. *Bemisia tabaci* first invaded this industry in 1988 (Villar *et al.*, 1998) and Tomato yellow leaf curl virus (TYLCV) was found in 1992 (Polston *et al.*, 1994). The industry was devastated, with harvested hectares dropping from 8805 in 1989 to 3729 in 1993, and yield decreasing from 21.6 to 11.3 t/ha over the same interval. To overcome this crisis, cultural management practices were supported by regulatory measures that banned cultivation of whitefly reproductive hosts 90 days before the main growing season (Alvarez and Abud-Antún, 1995; Villar *et al.*, 1998). Approximately 600 ha of unauthorized crops and volunteer plants were eradicated every year during the implementation

phase of these regulatory measures, and sorghum was promoted as a rotational crop during summer. By 1997, the area of tomato harvested had increased to 8940 ha and yields to 30.4 t/ha. Compliance with the ordinances, along with deployment of tolerant hybrids and judicious insecticide use, was credited with allowing the local industry to prevail (Villar *et al.*, 1998). Similar measures were adopted in Arizona and Mexico in 1990s crisis (Ellsworth, 2001).

Growers may be reluctant to adopt cultural practices that require significant changes in conventional cropping practices. Nonetheless, some cultural practices such as plantings systems where the crop is covered with insect proof screens are feasible at reasonable cost (Hilje, 2001).

1. 6. 6. Plant Breeding for resistance to *B. tabaci.*

Host plant resistance (HPR) to whiteflies is rare in cultivated crops. A literature search revealed that HPR research with the *Bemisia tabaci/Bemisia argentifolii* complex has increased considerably in recent years, but as far as could be determined, in most cases these are not cultivars that were developed for whitefly resistance; rather they are cultivars or breeding lines that happened to have resistants traits and were selected in the field or greenhouse trials (Bellotti and Arias, 2001). In several cases, antixenosis (non preference for oviposition or feeding) or tolerance appears to be the resistance mechanism in operation (Laska *et al.*, 1982; 1986). Glabrousness, trichome density, latex, acylsugars and glossy foliage have also been linked to resistance (Pollard and Saunders (1956). Glabrous cotton cultivars resulted in lower oviposition and few nymphs (Butler *et al.*, 1992; Navon *et al.*, 1991), while glabrous-leafed melons (*Cucumis melo*) were found to reduce numbers of whitefly stages (adults and nymphs), when compared to commercial pubescent-leafed cultigens (Riley *et al.*, 2001). Higher phenolic and odihydroxy phenolic content of cotton cultivars resulted in fewer eggs oviposited by *Bemisia tabaci* (Butler *et al.*, 1992), and vascular bundle depth was negatively related to

B. tabaci adult and nymph densities (Chu *et al.*, 1998). In tomato, Gentile *et al.* (1968) identified *Lycopersicon hirsutum* and *Solanum pennellii* as resistant due to a heavy vesture of sticky glandular exudate on leaves and stems.

1. 6. 7. Breeding for resistance to *Bemisia tabaci*-transmitted viruses.

Conventional selection of plant genotypes for begomovirus resistance started in Kenya as early as 1929, where some local cassava (*Manihot esculenta L.*) cultivars were tolerant to African Cassava Mosaic Virus (ACMV). Attempt at increasing the initial level of resistance failed, but the resistance trait was successfully introduced into susceptible cultivars using interspecific hybrydization (Nichols, 1947). This breeding technique allowed the incorporation of TYLCV tolerance gene into tomato (*Lycopersicon lycopersicum* L.) (Vidavski and Czosnek, 1998), and of BGMV, BGYMV, BcaMV, and BDMV high level resistance gene into common bean (*Phaseolis vulgaris* L.) (Lapidot, 1997; Morales, 2000).

Genetic engineering is the current method of choice to incorporate resistance to plant viruses into commercial crops, including cassava, common bean, and tomato (Hong *et al.* 1996; Aragao et al.1998). This technique has allowed the creation of increased numbers of commercial cultivars expressing high level of resistance to begomoviruses (Morales, 2001).

Preface to chapter 2

The high propensity of *B. tabaci* to develop resistance to insecticides requires that pest management specialists consistently check the susceptibility to the array of insecticides used against it in agricultural systems, especially where management problems are reported. This is recommended because repeated usage of an insecticide against resistant pest may cause population outbreaks and significant economic losses to the pesticide industry and to growers.

For the success of a *B. tabaci* management program, it is also instrumental that the natural enemy fauna that contribute to its suppression incurs minimal damage from the use of insecticides. Thus it is of particular importance to answer questions such as "can natural enemies (e.g., parasitoids) be conserved or augmented against the pest in crops where insecticides are applied"? This question can be answered by conducting bioaasays of key natural enemies.

In Chapter 2, we documented the susceptibility of field populations of *B. tabaci* and those of one of its most frequent parasitoids to the insecticides that were routinely sprayed in cotton. This chapter 2 has been published in Pest Management Science in 2003 and has been co-authored as outlined in the manuscript and authorship section. The format has been changed to allow constitency of presentation throughout this thesis.

Chapter 2.

Susceptibility of Field Populations of Adult *Bemisia tabaci* Gennadus (Homoptera: Aleyrodidae) *Eretmocerus* spp. and *Encarsia spp.* (Hymenoptera: Aphelinidae) to Cotton Insecticides in Burkina Faso (West Africa).

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Published in Pest Management Science 2003. **59**: 97-106

2. 1. Abstract

Research was conducted in 14 cotton fields (3 to 10 ha) selected in 7 localities (two fields per locality) in Burkina Faso, with the objectives to: 1) estimate *Bemisia tabaci* Gennadus (Homoptera: Aleyrodidae) population abundance, 2) assess the levels of parasitism by *Encarsia* spp. and *Eretmocerus* spp. (Hymenoptera: Aphelinidae) and, 3) estimate the susceptibilities of the pest and those of an *Eretmocerus* sp. to the insecticides currently sprayed on cotton. Yellow sticky cards and a leaf turning technique were used to estimate adult *B. tabaci* population densities. Yellow sticky cards were also used to estimate the densities of adult *Eretmocerus* sp. and the susceptibilities of *B. tabaci* to insecticides. Leaf disk techniques were used to estimate *B. tabaci* red eye nymph populations, and parasitism by *Encarsia* spp. and *Eretmoceus* spp. were evaluated using stereomicroscopy. A leaf cage technique was used to estimate the susceptibilities of *Eretmocerus* sp. to insecticides. A mean of 6.5 to 27.4 adult *B. tabaci* were trapped per yellow sticky card and 5.5 to >34.9 were counted per leaf using the leaf turning technique. There were 0.14 to 13 *Eretmocerus* sp. trapped per yellow sticky card. The levels of parasitism varied between 36 and 87% by the end of the season and parasitism by *Eretmocerus* sp. predominated in most of the fields. The susceptibilities of *B. tabaci* and *Eretmocerus* sp. varied from field to field and with the insecticide tested.

Key words: *Bemisia tabaci* (Gennadus), *Encarsia* spp., *Eretmocerus* spp., cotton, insecticides, parasitism.

2. 2. Introduction

The whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) has become one of the most economically important pests in the world. The epizootic outbreaks of the pest populations began in the seventies in the Sudan and the Middle East (Dittrich *et al.*, 1985), reached the USA in the eighties (Jonhson *et al.*, 1982) and is expanding to most tropical and, subtropical countries including Australia, and temperate countries like Japan, Canada, and the Netherlands (Gerling and Mayer, 1996). *Bemisia tabaci* causes severe damage amounting to hundreds of millions of dollars annually (Menn, 1996). Damage is due to feeding and deposition of honeydew (Schuster *et al.*, 1996), physiological disorders (Yokomi *et al.* 1990) and transmission of geminiviruses (Bedford *et al.*; 1994, Markham *et al.*, 1996). No significant economic damage had been caused to crops in West Africa until population outbreaks started in 1998 in cotton crops in Burkina Faso, the Republic of Mali, and Ivory Coast. Outbreaks are often associated with intensive use of broad-spectrum insecticides against other pest (e.g. *Helicoverpa armigera* Hübner) that induce resistance development (Dittrich *et al.*, 1985; Prabhaker *et al.* 1985,1989) and/or disrupt control by natural enemies (Eveleens, 1983).

To efficiently manage the pest, resistance management strategies should be implemented. This highlights the need to assess and monitor the responses to insecticides in the target population, to enable the timely use of alternative control measures such as rotation of different insecticides, reduction in the number of insecticide applications, or the use of synergists. The use of chemicals, although necessary in multiple pests crop systems such as cotton, should not compromise the activity of a diverse fauna of parasitoids and predators that attack and sometimes effectively control the pest (Stansly *et al.*, 1997; McAuslane *et al.* 1993). To achieve this goal the susceptibilities of natural enemies should be known for each candidate insecticide.

Parasitism by two genera of aphilinids, *Encarsia* spp. and *Eretmocerus* spp., has been shown to reach levels as high as 70 to 80% (Bellows and Arakawa 1998, Sundaramurthy 1992). Species of *Eretmocerus* spp. specially could be of great importance because they are a major component of whitefly-parasite complexes (Rose *et al.*, 1996), and they may develop resistance to insecticides (Jones *et al.* 1995), allowing their integration in IPM using selective insecticides (Hoelmer 1996).

Our objectives were to: 1) estimate *B. tabaci* population abundance in Burkina Faso (West Africa), 2) assess the levels of parasitism by *Eretmocerus* spp. and *Encarsia* spp., and 3) estimate the susceptibilities of field populations of the pest and those of one of its parasitoids, an *Eretmocerus* sp. to insecticides currently used in cotton.

2. 3. Materials and Methods

2. 3. 1. Estimation of population densities

Investigations were conducted from September to December in farmers' fields at Dédougou, Solenzo, Safané, Matourkou, Farako-Ba, Yuéguéresso, and Kotédougou, respectively located at 171 km northeast, 153 km and 231 km northwest, 10 and 12 km west and 10 and 20 km south of Bobo-Dioulasso, Burkina Faso (West Africa). The fields, ranging in size from 3 to 10 ha, were sown with the local cotton cultivar FKB 290 and the commercial protection programs shown in Table 3 were applied. Voucher specimens of insects were deposited at the Laboratoire de Recherches, Institut de l'Environnement et des Recherches Agricoles, Bobo-Dioulasso, Burkina Faso and at the Biological Control Laboratory of Kansas State University, Manhattan, Kansas.

We used a leaf turning technique and binomial count described by Ellsworth *et al.* (1994) and the yellow sticky card (7.5 by 12.5 cm) method described by Gerling and Horowitz (1984) to estimate adult *B. tabaci* populations densities. The leaf turning and binomial count technique consist of

selecting a leaf situated on the 5th node position of a plant and gently turning it to count the number of *B. tabaci* adult on its lower surface; if less than 3 adults are found, the leaf is considered uninfected; if 3 or more adults are found, the leaf is considered infected. The yellow sticky card technique was adapted to estimate adult *Eretmocerus* populations. One square of 6.25 cm^2 was delimited at the middle of each of fifty yellow sticky cards used per field. The cards were held for 5 minutes in the field while disturbing nearby cotton foliage. Whiteflies and *Eretmocerus* sp. took flight when cotton foliage was disturbed. Highly attracted to yellow, they readily landed on the individually held yellow sticky cards. Adult whiteflies counts were made at random with the naked eye while hand magnifying lenses were used to count adult parasitoids in situ in the 6.25-cm^2 area of each card. Fourth instar nymph (also called red eye nymphs) densities were estimated in situ using hand magnifying lenses and the method of McAuslane *et al.* (1993) was used to estimate parasitoids immature population densities. Fifty cotton leaves on the 7th node position were randomly collected per field walking on a diagonal and picking leaves at about 5 m apart on each sampling date. The leaves were brought back to the laboratory and one leaf disk of ca. 4 cm^2 with nymphs was cut per leaf and the nymphs on the disk examined under a stereomicroscope to evaluate natural parasitism. Parasitism without meconia (i.e. residues of mycetome digested by the parasitoid) was ascribed to *Eretmocerus* spp., and parasitism with meconia in the parasitized nymphs to *Encarsia* spp.

2. 3. 2. Laboratory Bioassays

2. 3. 2. 1. *Bemisia tabaci*

We used a yellow sticky trap technique initially developed by Prabhaker *et al.* (1992) and subsequently adopted by Castle *et al.* (1996) to monitor the susceptibility of *B. tabaci* to endosulfan, cypermethrin, methamidophos, omethoate and to the mixtures cypermethrin + methamidophos and

cypermethrin + omethoate supplied by Saphyto S.A. (Bobo-Dioulasso, Burkina Faso) the local representative of the agrochemical company Calliope. Each trap consisted of a yellow sticky card (7.5 by 12.5 cm) attached to a plastic pad (1.5 by 12.5 cm). One side of the cards was coated with a thin layer of insect adhesive (Tanglefoot Company, Michigan, USA), and aqueous dilutions (Table 4) of each insecticide were sprayed on the yellow sticky cards with a Mataby domestic sprayer. The cards were air dried and transported to the fields in ice coolers. Controls consisted of yellow sticky cards treated with water alone.

The cards, coated with insecticides, were put in ice chests when ca. 60 whiteflies were trapped per card. Back in the laboratory, the cards were incubated at 26°C during 24h (12L:12D) in containers in which ca. 4 liters of water were added in the bottom to maintain high humidity. Mortality was determined by checking the adults under stereomicroscope for movement by probing them with a needle.

2. 3. 2. 2 *Eretmocerus* spp.

We used a leaf-cage technique adapted from Kishaba *et al.* (1976). The cages had four ventilation holes of ca. 10 mm diameter and six other ventilation holes of ca. 2 mm diameter respectively cut on the upper and the lower parts of plastic petri dishes (Falcon 1008 Easy Grip 35 X 10 mm style). The holes on the upper side were covered with an extremely fine nylon screen to prevent the escape of insects. The two sides were assembled with aluminum clips using Araldite glue. The edges of the two components of the leaf-cages were lined with a thin layer of sponge that kept cotton leaves undamaged during the experiment. The cages were positioned on the lower side of leaves, the leaves closing the ventilation holes of the upper side of the cages. A small opening made on the side of the cages was closed with a cork after parasitoids introduction.

On the day of *Eretmocerus* spp. collection, potted cotton plants were dipped for five seconds into a given dose of insecticide. Cypermethrin, methamidophos, omethoate and the mixtures cypermethrin + methamidophos and cypermethrin + omethoate were tested (Table 4). Controls consisted of plants dipped into water alone. After treatment the plants were air-dried and drops of honey were deposited on the leaves to serve as food for the parasitoids. The leaf-cages were clipped on the lower side of leaves.

Yellow sticky cards were used to trap *Eretmocerus* spp. in the fields. To aspirate alive and undamaged parasitoids, the cards were coated with an extremely fine layer of insect adhesive (Tanglefoot Company, Michigan, USA). A smooth cloth was used to wipe the glue to the point that the parasitoids that landed on the cards were unable to take off immediately but could walk to escape. To prevent their escape, they were quickly aspirated using Eppendorf® pipette tips (101-1000 µl) cut to a diameter of ca. 2 mm and mounted on Tygon plastic tubes. One of the openings of the plastic tubes to be mounted on the large opening of the pipette tips was covered with a thin nylon tissue mesh to retain parasitoids in the pipette tips. Ten parasitoids were aspirated per pipette tip. Next the pipettes tips containing the parasitoids were placed on a piece of pharmaceutical cotton in an ice cooler where a piece of ice was placed to slightly chill the parasitoids during their transport to the laboratory. In the laboratory, the parasitoids were released into the leaf-cages through a small opening. An experimental unit consisted of a leaf cage containing 10 parasitoids and positioned on the lower side of a cotton leaf treated with a given dose as described above. There were four experimental units per dose making a total of 40 parasitoids tested per dose and 240 per insecticide.

The experimental units were incubated for 24 hours at 25°C and then were chilled for ca. 2 min in a refrigerator. The parasitoids were emptied into a petri dish and were examined for movements under a stereomicroscope. Dead parasitoids were immobile and shriveled while living parasitoids

recovered quickly from chilling and were active. LC_{50} values for each insecticide were calculated with POLO-PC (LeOra Software 1987).

2. 4. Results

2. 4. 1. *Bemisia tabaci*

In October in all the fields, the densities of red eye nymphs and adult *B. tabaci* by far exceeded the economic injury levels for *B. tabaci* in cotton, i.e. 6.8-18.8 nymphs/leaf disk and 5.9-15.2 adults/leaf (Stam *et al.*1994, Naranjo *et al.* 1996,). The levels of infestation exceeded 34.9 adults per leaf in 5 out of 10 fields and mean densities of 4.5 to 27.5 red eye nymphs were recorded per leaf disk (Table 5). Mean numbers of 5.5 to 27.36 adults were trapped per sticky yellow cards after five minutes of exposure (Table 6).

The LC_{50} values for endosulfan ranged from 1970 to 5830 mg l^{-1}, 100 to 710 mg l^{-1} for cypermetrin (Table 7), while those for methamidophos and omethoate ranged respectively from 1300 to 8230 and 1160 to 5100 mg l^{-1} ($P < 0.05$, Table 8). The LC_{50} values for cypermethrin + methamidophos and those for cypermethrin + omethoate ranged respectively from 130 to 1630 mg l^{-1} and 270 to 4390 mg l^{-1} (Table 9).

2. 4. 2. Eretmocerus spp.

In October parasitism averaged 4-34.7% (Table 6) and average numbers of 0.08 to 1.36 adult *Eretmocerus* spp. were trapped per sticky yellow card. In November parasitism dramatically increased (min 23%, max. 93%). Similarly adult *Eretmocerus* spp. densities increased in all fields, reaching levels as high as 2.08 to 13 adult *Eretmocerus* spp. per sticky yellow cards. *Eretmocerus* spp. parasitism exceeded 50% in nine fields out of 10 (Table 6).

In general adult *Eretmocerus* spp. were tolerant to insecticides. The levels of susceptibility varied from one field to another and depended on the insecticide used (Table 10). For example, *Eretmocerus* spp. population from Farako-Ba was more tolerant to the insecticides tested than the populations from the other fields. For the population from Yéguéresso, the LC_{50} values were 1960 mgl^{-1} for omethoate and 1390 mgl^{-1} for methamidophos while for those from Kotédougou, the LC_{50} values were 1610 mg/ml for omethoate, and 1688 mg/l for methamidophos. The same pattern was observed with insecticide mixtures. For example the parasitoids from Kotédougou were more susceptible to cypermethrin + omethoate (LC_{50} = 274 mg l^{-1}) than to cypermethrin + methamidophos (LC_{50} = 890 mg l^{-1}). Mixtures of cypermethrin with either methamidophos or omethoate were highly toxic to the parasitoids.

2. 5. Discussion

The levels of infestation varied from field to field and *B. tabaci* populations declined or stagnated in all the fields in November. We observed that early sown fields were first infested, followed by late sown fields. Early sown fields received two to three insecticide treatments more than the fields sown at later dates. In addition, after maturation cotton foliage deteriorates and whiteflies migrate to greener fields (Byrne and Blackmer, 1996). The extent to which the differing dates of sowing, insecticide treatments and migrations from senescing cotton fields to greener ones impacted on the different levels of infestation observed is not known. The decline or stagnation of *B. tabaci* populations was partly attributable to the increase of parasitism with parasitoid densities nearly equaling those of *B. tabaci* in many fields. Stansly *et al.* (1997) also observed the decline of *Bemisia argentifolii* (formerly *B. tabaci* strain B) population densities in eggplant and tomato following the increase of parasitism in the field.

The susceptibility of pest populations to a given pesticide is an evolutionary phenomenon due to changes in frequencies of resistant and susceptible alleles in pest populations (Dobzhanky, 1951). These changes in allele frequencies result from the complex interaction of mutation, selection, gene flow, and random genetic drift (Roush and Daly, 1991). Once a pesticide use has become widespread against a pest, selection and gene flow have major influence on the evolution of the susceptibility of the populations of this pest to the pesticide, and are the most likely factors that can be manipulated to achieve a desired goal (Roush and Daly, 1991), e.g. slowing down the magnitude of resistance to this pesticide in the pest populations. The manipulation of selection to influence evolution of susceptibility requires monitoring for resistance which traditionally involved comparisons of LD_{50}s, LD_{90}s, or slopes of dose-response curves between field and laboratory populations (Staetz, 1985). In the absence of a laboratory population (or reference strain), the LD_{50}s of field populations can be compared against specific insecticide levels, e.g. the doses recommended in the field. In this case a population is considered as "susceptible" to the pesticide used against it if the LD_{50} and slope of dose-response of this population are below those recommended in the field. Conversely this population is considered as "resistant" to the pesticide used if its LD_{50} and slope of dose-response are above the dose recommended (Roush and Milner,1986).

In our study while the LC_{50} values for endosulfan did not exceed the recommended dose in any field, those for cypermethrin were 1.4 to 2-fold the recommended dose in 5 out of 13 fields. LC_{50} values for methamidophos and omethoate exceed the recommended doses respectively in 6 and 2 out of 8 fields. LC_{50} values for methamidophos were 1.2 to 2.9 fold higher than those for omethoate in 5 fields out 8 suggesting that methamidophos was less effective at killing whiteflies than omethoate in these fields. Mixtures were more lethal than the individual insecticides. The LC_{50}s values for

cypermethrin + methamidophos were below the recommended rates in all the fields while those of cypermethrin + omethoate were above the recommended rate in one out 14 fields.

Generally, the magnitude of resistance to an insecticide may vary from one year to another and between geographical regions. For example, LC_{50} values for cypermethrin ranged from 16840 to 39340 mg l^{-1} among 19 populations in 1987, and from 27140 to 75050 mg l^{-1} among 13 populations in 1988 in California (Prabhaker *et al.* 1992). In Guatemala, LC_{50} values for cypermethrin were 1485 mg l^{-1} in 1986 and 2052mg l^{-1} in 1987 (Dittrich *et al.* 1990), In the Sudan, LC_{50} values for methamidophos were 1593 in 1986 and 3894 mg l^{-1} in 1987 while those for endosulfan were 17.6 and 33.6 mg l^{-1} respectively in 1986 and 1987 (Dittrich and Ernst 1983).

The LC_{50} values for cypermethrin in Burkina Faso were lower than those found in California by Prabhaker *et al.* (1992) and in Guatemala by Dittrich *et al.* (1990), but equivalent to those found in the Sudan by Dittrich and Ernst (1983) However LC_{50} values for endosulfan was lower in the Sudan than in Burkina Faso.

In Burkina Faso endosulfan was used twice at the beginning of the insecticide program. At that time *B. tabaci* populations were low in the fields. Conversely cypermethrin was consistently sprayed from the flowering stage until the open boll stage. The mixtures cypermethrin + methamidophos or cypermethrin + omethoate were sparingly sprayed in some fields in the flowering stage, then consistently in all the fields from the green to the open boll stages. It is in these periods that *B. tabaci* populations usually explode in cotton fields. Thus selection for resistance to cypermethrin, methamidophos and omethoate was more likely as large populations of the pest were treated. However mixtures were more active than individual insecticides, hence LC_{50} values for individual compounds were 3 to 27 folds greater than those for mixtures. Castle *et al.* (1996) also observed that mixtures of pyrethroids with insecticides from a different class were more effective than single insecticides. Cahill

et al. (1994) showed that susceptible *B. tabaci* adults were rapidly killed by recommended field rates of cypermethrin, profenofos and a commercial mixture of these two chemicals in field simulators while adults of resistant strains having resistance factors (RF) (RF = LC_{50} field strain/ LC_{50} sensitive strain) varying from 23 to 44 were much less affected. Hence *B. tabaci* population outbreaks in Burkina Faso could partly be associated with the development of resistance to the field rates of the insecticides sprayed. Control failures attributable to pesticide resistance were also documented by Dittrich *et al.* (1985) who demonstrated that control levels of *B. tabaci* were broadly correlated with a resistance factor, aldicarb (RF =2) providing the best control and monocrotophos (RF= 150) not only failing to control the pest, but causing an increase in whitefly numbers compared to the untreated plots. The increase was due to the improved fecundity of resistant *B. tabaci* females that laid 1.5 to 3 fold more eggs than susceptible females (Dittrich *et al.* 1990).

The low levels of parasitism in the flowering and green boll period when insecticides were frequently sprayed and their increase in the open boll period after insecticide spraying ceased, suggest that natural enemies were suppressed. Eveleens (1983) observed the suppression of aphelinid parasitoids and other beneficial insects due to repeated usage of DDT in the Sudan and Gerling and Naranjo (1998) observed that parasitism was lower in experimental plots treated with monocrotophos. However, parasitism following application of buprofezin and pyriproxyfen has been found to be higher in treated plots than in the control (Naranjo and Hagler, 1997) or to remain at or near pretreatment levels (Bellows and Arakawa, 1988).

LC_{50} values and slopes of regression lines indicated the presence of *Eretmocerus* spp. individuals tolerant to insecticides within the populations studied. In general the parasitoid was more tolerant to individual insecticides than to mixtures. Higher tolerance to insecticides corresponded to higher percentages of parasitism in the fields. For example at Farako-Ba where tolerance to

insecticides was the highest, 93% of parasitism were recorded, indicating the potential for conservation of parasitoids or achievement of a favorable host-natural enemy ratio through judicious timing of application or manipulation of dosage coupled with release of the more resistant parasitoids strains.

Our results support those of Jones *et al.* (1995) that suggested that resistance to insecticides may exist in some field populations of *Eretmocerus* spp. after having observed that 40% of a strain from Spain survived 48 hours confinement on cotton leaves sprayed to runoff with 7370, 9740 or 2100 mg l^{-1} respectively of amitraz, thiodicarb and cypermethrin.

2. 6. Conclusion

Although to our knowledge no reference data on *B. tabaci* and *Eretmocerus* sp. population susceptibility to the insecticides used in this study are available in West Africa to provide a comparison of the evolution of the magnitude of their susceptibility in this area, we found that *B. tabaci* populations in Burkina Faso were resistant to cypermethrin, methamidophos and omethoate as the LD_{50}s of most populations were well above the dose recommended in the field. Selection for resistance likely occurred among the populations as a consequence of repeated usage of insecticides against the complex of cotton pests. This selection pressure resulted in poor management of most *B. tabaci* populations in cotton fields when cypermethrin, methamidophos and omethoate were sprayed. Parasitism was suppressed during insecticide spraying period in farmers' fields, and dramatically increased after the cessation of insecticide sprayings. Parasitism by *Eretmocerus* spp. predominated in most of the cotton fields and *Eretmocerus* spp. showed tolerance to insecticides. LC_{50} values for the insecticides tested on *Eretmocerus* spp. differed from field to field, suggesting higher tolerance to insecticides in the populations of Farako-Ba. We suggest: 1) that broad spectrum insecticides (cypermethrin, methamidophos and omethoate) be replaced by more selective insecticides, or 2) they

be placed at the end of the season (i.e. open boll period) and be used only twice to allow the parasitoids to act, 3) that insecticides from different classes be alternated to reduce the risk of resistance buildup.

2. 7. Acknowledgements

This research was supported by the Institut de l'Environement et des Recherches Agricoles (INERA) and the Société des Fibres Textiles (Sofitex) du Burkina Faso. We are indebted to Georges Yaméogo and Dakuo Déhou, respectively Director and Adjunct Director of the Direction du Développement de la Production cotonnière (DDPC) of Sofitex for facilitating diverse research activities. Many thanks to Paco Sereme, Konaté Gnissa and Abdoussalam Sawadogo for administrative support. We thank SAPHYTO, the local representative of the agrochemical company CALLIOPE for supplying the insecticides. We are grateful to Bambara Issa, Dakuo Patrice and Sinon El Adj Adama for technical assistance.

2. 8. REFERENCES

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Table 3. Insecticide program for managing cotton pests (including *B. tabaci*) in the fields sampled in Burkina Faso from September to October 2000 (all recommendations are per ha).

Localities (Surfaces inha)	Sowing dates (m/d/y)	Cotton phenology		
		Period I (Emergence-squares)	Period II (Flowers- green bolls)	Period III (Green bolls- open bolls)
Dédougou 1 (7)	5/28/00	45 DAS[a]: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	67 DAS: 36g of Cypermethrin + 200 g of Profenophos 75 DAS: 36g of Cypermethrin + 300 g of methamidophos 82 DAS: 30g of cypermethrin +200 g of Profenophos	88 DAS: 36 g of Cypermethrin + 300 g of Omethoate 95 DAS: 36 g of Cypermethrin + 300 g of Methamidophos 102 DAS : 36 g of Cypermethrin + 250 g of Benfuracarb
Dédougou 2 (10)	5/12/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	72 DAS: 12 g of Alphacypermethrin + 200 g of profenophos. 77 DAS: 36g of Cypermethrin + 300 g of Omethoate 82 DAS: 36g of cypermethrin + 200 g of Profenophos	88 DAS: 36 g of Cypermethrin+ 400 g of Dimethoate 93 DAS: 36 g of Cypermethrin+ 400 g of Dimethoate 100 DAS: 36 g of Cypermethrin + 300 g of Methamidophos 105 DAS: 36g of Cypermethrin + 250 g of Benfuracarb
Solenzo 3 (3)	6/12/00	45 DAS[a]: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	70 DAS: 12 g of Deltamethrin + 200g of Triazophos 77 DAS: 36g of Cypermrthrin +300g of Methamidophos 84 DAS: 30g of cypermethrin +200g of Profenofos	88 DAS: 36 g of Cypermethrin + 300 g of Omethoate 95 DAS: 36 g of Cypermethrin + 300 g of Methamidophos 102 DAS: 300 g of Diafenthiuron 107 DAS : 300 g of Diafenthiuron
Solenzo 4 (4)	7/12/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Alphacypermethrin. + 200 g of profenofos. 85 DAS: 36g of cypermethrin. + 200 g of Profenophos.	95 DAS: 36 g of Cypermethrin + 400 g of Dimethoate 102 DAS: 36 g of Cypermethrin + 250 g of Benfurararb

[a] DAS: Days after sowing

Table 3. (continued)

Localities (Surfaces in ha)	Sowing dates (m/d/y)	Cotton phenology		
		Period I (Emergence-squares)	Period II (Flowers-green bolls)	Period III (Green bolls-open bolls)
Safané 5 (6)	6/2/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	70 DAS: 36 g of Cypermethrin + 200 g of Chlorpyriphos-ethyl 76 DAS: 36g of Cypermethrin + 300 g of Methamidophos 83 DAS: 30 g of cypermethrin + 200 g of Profenophos	88 DAS: 36 g of Cypermethrin + 300 g of Omethoate 95 DAS: 300 g of Diafenthiuron 102 DAS: 300 g of Diafenthiuron
Safané 6 (4)	7/17/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g/ha of Endosulfan	74 DAS: 12 g of Alphacypermethrin + 200 g of profenophos 81 DAS: 12 g of Deltamethrin +200 g of Triazophos.	88 and 95 DAS: 36 g of Cypermethrin + 400 g of Omethoate 105 DAS: 36 g of Cypermethrin + 250 g of Benfuracarb
Matourkou 7 (3)	6/15/00	45 DAS[a]: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 36 g of Cypermethrin + 200 g of Profenophos/ha 81 DAS: 30 g of cypermethrin. +200 g of chlorpyrifos-ethyl/ha	88 DAS: 36 g of Cypermethrin + 300 g of Omethoate; 95 DAS: 36 g of Cypermethrin + 300 g of Methamidophos; 102 DAS: 36 g of Cypermethrin + 400 g of Dimethoate; 109 DAS: Cypermethrin + 250 g of Benfuracarb
Matourkou 8 (5)	7/18/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Alphacypermethrin+ 200 g of profenophos. 81 DAS: 12 g of Deltamethrin + 200 g of Triazophos	88 DAS: 36 g of Cypermethrin + 400 g of Dimethoate 95 DAS: 36 g of Cypermethrin + 250 g of Benfuracarb
Yéguéresso 9 (7)	7/8/00	45 DAS[a]: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Deltamethrin + 200 g of Triazophos 81 DAS: 12 g of Lamdacyalothrin +200 g of Profenophos	88 DAS: 36 g of Cypermethrin + 300g of Omethoate 95 DAS: 36 g of Cypermethrin + 300 g of Methamidophos
Yéguéresso 10 (10)	7/19/00	45 DAS: 700g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Alphacypermethrin+ 200 g of profenophos 81 DAS: 30 g of Lamdacyalothrin + 200 g of Profenophos	88 and 95 DAS: 36 g of Cypermethrin + 400 g of Dimethoate 105 DAS: 36 g of Cypermethrin + 250 g of Benfuracarb

[a] DAS: Days after sowing

Table 3. (continued)

Localities (Surfaces in ha)	Sowing dates (m/d/y)	Cotton phenology		
		Period I (Emergence-squares)	Period II (Flowers- green bolls)	Period III (Green bolls- open bolls)
Farako-Ba 11 (8.5)	7/03/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Deltamethrin + 200 g of Triazophos; 81 DAS: 36g of cypermethrin +200g of Profenophos	88 DAS: 36 g of Cypermethrin + 300 g of Omethoate 95 DAS: 36 g of Cypermethrin + 300 g of Methamidophos
Farako-Ba 12 (6.5)	6/25/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Alphacypermethrin+ 200 g of profenophos; 81 DAS: 12 g of Deltamethrin + 200 g of Triazophos	88 and 95 DAS: 36g of Cypermethrin + 400 g of Dimethoate 105 DAS: 36 g of Cypermethrin + 250 g of Benfuracarb
Kotédougou 13 (3)	7/20/00	45 DAS[a]: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	74 DAS: 12 g of Deltamethrin + 200 g of Triazophos 81 DAS: 30 g of cypermethrin +200 g of Chlorpyriphos-ethyl	88 DAS: 36 g of Cypermethrin + 300 g of Omethoate; 95 DAS: 36 g of Cypermethrin + 300 g of Methamidophos
Kotédougou 14 (4)	7/9/00	45 DAS: 700 g of Endosulfan 60 DAS: 700 g of Endosulfan	70 DAS: 12 g of Alphacypermethrin+ 200 g of profenophos 77 DAS: 36 g of Cypermethrin + 300 g of Omethoate 85 DAS: 30 g of Lamdacyalothrin + 200 g of Profenophos	92 DAS: 36 g of Cypermethrin + 400 g of Dimethoate; 100 DAS: 36 g of Cypermethrin + 250 g of Benfuracarb; 107 DAS: 36 g of Cypermethrin + 300 g of Methamidophos

[a] DAS: Day after sowing.

Table 4. Insecticide dosages recommended in the fields and the doses evaluated for their effects on *B. tabaci* and *Eretmocerus* sp.

Insecticide and field dosage (g/ha)	Concentrations of the formulations ($mg \times l^{-1}$)	Concentrations under experimentation [b] ($mg \times l^{-1}$)
Endosulfan: 700 (EC)	7000	875, 1750, 3500, 7000, 14000
Methamidophos: 300 (EC)	3000	375, 750, 1500, 3000, 6000
Omethoate: 300 (EC)	3000	375, 750, 1500, 3000, 6000
Cypermethrin: 36 (EC)	360	45, 90, 180, 360, 720
36g of Cypermethrin.+ 300g of Omethoate. (EC)	360:3000	45:375, 90:750, 180:1500, 360:3000, 720:6000
36g of Cypermethrin +300g of Methamidophos (EC)	360:3000	45:375, 90:750, 180:1500, 360:3000, 720:6000
Control	0	water

[b] The concentrations under experimentation ranged from 0.125 to 2 fold the doses recommended in the field.

Table 5. Average number of adults *B. tabaci* in cotton field in October using binomial count method (Ellsworth *et al.* 1994) for adults and individual count for immatures (30 leaves or disks, were inspected in each field respectively for adults and nymphs).

Localities + field number	Adults			Nymphs	
	% infested	Average/leaf in the field	Nb. infested leaf disks (n= 30)	% infested	Average/ leaf disk
Dédougou 1	100	> 34.9	28	93	24.0
Dédougou 2	100	> 34.9	27	90	19.5
Solenzo 3	100	> 34.9	22	73	15
Solenzo 4	100	> 34.9	16	53	7.5
Safané 5	93	14.9	17	57	7.8
Safané 6	100	18.4	30	100	27.5
Matourkou 7	87	11.3	18	60	9.0
Matourkou 8	90	12.8	16	53	8.2
Kotédougou 13	60	5.5	8	27	4.5
Yéguéresso 10	100	> 34.9	25	83	18.6

Table 6. Mean Numbers of adult *B. tabaci* and *Eretmocerus* sp. per yellow sticky cards (n =50) after 5 minutes trapping and percentages of parasitism (n= 250 nymphs per field) during insecticide sprayings (October), and after cessation of insecticide sprayings (November) in 2000.

Period of estimate	Localities/ field number	Mean No. of *B. tabaci*	Mean No of *Eretmocerus*	% parasitism	% *Encarsia*	% *Eretmocerus*
October	Dédougou 1	16.12	1.36	26.7	13.5	86.5
	Dédougou 2	14.70	0.14	8.9	48.0	52.0
	Solenzo 3	16.26	0.08	12.0	0.0	100.0
	Solenzo 4	15.08	0.52	4.0	52.4	47.6
	Safané 5	27.36	0.14	19.0	11.0	89.0
	Safané 6	14.56	0.52	10.5	8.3	91.7
	Farako-Ba 11	21.46	1.30	34.0	7.5	92.5
	Matourkou 7	17.42	1.00	7.2	0.0	100.0
	Kotédougou 13	16.26	0.92	17.0	15.0	85.0
	Yéguéresso 9	6.50	1.18	20.6	4.5	95.5
November	Dédougou 1	14.30	9.76	83.0	31.5	68.5
	Dédougou 2	12.36	3.58	45.0	58.0	42.0
	Solenzo 3	16.90	2.28	47.8	22.8	77.2
	Solenzo 4	14.64	3.96	36.0	72.4	27.6
	Safané 5	16.58	2.08	78.0	41.0	59.0
	Safané 6	10.86	3.00	62.0	38.3	61.7
	Farako-Ba 11	15.34	13.00	93.0	27.9	72.1
	Matourkou 7	13.92	4.48	42.0	11.0	89.0
	Kotédougou 13	17.56	5.28	64.0	45.0	55.0
	Yéguéresso 9	12.36	5.08	87.0	24.5	74.5

Table 7. Toxicities of endosulfan and cypermethrin to adult *B. tabaci* in Burkina Faso.

Localities/ field #	Endosulfan(df =3)				Cypermethrin(df = 4)			
	Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	χ^2 [a]	t [b]	Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	χ^2 [a]	t [b]
Dédougou 1	2.37 (0.35)	2418 (1546-3336)	2.69	3.94	1.14 (0.23)	330 (178-1289)	0.63	2.30
Dédougou 2	1.97 (0.31)	2920 (1751-4.238)	1.95	4	0.92 (0.21)	340 (154-2567)	2.35	2
Solenzo 3	2.70 (0.35)	2970 (1740-4530)	4.52	5.69	1.28 (0.24)	230 (134-536)	0.79	3.42
Solenzo 4	3.21 (0.55)	3960 (2604-5200)	2.71	4.52	0.92 (0.21)	490 (211-2140)	2.14	1.98
Safané 5	4.09 (0.88)	5830 (2720-8040)	3.11	4	1.05 (0.29)	590 (310-3210)	0.69	2.9
Safané 6	1.84 (0.29)	3290 (2007-4852)	1.13	4.32	0.74 (0.19)	710 (300-6550)	0.84	2.49
Matourkou 7	2.51 (0.38)	2160 (1342-2980)	1.48	3.5	1.12 (0.23)	280 (170-630)	1.64	2.6
Matourkou 8	2.39 (0.32)	1970 (870-3234)	9.54	3.79	1.62 (0.24)	100 (71-140)	1.60	7
Yéguéresso 9	2.23 (0.33)	3060 (1960-4276)	0.74	4.46	0.70 (0.18)	320 (160-1690)	1.59	6.19
Yéguéresso 10	2.72 (0.37)	2900 (1298-4975)	11.36	5.20	0.97 (0.26)	670 (320-4500)	3.35	2.91
Farako-Ba 11	1.81 (0.31)	3260- [c]	9.57	3.77	0.80 (0.21)	580 (260-4130)	0.64	2.82
Farako-Ba 12	2.09 (0.31)	2550 (1290-4050)	3.39	5	0.98 (0.21)	230 (130-550)	1.40	2.67
Kotédougou 13	1.48 (0.24)	2530 (1730-3460)	2.95	3.6	0.79 (0.18)	250 (100-4270)	4.66	2.19

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,3}$: 7.85; $\chi^2_{0.05,4}$: 9.48

[b] For t-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05, $t_{0.05,\infty}$ =1.96

[c] 95% CL value not presented because the index of significance for potency estimation, 'g' exceeded 0.5 as calculated with Polo-PC.

Table 8. Toxicity of Methamidophos and Omethoate to adult *B. tabaci* in Burkina Faso.

Localities/ field #	Methamidophos(df=4)				Omethoate(df=4)			
	Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	$\chi^{2\,a}$	t^{b}	Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	$\chi^{2\,a}$	t^{b}
Dédougou 1	2.2 (0.7)	7350 (5130-21500)	0.7	4.12	2.1 (0.28)	3680 (2200-10540)	10	8.29
Dédougou 2	1.8 (0.6)	8230 (5340-47660)	2.1	4.03	2.5 (0.65)	1160 (2773-7279)	2.8	
Solenzo 3	2.5 (0.6)	4169 (3143-5900)	2.8	3.83	1.6 (0.2)	1420 (673-3829)	4.8	2.66
Solenzo 4		not tested in this field			1.6 (0.23)	1160 (330-2800)	12	1.97
Safané 5	2.1 (0.7)	7160 (5169-18312)	0.4	3.47	3.1 (0.96)	4643 (2843-11549)	0.43	5.08
Safané 6	1.7 (0.3)	2460 (1590-3930)	1.4	4.30	3.3 (1.23)	2410[c]	7.7	1.86
Matourkou 7	0.9 (0.2)	4890 (2563-22016)	0.5	7	1.1 (0.19)	5100 (2818-18109)	0.3	7.8
Matourkou 8	1.6 (0.4)	4640 (2843-11549)	0.4	5		not tested in this field		
Yéguéresso 9	1.8 (0.2)	1303 (956-1810)	0.3	2.22	1.9 (0.21)	1613 (747-4950)	6.4	3.96

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,4}$: 9.48

[b] For *t*-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05. $t_{0.05,\infty}$ =1.96

[c] 95% CL value not presented because the index of significance for potency estimation, 'g' exceeded 0.5 as calculated with Polo-PC.

Table 9. Toxicities of two insecticide mixtures to adult *B. tabaci* in Burkina Faso.

Localities/ field #	Cypermethrin + Methamidophos (df=4)				Cypermethrin + Omethoate (df=4)			
	Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	χ^{2a}	t^{b}	Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	χ^{2a}	t^{b}
Dédougou 1	2.60 (0.35)	270 (184-358)	2.0	7.61	2.24 (0.25)	473 (345-629)	2.8	5.85
Dédougou 2	1.65 (0.23)	370 (205-556)	2.2	5.71	2.02 (0.25)	617 (421-860)	2.9	3.64
Solenzo 3	1.20 (0.19)	440 (190-810)	5.2	4.05	1.59 (0.28)	1492 (654-1887)	0.5	2.71
Solenzo 4	3.04 (0.57)	300 (161-415)	3.9	6.74	1.18 (0.24)	1490 (785-3335)	2.7	2.68
Safané 5	2.82 (0.41)	240 (70-426)	4.5	7.41	1.37 (0.24)	940 (330-2170)	6.4	2.31
Safané 6	2.84 (0.35)	255 (103-446)	4.99	7.90	2.56 (0.31)	270 (110-493)	5.8	7.80
Matourkou 7	2.67 (0.44)	134 (81-179)	1.4	7.20	1.29 (0.21)	1500 (893-2963)	3.8	2.01
Matourkou 8	3.53 (0.75)	830 (463-1090)	3.9	2.68	1.58 (0.24)	1250 (813-2050)	2.2	3.37
Yéguéreso 9	1.16 (0.24)	1630 (837-4034)	0.9	2	1.37 (0.26)	1026 (507-1835)	2.6	2.18
Yéguéresso 10	1.64 (0.23)	460 (210-810)	6.1	4.53	0.9 (0.21)	962 (412-1559)	3.6	3.05
Farako-Ba 11	1.61 (0.26)	685 (370-1076)	0.5	2.16	0.98 (0.18)	4390 (2250-18330)	1.9	2.27
Farako-Ba 12	1.23 (0.20)	890 (410-2000)	6	2.57	1.02 (0.23)	1900 (1100-4110)	1.6	3.61
Kotédougou 13	1.91 (0.25)	390 (150-690)	9.6	2.42	1.77 (0.28)	560 (190-1010)	6.9	3.57
Kotédougou 14	1.58 (0.23)	800 (460-1330)	4.7	6.19	1.70 (0.36)	960 (560-1390)	1.9	5.18

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,4}$: 9.48

[b] For *t*-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05, $t_{0.05,\infty}$ =1.96

Table 10. Toxicities of three insecticides and two mixtures to adults *Eretmocerus* sp. in Burkina Faso.

Localities/field #	Insecticide or mixture (df=3) Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	$\chi^{2\,a}$	t^{b}
	Omethoate			
Matourkou 7	1.95	930	4.84	4.63
	(0.29)	(370-1600)		
Yéguéresso 9	1.63	1960	1.81	3.20
	(0.26)	(1120-3450)		
Farako-Ba 11	1.15	3740	0.94	7.13
	(0.32)	(2050-8730)		
Kotédougou 13	1.28	1610	0.79	4.96
	(0.22)	(1150-2310)		
	Cypermethrin			
Matourkou 7	1.91	110	2.89	7.17
	(0.29)	(63-158)		
Yéguéresso 9	0.85	48	1.47	5.20
	(0.24)	(8-93)		
Farako-Ba 11	1.13	670	0.68	2.93
	(0.30)	(321-5562)		
Kotédougou 13	1.48	130	2.94	6.22
	(0.24)	(75-197)		
	Methamidophos			
Matourkou 7	2.04	680	5.40	3.41
	(0.23)	(288-1334)		
Yéguéresso 9	1.51	1390	3.21	3.03
	(0.21)	(711-2762)		
Farako-Ba 11	1.45	1957	0.59	3.72
	(0.21)	(1265-3219)		
Kotédougou 13	1.31	1688	7.22	2.07
	(0.25)	(838-2997)		

Table 10 (continued)

Localities/field #	Insecticide or mixture Slope (±SEM)	LC_{50} (mg l^{-1}) (95%CL)	χ^2 [a]	t [b]
	Cypermethrin + Omethoate			
Matourkou 7	1.30	410	1.34	4.01
	(0.29)	(140-680)		
Yéguéresso 9	2.24	800	2.09	2.69
	(0.30)	(470-1260)		
Farako-Ba 11	1.45	1000	0.47	3.39
	(0.23)	(620-1740)		
Kotédougou 13	2.76	274	1.36	5.19
	(0.36)	(50-530)		
	Cypermethrin + Methamidophos			
Matourkou 7	4.62	460	0.28	5.60
	(0.93)	(360-540)		
Yéguéresso 9	2.74	1130	3.76	2.97
	(0.38)	(620-1680)		
Farako-Ba 11	1.78	1120	2.50	3.65
	(0.28)	(750-1510)		
Kotédougou 13	3.72	890	2.47	2.06
	(0.65)	(670-1590)		

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,3}$: 7.85

[b] For t-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05, $t_{0.05,\infty}$ =1.96

Preface to chapter 3.

When an insect management crisis happens, new and effective insecticides are usually sought. Pyriproxyfen, acetamiprid, and diafenthiuron are new types of insecticides. Following the *B. tabaci* crisis in Burkina Faso they were granted a provisory authorization for use in cotton in 1999 and are being used since then. Given the high propensity of *B. tabaci* to develop resistance against several insecticides, the optimum procedure to preserve the effectiveness of these new compounds is to minimize selection pressure that their widespread use will exert on the pest populations. This required implementing a resistance management strategy at the inception of their use to delay the building of resistance against them to ensure their long lasting efficacy. Resistance management necessitates careful planning of insectide use and monitoring the field efficacy and responses of the pest to the compounds in the course of their use, to enable timely shift to new compounds acting differently to minimize selection pressure.

Because natural enemies contribute, along with other mortality agents e.g. insecticides, to inflict high levels of mortality on populations of *B. tabaci*, it is desirable that the use of insecticides be compatible with their activities. This compatibility is possible because several selective insecticides have been engineered in the past decade and their appropriate use may allow the integration of conservation biological control into IPM systems. In chapter 3, I investigated the effectiveness of pyriproxyfen, acetamiprid, and diafenthiuron against *B. tabaci* and their impact on its parasitism. The chapter has been submitted to the Journal of Environmental Sciences and Health: Part B. Pesticides, Food contaminants, and Agricultural. Wastes.

Chapter 3.

Field efficacy and baseline toxicities of pyriproxifen, acetamiprid, and diafenthiuron against *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) in Burkina Faso (West Africa).

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Submitted to: J. Environ. Sci. Health: Part B. Pest. Food cont., Agric. Wastes.

3. 1. Abstract.

Bioassays were conducted in 2001 and 2002 to estimate toxicities and dose-response relationships of 24 *Bemisia tabaci* Gennadius populations to pyriproxifen, acemitaprid, and diafenthiuron. LC_{50s} ranging from 0.014 to 0.096 mgl^{-1}, 0.60 to 1.3 mgl^{-1}, and 3.5 to 6.7 mgl^{-1} were observed respectively for pyriproxifen, acemitaprid, and diafenthiuron. These LC_{50s} were much lower than the field doses recommended for each compound. A fast increase in rates of mortality within a narrow range of lethal concentrations was observed for each compound, indicating that all three compounds were highly effective at killing whiteflies.

In a separate experiment, pyriproxifen, acemitaprid, and diafenthiuron were tested in 2001 and 2002 to compare their effectiveness and assess their impact on parasitism in the field. In both years all three compounds significantly prevented *B. tabaci* populations from reaching economic injury levels in cotton and minimized adverse effects on parasitism. Our results provide for the first time baseline toxicological, field efficacy, and effect on parasitism data of pyriproxifen, acemitaprid, and diafenthiuron against *B. tabaci* in West Africa. These compounds should be included in a resistance management program of the cotton pest complex and their use should be restricted to prevent the building of resistance in *B. tabaci* populations.

Key words. *Bemisia tabaci* Gennadius, pyriproxifen, acemitaprid, diafenthiuron, toxicity, dose-response relationships, parasitism.

3. 2. Introduction

The whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), has become one of the most economically important pests worldwide (Oliveira *et al.*, 2001). Losses estimated to hundreds of millions of US$ annually (Gerling and Mayer, 1996) have been reported in several agricultural systems, including greenhouse and ornamental productions, in tropical and subtropical areas of all continents except Antarctica. Damage is due to feeding and deposition of honeydew (Schuster *et al.*, 1996), physiological disorders (Yokomi *et al.* 1990) and, transmission of geminiviruses (Bedford *et al.*, 1994, Markham *et al.*, 1996). Typically, heavy infestations in cotton fields are often followed by dispersal to other field and vegetable crops following termination of the cotton crop (Byrne and Blackmer, 1996).

Insecticide applications are the main control tactics against this pest (Horowitz and Ishaaya, 1996). However insecticides may select for resistant *B. tabaci* populations (Palumbo *et al.*, 2001, Otoidobiga *et al.*, 2003) and suppress natural enemies (Naranjo, 2001). When an insect management crisis happens, new and effective insecticides are usually sought. Pyriproxyfen, acetamiprid, and diafenthiuron are new types of insecticides. Following a *B. tabaci* crisis in West Africa they were granted a provisional authorization for use in cotton in 1999 and since then are being used. Pyriproxyfen is an insect growth regulator acting to suppress adult emergence when sprayed on nymphs, and embryogenesis by direct contact with eggs deposited on lower leaf surfaces or indirectly through adult contact on treated foliage (Ishaaya and Horowitz, 1992). Acetamiprid belongs to a new class of insecticides referred to as nicotinoids, nitroquanidines, nitromethylenes, or chloronicotinyls. It is a systemic neurotoxin that is recommended for foliar spray against adults. Diafenthiuron belongs to

the thiourea derivative class. Used as foliar spray it affects insect respiration through the inhibition of oxidative phosphorylation and disruption of mitochondrial synthesis (Ruder and Kayser, 1993).

When wide usage of given pesticides is envisioned against a pest, resistance management strategies are required at the inception of the control program to delay the building of resistance and ensure long lasting efficacy o the compounds (Toscano *et al.*, 2001). Resistance management necessitates monitoring the field efficacy and responses of the pest to the compounds in the course of their uses. Of equal importance is the preservation of natural enemies that contribute to the pest control. In that context we document here 1) their LC_{50} and LC_{90} at the very earliest stage of their uses in Burkina Faso, 2) the field performance of pyriproxyfen, acetamiprid, and diafenthiuron on whiteflies and, 3) their impact on parasitism, and

3. 3. Materials and Methods

3. 3. 1. Laboratory Bioassays

3. 3. 1. 1. Insects

Bemisia tabaci populations were collected from August to November in cotton fields in 12 localities (Tables1-3) in the region of Bobo-Dioulasso (11°10'N, 4°19'W), Burkina Faso. Cotton plants were grown individually in 10-cm plastic pots and transported to the fields in 20x20x50 cm metal frame cages covered with 3-meshes/mm-nylon screen. In each field, insects were vacuumed using a Black and Decker® hand-portable battery-operated suction sampler. Approximately 5000 whiteflies were collected per field and released on five untreated cotton plants (*Gossypium hirsitum* L.). The samples were transported to the laboratory, and the plants with insects were placed into (1x1x1m) metal frame cages. The rearing cages were covered with 3-meshes/mm nylon screen and

contained 15 untreated cotton plants individually grown to the sixth true leaf stage in 10-cm plastic pots. *Bemisia tabaci* were held into these cages and used for bioassays the following day.

3. 3. 1. 2. Chemicals

The insecticides pyriproxyfen (10% emulsifiable concentrate, Tiger® 10 EC, Sumitomo Co Japan), acetamiprid (Mospilan®, 20 soluble pooder, Nippon Soda Co. Japan, and diafenthiuron (Polo® 500 concentrated solution, Ciba-Geigy, Basel, Switzerland) were bioassayed against *B. tabaci.* Sofitex, a Burkinabe cotton company, supplied all three insecticides. The insecticides were diluted with water to obtain the desired concentrations.

3. 3. 1. 2. 1. Pyriproxifen tests.

Pyriproxifen was tested against first instar nymphs. Unsprayed plants were first exposed for a 24-h egg laying period in a 1 x 1x 1 m cages where whiteflies from a given field were reared. Adult whiteflies were discarded and the plants containing eggs were held in a growth chamber under 26.7± 2°C, 30-40% RH, and 12:12 (L: D) photoperiod. Most eggs hatched on day 8 after oviposition and all immature stages were removed, leaving only 40 first instar nymphs to be treated for each concentration and replicate per field. Using a Matabi® hand sprayer, the plants were sprayed to run off with the various concentrations of the formulated compounds or water (control) and allowed to air dry. Each bioassay was done with five concentrations, and each concentration was replicated three times. Control plants were sprayed with water and allowed to dry under sunlight. The treated plants were placed in a different growth chamber under the same conditions mentioned above. Adult emergence was assessed on day 21 after oviposition. Nymphs from which no adult emerged were considered dead.

3. 3. 1. 2. 2. Acetamiprid tests.

Acetamiprid was tested against *B. tabaci* adults. Cotton plants were individually grown to the sixth true leaf stage in 10-cm plastic pots. Using a Matabi® hand sprayer, the plants were sprayed to run off with the various concentrations of the formulated compounds or water (control) and allowed to air dry. For each field and each concentration replicate, 40 whiteflies were aspirated from rearing cages and confined in leaf clip-on cages as described in Otoidobiga *et al.* (2003). Each cage was clipped on the lower surface of the fifth fully developed leaf on the plant. The leaf cages were incubated for 48 hours at 25°C. After the 48h, the experimental units, (i.e. leaf cages enclosing whiteflies) were detached from plants and whiteflies were emptied in a petri dish and examined for movements under a stereomicroscope. Dead whiteflies were immobile and shriveled while living whiteflies were active. Each bioassay was done with five concentrations, and each concentration was replicated three times. Control plants were dipped into water and allowed to dry under sunlight.

3. 3. 1. 2. 3. Diafenthiuron tests.

Diafenthiuron was tested against second instar nymphs. Untreated test plants were exposed to whiteflies of a given field in the rearing cage where they were swarming. After a 24-h egg laying period, the plants containing eggs were placed in a growth chamber at 26.7± 2°C, 30-40% RH, and 12:12 (L: D) photoperiod. The leaves were examined every second day. In general the majority of nymphs on leaves reached the second instar stage on day 13 after oviposition. All immature stages were then removed from test leaves, leaving only 40-second instar nymphs before treatment. Leaves infested with nymphs were sprayed to run off with the solutions using a Matabi® hand sprayer, and the plants were exposed to sunlight for two hours to allow the transformation of diafenthiuron to its more active carbodiimide derivative. Nymph mortality was determined 48h after treatment. Each bioassay was done

with five concentrations, and each concentration was replicated three times. Control plants were dipped into water and allowed to dry under sunlight. LC_{50} and LC_{90} values for each insecticide were calculated with POLO-PC.

3. 3. 2. Field experiments.

Experiments were conducted at the agricultural experimental station of Farako-Ba, located 10 km west of Bobo-Dioulasso, Burkina Faso, in cotton fields measuring 4 and 2.5ha respectively in 2001 and 2002. The fields were subdivided in plots (0.02 to 0.4ha) laid 3m apart in a completely randomized design, and sown with the local cotton cultivar FKB 290. Two applications of Endosulfan (EC 700g/ha) were done during the young plant- square period in all treatments including controls with the objective to protect cotton against key pests e.g. *Heliothis armigera* Hübner (Noctuidae), and *Aphis gossypii* Glover (Aphidae). The chemicals under experiment were pyriproxyfen (Tiger® 60g a.i./L/ha), acetamiprid (Mospilan® 60g a.i/L/ha), and diafenthiuron (Polo®, 300g a.i./L/ha). The commercial products were sprayed as recommended by the manufacturer, i.e. in 100L of water per ha at dates shown in Figs.1A, B, C, and D. Control plots were sprayed with water.

When *B. tabaci* infestations begun, 30 leaves situated on the fifth node position down from the terminal were randomly sampled per plot using the method described by Ellsworth *et al.*, (1996). The leaves were brought back to the laboratory in an ice cooler. In the laboratory they were transferred into a refrigerator and, the following day, a leaf disk of ca. 4 cm^2 was cut between the main veins of each leaf. All the nymphs on the leaf disks were counted and examined with a stereomicroscope to assess apparent parasitism, hereafter mentioned as parasitism. Insecticide treatments against *B. tabaci* started when a mean of 1 nymph/leaf disk (i.e. the action threshold of Ellsworth *et al.*, 1996) was observed in the plots. The abundance of the pest and its parasitoids was assessed every week. Fisher's LSD test was used to compare weekly and seasonal densities of *B. tabaci* % parasitism. Voucher specimens of

insects were deposited at the Laboratoire de Recherches, Institut de l'Environnement et des Recherches Agricoles, Bobo-Dioulasso, Burkina Faso, and at the Kansas State University, Manhattan, Kansas, and the University of Tucson, Arizona, where identification was made.

3. 4. Results

3. 4. 1. Bioassays

3. 4. 1. 1. Baseline susceptibility to pyriproxifen.

In 2001 LC_{50s} varied from 0.03 to 0.10 mgL^{-1} (1-to 3 fold), and LC_{90s} from 0.24 to 1.59 mgL^{-1} (1-to 6.6 fold). In 2002 LC_{50s} varied from 0.01 to 0.09 mgL^{-1} (1-to 6.43 fold), and LC_{90s} from 0.11 to 0.9 mgL^{-1} (1-to 8.18 fold) (Table 1). Among the fields surveyed, LC_{50s} and LC_{90s} were lower in 2002 compared to 2001 in 5 and 6 fields out 8 respectively. The slopes of regression lines denoted a fast increase in rates of mortality, and the 95% confidence delimited a narrow range of lethal concentrations.

3. 4. 1. 2. Baseline susceptibility to acetamiprid.

In 2001 LC_{50s} varied from 0.60 to 1.80 mgl^{-1} (1-to 3 fold), and the LC_{90s} from 4.30 to 16.30 mgL^{-1} (1-to 3.8 fold). In 2002 the LC_{50s} varied from 0.62 to 1.30 mgl^{-1} (1-to 2 fold), and LC_{90s} from 1.53 to 4.59 mgL^{-1} (one to 3 fold) (Table 2). Among the fields surveyed, LC_{50s} in 8 out of 12, and all LC_{90s} were lower in 2002 compared to 2001. The slope of regression lines denoted a fast increase in the rates of mortality, and the 95% confidence limits indicated a narrow range of lethal concentrations.

3. 4. 1. 3. Baseline susceptibility to diafenthiuron.

In 2001 LC_{50s} varied from 5.10 to 8.80 mgL^{-1} (1-to 1.72 fold), and LC_{90s} from 15.10 to 25.40 mgL^{-1} (1-to 1.68 fold). In 2002 LC_{50s} varied from 3.50 to 6.70 mgL^{-1} (1-to 1.91 fold), and LC_{90s} from 11.10 to 31.30 mgL^{-1} (1-to 2.81 fold) (Table 3). Among the fields surveyed, the LC_{50s} in 8 out of 12 were lower in 2002 compared to 2001, but LC_{90s} were higher in 7 fields out of 12 in 2002 compared to 2001. The slope of regression lines varied from 2.05 to 3.30 in 2001 and from 1.80 to 3.16 among fields in 2002 and denoted a fast increase in mortality rates, with the 95% confidence limits indicating a narrow range of lethal concentrations.

3. 4. 2. Field experiments.

In both 2001 and 2002 there were significantly fewer whiteflies in acetamiprid and diafenthiuron treated plots compared to the control one week after the first spray of these insecticides. From the second week after spray with the three insecticides to the end, there were significantly fewer whiteflies in all insecticides treated plots compared to the control except on October 31, 2002 where pyriproxifen were not significantly different from the control. No significant differences were observed among weekly and seasonal means of the three insecticides. However there were on average fewer whiteflies in diafenthiuron treated plots compared to the other insecticides from October 18 to November 15, 2001 and from October 17 to October 31, 2002 (Fig. 1).

No significant differences were observed in the levels of parasitism among treatments in both 2001 and 2002. However average parasitism in acetamiprid treated plots was less than in diafenthiuron, pyriproxifen and control plots. Similarly average parasitism in diafenthiuron treated plots was less than in pyriproxifen and control plots. Parasitism in pyriproxyfen was higher than that in the control in four and one occasions respectively in 2001 and 2002.

3. 5. Discussion.

3. 5. 1. Bioassays

It is generally assumed that the frequency of resistance genes in pest populations to a given insecticide is low in the absence of exposure of the pest populations to the chemical (Roush and Daly, 1991). Once a given pesticide is widely used against a pest, frequencies of resistant alleles may increase as a result of selection (Dobzhanty, 1951, Roush and Daly, 1991). Selection has major influence on the evolution of the susceptibility of the populations of this pest to the pesticide, and is the most likely factor that can be manipulated to maintain the efficacy of the pesticide. The manipulation of selection to influence evolution of susceptibility requires monitoring for resistance, which traditionally involves comparisons of LC_{50s}, LC_{90s}, or slopes of dose-response curves between field and laboratory populations (Staetz, 1985). In the absence of laboratory population (or reference strain), the LD_{50}s of field populations can be compared against specific insecticide levels, e.g. the doses recommended in the field. In this case a population is considered as "susceptible" to the pesticide used against it if the LD_{50} and slope of dose-response of this population are below those recommended in the field. Conversely this population is considered as "resistant" to the pesticide used if its LD_{50} and slope of dose-response are above the dose recommended (Roush and Miller, 1986).

Our study was initiated to begin field efficacy and resistance monitoring of whitefly populations in Burkina Faso to the newly introduced compounds with the requirement to prevent the building of resistance gene pools in *B. tabaci* populations and ensure long lasting susceptibility of the pest populations to the compounds. In both years the LC_{50s} and LC_{90s} of pyriproxifen, acetamiprid, and diafenthiuron were much lower than their respective recommended field doses, indicating that the compounds were highly effective at killing whiteflies.

The differences observed in LC_{50s} and $LC9_{0s}$ between fields and year may be due to natural variation or past exposure to insecticides. The decrease in LC_{50s} and LC_{90s} observed during the study period indicates a loss of gain in tolerance between the two seasons. This change in susceptibility of *B. tabaci* populations between seasons has also been observed for pyriproxyfen in Israel (Horowitz *et al.* 1999, 2002), and in the USA (Toscano *et al.,* 2001) and may reflect fitness costs (Horowitz *et al.*, 2002).

Although the levels of variations of susceptibility were low (< 10 fold) among fields for all three chemicals, and in general increased or remained much the same in 2002 compared to 2001, the potential for resistance building exists. This is particularly true for pyriproxyfen and acetamiprid. *B. tabaci* population developed > 500 fold resistance to pyriproxyfen after three consecutive applications in a greenhouse (Horowitz and Ishaaya, 1994; Horowitz *et al.* 2002), and Elbert and Nauen (2000) demonstrated strong cross-resistance between acetamiprid, imidacloprid and thiamethoxan (insecticides of the same nicotinoid class) in Spain although acetamiprid was not used in that country. Evidence of the high propensity of *B. tabaci* for developing nicotinoid resistance was further demonstrated by 9-fold increase in tolerance in the fifth generation and > 80-fold resistance to imidacloprid after 24 generations in *B. tabaci* population placed under continuous laboratory exposure (Prabhaker *et al.*, 1997). To our knowledge no resistance to diafethiuron has been reported to date. This may be due to the fact that the compound is not yet widely used.

Baseline LC_{50s} and LC_{90s} of *B. tabaci* have been established at 0.01 and 0.04 mgL^{-1} in nymphs for pyriproxifen (Horowitz and Ishaaya, 1994), 1.6 and 12.8 mgL^{-1} for acetamiprid against adults (Horowitz *et al.*, 1998), and 6.5 and 49.2 mgL^{-1} for diafenthiuron against 2nd instar nymphs (Ishaaya *et al.*, 1993) in Israel. Subsequently *B. tabaci* developed resistance to pyriproxifen in Israel, with LC_{50s} and LC_{90s} respectively rising to 14.41 and 107.21 mgL^{-1} (Horowitz and Ishaaya, 1994). In California,

LC_{50s} for pyriproxifen were lowest (0.03 mgL^{-1}) in the San Joaquin valley and highest (10.02 mgL^{-1}) in the Imperial Valley in 1997. In 1999, the LC_{50} for pyriproxifen was restored to 0.03 in the Imperial Valley (Toscano *et al.*, 2001). The LC_{50s} and LC_{90s} observed in Burkina Faso were lower than those reported in the USA and Israel, where the compound has been in use for about a decade. The current status in Burkina Faso reflects the high susceptibility of naïve *B. tabaci* to these newly introduced compounds.

The success of the Burkina Faso program to maintain the susceptibility of *B. tabaci* from the introduction of the compounds in 1999 to 2002 could be partly attributed to the fact that the compounds were sprayed only when pest populations reached the threshold levels of 1 nymph/leaf disk. Because levels of infestations on cotton usually vary from field to field (Naranjo *et al.*, 1996a, 1997), treating at threshold level suited the financial concerns of small holding farmers associated with the high cost of these novel insecticides. Treating at threshold level differs fundamentally from time-scheduled treatment programs that oblige farmers to spray even if the levels of infestation of their farms are low (see Otoidobiga *et al.*, 2003).

The untreated fields may have also served as refuges, allowing the dilution of resistance gene pools from treated fields. In addition, after their sprays in cotton from September to October, the insecticides were not used in other crops, making insecticide free periods long enough to dilute resistance gene pools as the whiteflies reproduced on untreated host plants.

3. 5. 2. Field experiments

Although none of the insecticides was able to bring the pest populations under the action threshold (1 nymph/leaf disk, Ellsworth *et al.*, 1996), all three differed significantly from controls and prevented *B. tabaci* populations from reaching the economic injury level i.e: 6.8-18.8 nymphs/leaf disk

(Fig. 1A and C) (Naranjo *et al.*, 1996b). Diafenthiuron applied twice consecutively in 7day intervals better suppressed whitefly populations compared with pyriproxifen and acetamiprid although no significant differences were observed among treatments.

The fact that no significant differences were observed in % parasitism between control and insecticide treated plots indicates that all three insecticides were relatively benign to parasitoids. Pyriproxyfen was more selective than both acetamiprid and diafenthuiron, and acetamiprid was less selective than diafenthiuron. Pyriproxifen is species-and life stages-specific i.e. toxic to some species and life stages but benign to others (Naranjo, 2001). For example it was found more harmful to *Encarsia formosa* Gahan compared with *Encarsia pergandiella* Howard or *Encarsia transvena*, and toxic to early stages of *Eretmocerus eremicus,* but benign to young stages of three species of *Encarsia* (Liu and Stansly, 1997). In contrast little information is available on the toxicities of acetamiprid and diafenthiuron against natural enemies. However acetamiprid has a broader insecticidal spectrum (Takahashi *et al.*, 1992) than diafenthiuron.

3. 6. Conclusion

In this study, we have estimated the variations in responses and established dose-response relationships to pyriproxifen, acemitaprid, and diafenthiuron in 24 *B. tabaci* populations in Burkina Faso. These insecticides are likely to remain major components of *B. tabaci* suppression; our results should represent baseline references and provide a foundation for the management of the pest in cotton in Burkina Faso. The field experiment data indicate that the three compounds significantly delay *B. tabaci* populations from reaching economic injury levels in cotton and minimize adverse effects on parasitism. These new compounds should be included in a resistance management program of the

cotton pest complex and their use restricted in ways that prevent the building of resistance in *B. tabaci* populations against them.

3. 7. Acknowledgements

This research was supported by the Institut de l'Environement et des Recherches Agricoles (INERA) and the Société des Fibres Textiles (Sofitex) du Burkina Faso. We are indebted to Dakuo Déhou, Adjunct Director of the Direction du Développement de la Production cotonnière (DDPC) of Sofitex for facilitating diverse research activities. Many thanks to Paco Séréme, Konaté Gnissa and Abdoussalam Sawadogo for administrative support. We are grateful to Bambara Issa, Dakuo Patrice and Sinon El Adj Adama for technical assistance.

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Table 11. Probit analyses of first instar *B. tabaci* nymphs' susceptibilities to pyriproxifen (n=200; df =3) in Burkina Faso.

Localities/	2001					2002				
field #	Slope (±SEM)	t [b]	χ^2 [a]	LC_{50} (mg L^{-1}) (95%CL)	LC_{90} (mg L^{-1}) (95%CL)	Slope (±SEM)	t [b]	χ^2 [a]	LC_{50} (mg L^{-1}) (95%CL)	LC_{90}(mg L^{-1}) (95%CL)
Dédougou 1	2.56 (0.15)	6.95	5.83	0.03 (0.03-0.09)	0.50 (0.15-4.20)	2.14 (0.27)	4.15	0.19	0.05 (0.01-0.10)	0.6 (0.30-1.80)
Solenzo 2	2.12 (0.18)	6.33	5.01	0.03 (0.02-0.09)	0.41 (0.13-3.67)	2.15 (0.18)	6.48	2.96	0.03 (0.01-0.04)	0.32 (0.18-0.81)
Safané 3	2.22 (0.20)	5.92	4.72	0.10 (0.05-0.27)	1.06 (0.35-3.10)	1.89 (0.14)	6.18	1.48	0.02 (0.01-0.04)	0.56 (0.27-1.69)
Bayé 4	1.90 (0.13)	6.59	2.87	0.03 (0.02-0.05)	0.24 (0.14-0.56)	2.00 (0.15)	6.46	1.67	0.02 (0.01-0.04)	0.45 (0.24-1.22)
Yéguérésso5	2.40 (0.22)	6.35	1.05	0.03 (0.01-0.06)	1.1 (0.5-3.84)	2.30 (0.26)	4.97	0.55	0.09 (0.03-0.16)	0.90 (0.50-2.30)
Farakoba 6	1.90 (0.13)	6.59	2.87	0.06 (0.02-0.11)	1.59 (0.8-5.20)	1.50 (0.36)	4.16	0.82	0.07 (0.02-0.12)	0.50 (0.30-1.30)
Houndé 7	2.21 (0.17)	7.12	7.32	0.06 (0.004-0.22)	0.69 (0.19-3.70)	2.45 (0.42)	3.44	0.4	0.04 (0.01-0.09)	0.34 (0.20-1.00)
Boromo 8	2.12 (0.16)	7.03	1.58	0.09 (0.04-0.15)	1.29 (0.7-3.29)	2.06 (0.18)	6.00	3.16	0.02 (0.01-0.05)	0.31 (0.12-3.08)
Fô 9	Not tested					2.27 (0.20)	6.35	1.31	0.02 (0.01-0.02)	0.22 (0.13-0.53)
Pâ 10	Not tested					2.39 (0.27)	5.20	1.04	0.01 (0.01-0.02)	0.11 (0.07-0.29)
Dandé 11	Not tested					2.33 (0.23)	5.69	1.02	0.02 (0.01-0.03)	0.15 (0.09-0.38)

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,3}$: 7.85

[b] For t-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05, $t_{0.05,\infty}$ =1.96

Table 12. Probit analyses of first instar *B. tabaci* nymphs' susceptibilities to acemitaprid (n= 200; df =3) in Burkina Faso

Localities/	2001					2002				
field #	Slope (±SEM)	t [b]	χ^2 [a]	LC_{50} (mg L^{-1}) (95%CL)	LC_{90} (mg L^{-1}) (95%CL)	Slope (±SEM)	t [b]	χ^2 [a]	LC_{50} (mg L^{-1}) (95%CL)	LC_{90} (mg L^{-1}) (95%CL)
Dédougou 1	2.50	4.80	1.90	0.60	4.30	3.20	5.30	1.60	0.62	1.53
	(0.31)			(0.20-1.10)	(2.70-7.90)	(0.62)			(0.45-0.76)	(1.21-2.33)
Solenzo 2	2.49	5.90	4.80	1.07	7.74	2.60	6.10	1.20	0.68	2.11
	(0.25)			(0.05-2.50)	(3.50-6.23)	(0.43)			(0.50-0.85)	(1.62-3.29)
Safané 3	2.35	6.20	5.60	1.80	16.30	2.91	6.30	1.10	0.88	2.44
	(0.22)			(0.10-4.30)	(6.90-30.4)	(0.46)			(0.67-1.10)	(1.88-3.71)
Bayé 4	2.40	5.70	4.30	1.40	11.70	2.43	7.20	1.50	0.97	3.25
	(0.24)			(0.08-3.40)	(5.1-35)	(0.34)			(0.76-1.20)	(2.46-5.04)
Yéguérésso5	2.40	5.70	5.70	1.10	15.29	2.37	6.80	7.70	1.04- [c]	6.54- [c]
	(0.24)			(0.15-2.50)	(12.10-3.30)	(0.35)				
Farakobâ 6	2.30	5.90	4.10	1.40	13.60	2.51	6.90	0.50	1.12	3.63
	(0.22)			(0.12-3.20)	(6.2-39)	(0.36)			(0.83-1.42)	(2.72-5.63)
Houndé 7	2.45	5.40	3.30	0.90	7.10	2.55	7.00	0.30	0.88	2.81
	(0.27)			(0.06-2.13)	(3.40-32.50)	(0.36)			(0.7-1.10)	(2.14-4.29
Boromo 8	1.68	6.40	2.10	1.22	7.00	2.06	6.40	0.80	0.76	3.20
	(0.26)			(0.65-1.81)	(4.87-11.90)	(0.32)			(0.52-10)	(2.33-5.31)
Fô 9	1.72	5.10	0.7	0.80	4.30	2.76	6.70	1.20	1.18	3.44
	(0.34)			(0.3-1.27)	(2.80-7.70)	(0.41)			(0.89-1.50)	(2.62-5.23)
Pâ 10	2.46	6.10	4.60	1.30	9.40	2.16	6.70	2.68	1.17	4.59
	(0.24)			(0.09-2.8)	(4.30-25.00)	(0.32)			(0.83-1.53)	(3.31-7.6)
Dandé 11	2.32	6.40	1.50	0.80	4.20	2.47	7.50	2.49	1.07	3.55
	(0.36)			(0.3-1.30)	(2.50-10.40)	(0.33)			(0.85-1.32)	(2.68-5.46)
Bama 12	2.94	6.40	1.30	1.26	9.40	2.58	7.30	1.80	1.30	4.09
	(0.47)			(0.09-2.80)	(4.30-17.00)	(0.35)			(1.0-1.63)	(3.09-6.25)

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,3}$: 7.85

[b] For t-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05, $t_{0.05,\infty}$ =1.96

[c] 95% CL value not presented because the index of significance for potency estimation, 'g' exceeded 0.5 as calculated with Polo-PC.

Table 13. Probit analyses of first instar *B. tabaci* nymphs' susceptibilities to diafenthiuron (n=200; df =3) in Burkina Faso.

Localities/ field #	2001					2002				
	Slope (±SEM)	*t*	χ^2	LC_{50} (mg L^{-1}) (95%CL)	LC_{90} (mg L^{-1}) (95%CL)	Slope (±SEM)	*t*	χ^2	LC_{50} (mg L^{-1}) (95%CL)	LC_{90} (mg L^{-1}) (95%CL)
Dédougou 1	3.3 (0.61)	5.40	1.40	6.20 (4.70-7.60)	15.10 (12.00-22.60)	2.58 (0.65)	3.90	0.09	3.50 (1.5-50)	11.1 (8.40-18.30)
Solenzo 2	2.6 (0.52)	5.10	0.47	5.10 (3.30-6.60)	15.60 (12.00-24.70)	2.41 (0.47)	5.04	0.67	4.90 (2.90-6.50)	16.60 (12.60-27.00)
Safané 3	3.1 (0.63)	4.90	0.22	8.80 (6.70-10.96)	24.40 (18.80-37.10)	3.16 (0.64)	4.96	0.93	5.70 (3.90-7.20)	14.50 (11.30-22.60)
Bayé 4	3.38 (0.65)	5.20	0.27	5.70 (4.20-7.00)	13.70 (10.90-20.40)	3.00 (0.60)	5.40	0.81	6.20 (4.40-7.80)	16.60 (12.90-25.70)
Yéguérésso5	2.81 (0.46)	6.10	0.91	6.97 (5.30-8.50)	19.80 (15.40-30.30)	1.91 (0.31)	6.05	0.84	6.70 (4.20-8.97)	31.30 (22.40-53.90)
Farakobâ 6	2.05 (0.36)	5.60	0.28	5.43 (3.30-7.40)	22.90 (16.80-38.30)	2.03 (0.37)	5.41	0.40	5.60 (3.20-7.70)	23.70 (17.10-40.80)
Houndé 7	2.4 (0.42)	5.80	1.93	5.90 (4.00-7.60)	20.00 (15.20-31.60)	2.08 (0.40)	5.18	2.07	5.00 (2.80-6.90)	20.70 (15.10-35.30)
Boromo 8	2.72 (0.51)	5.30	2.10	5.70 (3.90-7.30)	16.90 (13.00-26.70)	1.80 (0.32)	5.67	0.65	5.50 (3.10-7.60)	27.50 (19.70-48)
Fô 9	2.05 (0.34)	6.00	0.70	6.40 (3.90-8.00)	25.40 (18.80-42.20)	2.01 (0.34)	5.90	1.87	6.70 (4.20-9.00)	29.10 (20.90-49.70)
Pâ 10	2.64 (0.47)	5.60	1.20	6.60 (4.60-8.40)	20.10 (15.30-31.90)	2.64 (0.45)	5.87	1.03	6.40 (4.60-8.00)	19.50 (15.00-30.40)
Dandé 11	2.62 (0.5)	5.20	2.50	5.40 (3.60-7.00)	17.00 (12.90-27.10)	2.61 (0.50)	5.20	2.50	5.50 (3.60-7.00)	16.90 (13.00-27.10)
Bama 12	2.24 (0.38)	5.90	0.21	6.00 (4-70.8.00)	22.50 (16.80-35.90)	2.05 (0.41)	5.03	2.40	4.90 (2.60-7.00)	20.80 (15.00-36.40)

[a] For χ^2 values <critical values, the model fits, $\chi^2_{0.05,3}$: 7.85

[b] For *t*-ratio values >critical values, the regression (i.e. the probit response) is significant at P=0.05, $t_{0.05,\infty}$ =1.96

Legend of figure.

Fig. 2. Abundance of *B. tabaci* and its parasitoids' Populations under different insecticide programs in 2001 (A and B), and 2002 (C and D). The dotted horizontal line denotes the action threshold for nymphs (Ellsworth *et al.* 1996). The arrow indicates the economic injury level (Naranjo *et al.* 1996b). Black circles indicate the dates of endosulfan applications against *Helicoverpa armigera* Hubner in all plots including controls. Cubes, black crosses and white cylinders indicate the dates of pyriproxifen, acetamiprid and diafenthiuron applications. Open circles indicate dates at which insecticide treated plots were significantly less infested than control plots.

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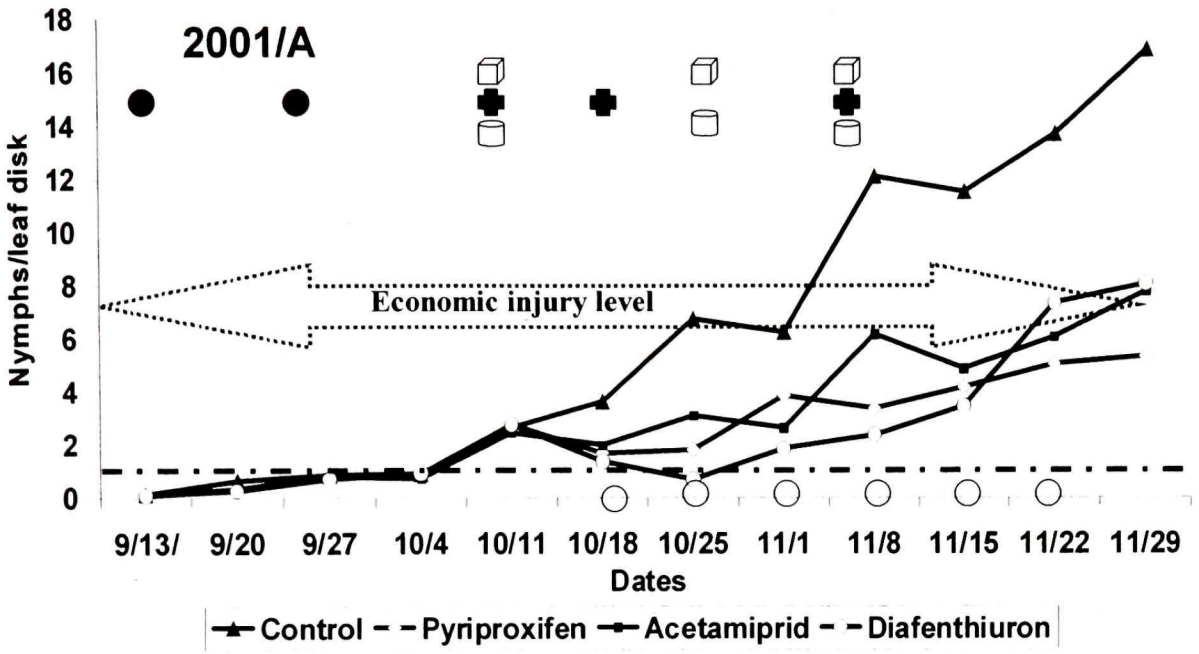
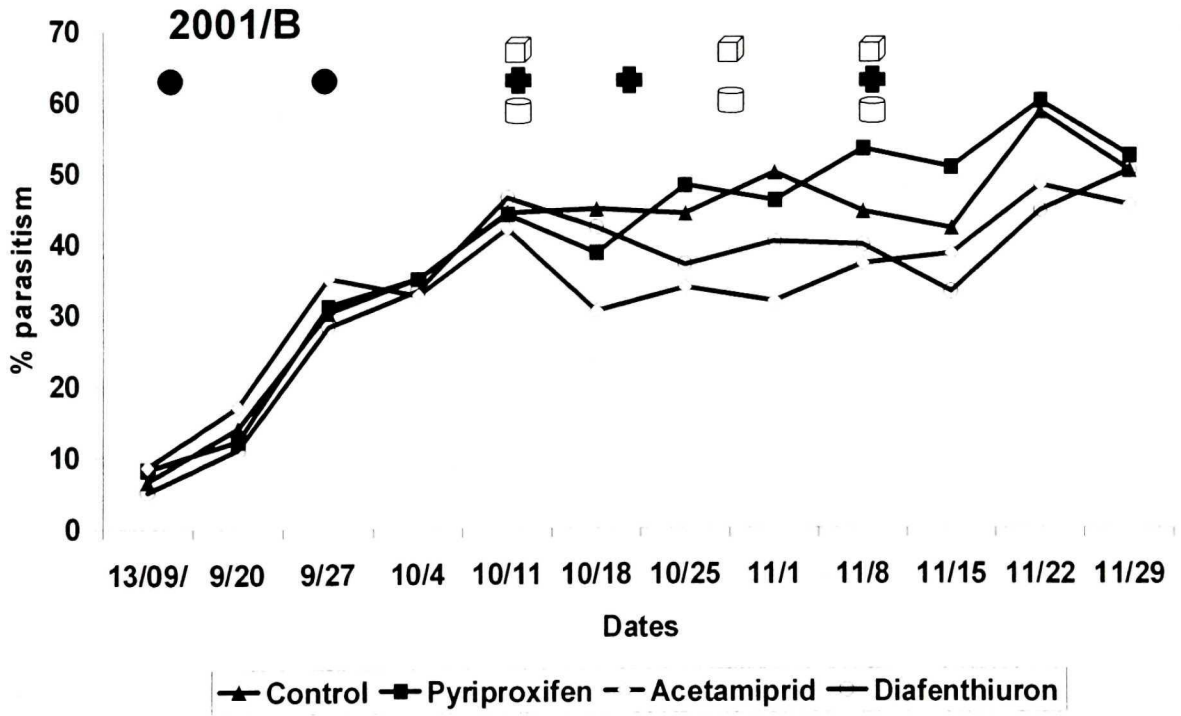
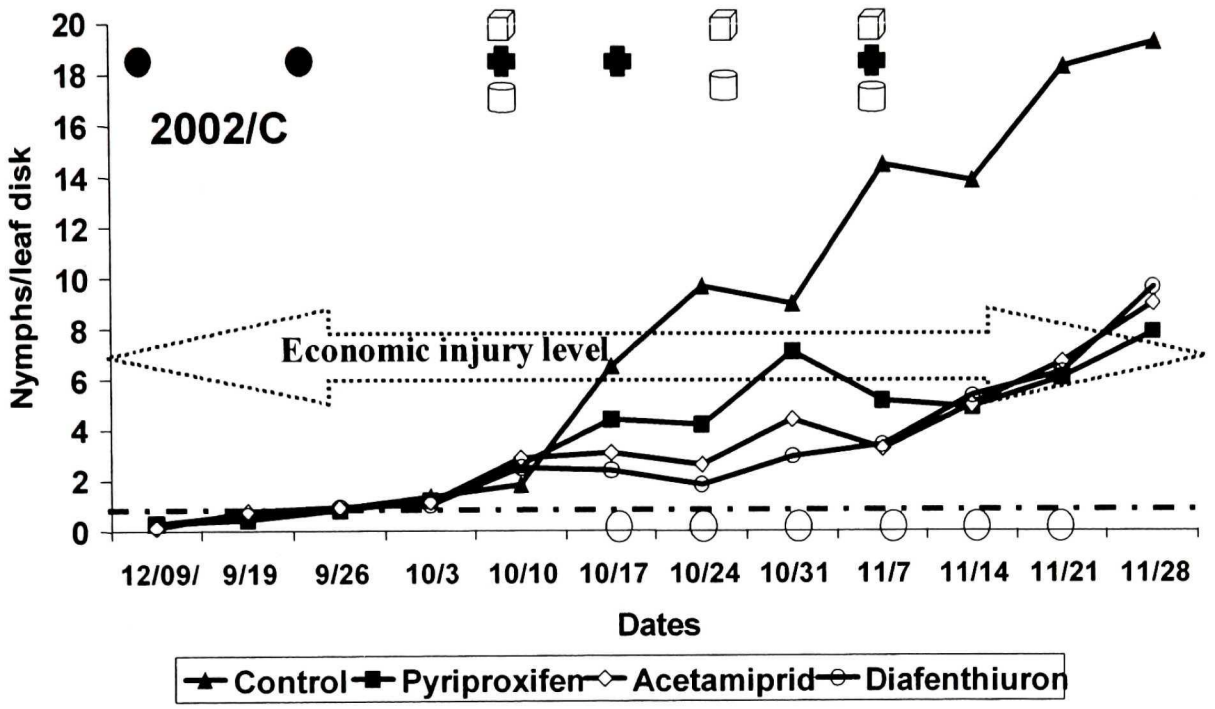
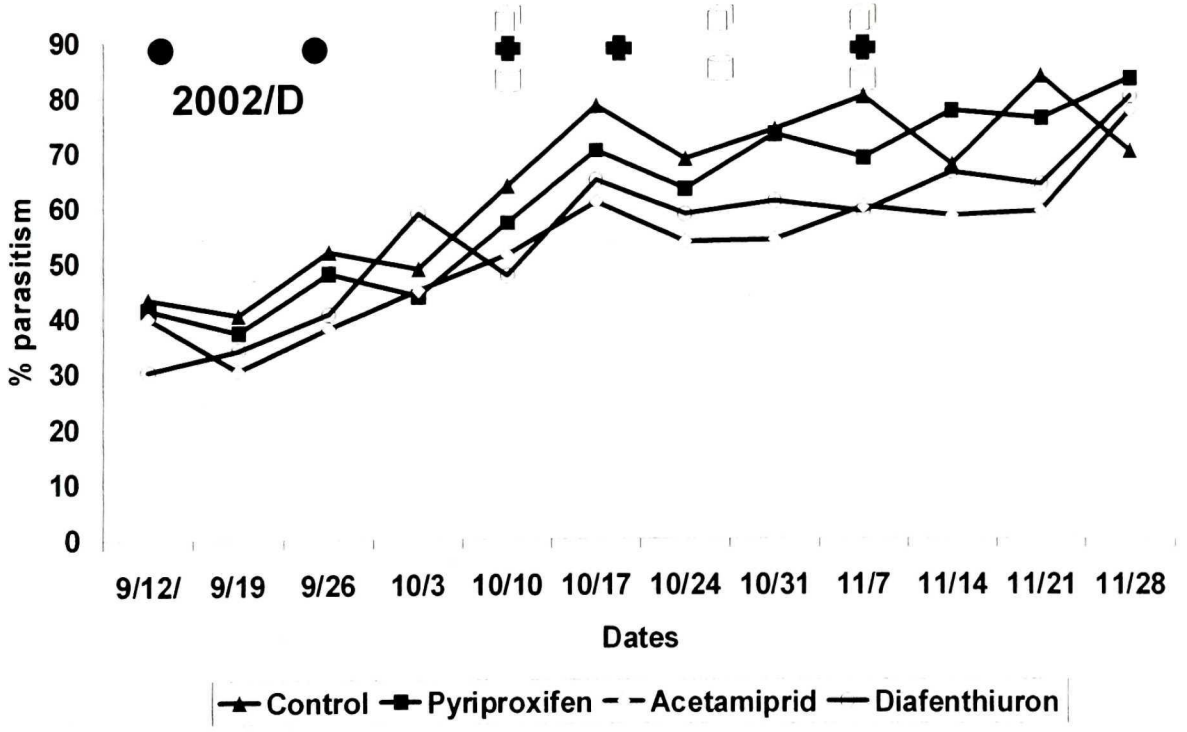

2001/A
Nymphs/leaf disk
18
16
14
12
10
8
6
4
2
0
Economic injury level
9/13/
9/20
9/27
10/4
10/11
10/18
10/25
11/1
11/8
11/15
11/22
11/29
Dates
Control
Pyriproxifen
Acetamiprid
Diafenthiuron


2001/B
% parasitism
70
60
50
40
30
20
10
0
13/09/
9/20
9/27
10/4
10/11
10/18
10/25
11/1
11/8
11/15
11/22
11/29
Dates
Control
Pyriproxifen
Acetamiprid
Diafenthiuron


2002/C
Nymphs/leaf disk
20
18
16
14
12
10
8
6
4
2
0
Economic injury level
12/09/
9/19
9/26
10/3
10/10
10/17
10/24
10/31
11/7
11/14
11/21
11/28
Dates
Control
Pyriproxifen
Acetamiprid
Diafenthiuron


2002/D
% parasitism
90
80
70
60
50
40
30
20
10
0
9/12/
9/19
9/26
10/3
10/10
10/17
10/24
10/31
11/7
11/14
11/21
11/28
Dates
Control
Pyriproxifen
Acetamiprid
Diafenthiuron

Preface to chapter 4.

The relative abundances of a pest and its natural enemy species at a given time are important components that pest management strategists seek to manipulate to achieve effective control of the pest. Because natural enemy species have proven effective components of *B. tabaci* control in many agricultural systems, their activity against the pest is exploited by employing proper conservation of extant fauna and, by augmentation of parasitoids and predators in greenhouses and in fields. However little research has addressed the evaluation of parasitoids effects on *B. tabaci* population dynamics in cotton fields. As a result information about how to better exploit the activities of extant natural enemies in the field is inadequate and, limits our ability to predict and exploit these activities to achieve greater suppression of the pest in this crop. For example little is known about parasitism levels year round in continous cultivation of a given crop, and particularly about the potential of African *Eretmocerus* against *B. tabaci.* There are still unexplored, potentially valuable species of natural enemies in many other areas of the world. The relative abundance of *B. tabaci* and its parasitoids and augmentation biological control as a key tactic in IPM of *B. tabaci* is the topic of chapter 4, which has been submitted to the International Journal of Pest Management in December 2002.

Chapter 4.

Relative Abundance of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and its Parasitoids and Impact of Augmentative Release of *Eretmocerus* spp. (Hymenoptera: Aphelinidae) on the Population Dynamics of the Pest in Burkina Faso (West Africa).

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Submitted to International Journal of Pest Management in November 2002.

4.1. Abstract

Whitefly infestations and parasitism were monitored year round in overlapping cotton crops sown at three dates in Burkina Faso. The relative abundance of *B. tabaci* (Gennadius) and its parasitoids, *Eretmocerus* spp. and *Encarsia* spp. was recorded in control and insecticide sprayed plots. Low *B. tabaci* populations developed during the first half of the rainy season. Pest populations increased when rainfall was ending and the levels reached were higher in insecticide treated plots (48 nymphs/leaf) than in control (25 nymphs/leaf) plots. Parasitism reached 88.7% in control plots, and 53.7% in insecticide treated plots. *Eretmocerus* spp. were more abundant than *Encarsia* spp. in both treated and control plots. A positive and significant curvilinear relationship was observed where % parasitism on a linear scale rose to a plateau with logarithmic increase in host density. In general % parasitism followed the abundance of pest populations except in March and April where parasitism increased while *B. tabaci* populations decreased.

In a separate experiment, adult *Eretmocerus* spp. were released into caged cotton plants to study the impact of augmentative releases of the parasites on the population dynamics of the pest. Pest densities increased from 1.47nymphs/leaf to 39.4 nymphs/leaf in the control, but were reduced to 0.8 and 0.6 nymphs/leaf in the cages where respectively four and height parasitoids have been released per plant. Parasitism is an important factor reducing *B. tabaci* populations during and after the cotton growing season and, *Eretmocerus* spp. are promising biological control candidates against the pest in cotton.

Key words: *Bemisia tabaci*, *Eretmocerus* spp., *Encarsia* spp., relative abundance, population dynamics, parasitism.

4. 2. Introduction

The Whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), colonizes most agricultural systems, including greenhouses, in tropical and subtropical areas of all continents except Antarctica (Oliveira *et al.*, 2001). Recently, damage attributed to the pest has increased dramatically to hundreds of millions of US$ annually worldwide (Gerling and Mayer, 1996). Damage is due to feeding, deposition of honeydew (Schuster *et al.*, 1996), physiological disorders (Yokomi *et al.*, 1990) and transmission of geminiviruses (Bedford *et al.*, 1994; Markham *et al.*, 1996). Heavy infestations in cotton fields are often followed by dispersal to other field and vegetable crops following harvesting of the cotton crop (Byrne and Blackmer, 1996; Oliveira *et al.*, 2001). Pest populations then decrease in these systems (Riley *et al.*, 1996).

No significant economic damage had been caused to crops in West Africa until population outbreaks started in 1998 in cotton crops in Burkina Faso, the Republics of Mali and Ivory Coast (Otoidobiga personal observation). Insecticide applications are the main control tactics against this pest (Horowitz and Ishaaya, 1996; Palumbo *et al.*, 2001), but control failures due to insecticide resistance (Palumbo *et al.*, 2001; Otoidobiga *et al.* 2003), and the suppression of natural enemies by wide spectrum insecticides have been frequently reported (Naranjo, 2001).

Among the numerous species of natural enemies (Gerling *et al.*, 2001), two genera of aphelinid parasitoids, i.e. *Encarsia* spp. and *Eretmocerus* spp., constitute major components of the whitefly-parasite complex (Rose *et al.*, 1996). Investigations conducted in different crops have frequently reported up to 80% parasitism in the field towards the end of the growing season (Bellows and Arakawa, 1988; Sundaramurthy, 1992). Efforts have been made in augmentation release i.e. release of natural enemies to increase the ratio natural enemies /host with objective to generate greater

suppression of *B. tabaci* populations in greenhouses (e.g., van Lenteren et al., 1997; Hoddle et al., 1998), and some research has examined the potential utility and efficacy of augmentation in field crops (e.g. Simmons and Minkenberg, 1994; Joyce and Bellows, 2000). Very little research has addressed the evaluation of natural enemy effects on *B. tabaci* population dynamics in any system. As a result our ability to predict and exploit these effects for pest suppression are limited. Little is known about parasite activity year round in continuous cultivation of a given crop. Our research aimed at filling these voids and at evaluating the potential of West African *Eretmocerus* spp. as biological control agents of *B. tabaci.* Here we 1) document the relative abundance of *B. tabaci*, *Encarsia* spp. and *Eretmocerus* spp. year round in insecticide treated and untreated cotton plots, 2) evaluate the effect of parasitism on the population dynamics of the pest, and 3) assess the impact of augmentative releases of *Eretmocerus* spp. on the population of the pest in field cages.

4. 3. Materials and Methods

4. 3. 1. Relative abundance of *B. tabaci* and its parasitoids and effect of parasitism on the population dynamics of the pest.

Whitefly infestations and parasitism were studied in cotton plots (0.02ha) three meters apart in a completely randomised design, and sown with the local cotton cultivar FK290 at Bobo-Dioulasso (11°10'N, 4°19'W), Burkina Faso. Two treatments (the commercial insecticide program and the control, i.e. water) were replicated five times. The commercial insecticide program was that recommended by the Office of Cotton Research of Burkina Faso, with the objective of protecting cotton against major pests e.g. *Heliothis armigera* Hübner (Noctuidae), *Aphis gossypii* Glover (Aphidae), and *B. tabaci.* It consisted of two sprays of endosulfan (EC 700g/ha) applied during the young plant- square period, two sprays of cypermethrin (EC) + profenophos (EC) (36g/ha + 300g/ha)

applied during the flowering-green boll period, one spray of cypermethrin (EC) + omethoate (EC) (36g/ha + 300g), and one of cypermethrin (EC) + methamidophos (EC) (36g/ha +300g/ha) applied during the green boll-open boll period. The insecticides were sprayed every second week starting from the 45th day after sowing.

To study parasitism throughout the year three sowing dates were used as follows: plots were sown on June 15, 2000 using the previously mentioned experimental design (i.e. 2 treatments and 5 replicates). On November 15, 2000 cotton seeds were sown between the lines of the senescing cotton plants, which were later uprooted (December 15, 2000). On February 15, 2001 cotton seeds were again sown between the lines of the senescing cotton plants of the second sowing date, which were also uprooted on March 15, 2001. This procedure allowed *B. tabaci* and its parasitoids to establish on younger cotton plants before the uprooting of the older senescing plants. Year round monitoring on cotton was then possible. For the first sown crop water was provided by natural rainfall, while the second and third were furrow irrigated. Rainfall and temperature were recorded at the local Bobo-Dioulasso meteorological station.

When *B. tabaci* infestations began, thirty leaves situated on the fifth node position down from the terminal were randomly sampled per plot using the method described by Ellsworth *et al.* (1996). The leaves were transported to the laboratory in an ice cooler. In the laboratory they were transferred to a refrigerator and the following day, all *B. tabaci* red eye nymphs on the entire surface of the leaves were counted. A leaf disk of ca. 6.25 cm^2 was cut between the main veins of each leaf. All the nymphs on the leaf disks were counted and examined with a stereomicroscope to assess apparent parasitism, hereafter referred to as parasitism. Parasitism without meconia (i.e. residues of mycetomes digested by parasitoids) was ascribed to *Eretmocerus* spp. and parasitism with meconia in parasitized nymphs, to *Encarsia* spp. Three not yet described species of *Eretmocerus* spp. were collected. One

species represented 80% of the parasitoids collected (Gregory Zolnerowich, personal communication). Pooled data of *Eretmocerus* and *Encarsia spp.* are presented here.

4. 3. 2. Augmentative release of *Eretmocerus* spp.

One plot (700m²) was sown at Bobo-Dioulasso on January 2, 2001 with the local cotton cultivar FK290 and irrigated. Thirty metal frames (1 X 2 X 1.5 m) enclosing six plants each were set up in a randomised complete block design, comprising five treatments and six replications. On February 2, 2001 the densities of *B. tabaci* immature populations were estimated, and 24 metal frames were covered at random with a thin nylon screen of three mesh/mm. Six metal frames were left uncovered to serve as a control. The treatments, replicated six times, consisted of zero, one, two, four or height adults of *Eretmocerus* spp. released per plant into the cages.

Yellow sticky cards coated with an extremely fine layer of insect adhesive (Tanglefoot Company, Michigan, USA) were used to trap *Eretmocerus* spp. adults in a cotton field at Matourkou (ca. 10 km west of Bobo-Dioulasso). *Eretmocerus* spp. took flight when the cotton foliage was disturbed. Highly attracted to yellow, they readily landed on the hand-held yellow sticky cards. To trap live and undamaged parasitoids, a fine cloth was used to remove the glue to the point that the parasitoids that landed on the cards were unable to take off immediately but could make a delayed escape. To prevent their escape they were quickly aspirated using Eppendorf® pipette tips (101-1000µl) cut to a diameter of ca. 2 mm and mounted on Tygon® R-3603 plastic tubes. One of the openings of the plastic tube to be mounted on the large opening of the pipette tip was covered with a thin nylon screen (3 meshes/mm) to retain parasitoids in the pipette tip. After aspiration of parasitoids, both openings of the pipette tip were closed with cotton. Next the pipettes tips containing the parasitoids were placed on a piece of pharmaceutical cotton laid on a piece of ice placed in a cooler to

slightly chill them during their transportation to the laboratory at Bobo-Dioulasso. In the laboratory the pipettes tips containing *Eretmocerus* spp. were placed in a refrigerator for two-three minutes to chill the parasitoids. Pipettes tips were emptied into petri dishes to allow sorting of *Eretmocerus* spp. and *Encarsia* spp. under a stereomicroscope.

Starting February 16, 2001 (i.e. two weeks after parasitoid releases), *B. tabaci* red eye nymphs were counted every week on five leaves per cage using a hand lens to assess nymph populations. The sample leaves were selected at the fifth node down from the terminal of the main stem of the plants where nymphs are most abundant (Naranjo, 1996). Observations ceased when whitefly populations became extinct in the cages that had received the highest rate of release, i.e. height parasitoids/plant.

Seasonal patterns of rainfall, temperature, and parasitoid and whitefly populations' levels were summarized by calculating mean monthly rainfall and temperatures, and mean host densities and % parasitism year round. Regressions were used to determine the best relationship between levels of parasitism and host densities, as expressed by R^2 values. MANOVA for repeated measures, PROC GLM, SAS institute Inc, 1989-1996 was used to compare densities of *B. tabaci* and % parasitism. Voucher specimens of insects were deposited at the Laboratoire de Recherches, Institut de l'Environnement et des Recherches Agricoles, Bobo-Dioulasso, Burkina Faso, and at the Kansas State University, Manhattan, Kansas, where identifications were made.

4. 4. Results and discussion

4. 4. 1. Relative abundance of *B. tabaci* and its parasitoids and effect of parasitism on the population dynamics of the pest.

From May to August i.e. the heavy rainfall period, *B. tabaci* populations developed slowly. Peterlin and Helman (1996) found that rainfall negatively impacts both nymph and adult populations.

From September to April, pest populations were significantly more abundant in insecticide treated (min: 3.1; max: 48 nymphs/leaf) than in control plots (min: 1.12; max: 25.2 nymphs/leaf) plots (fig.3A). Pest population increase coincided with the decrease of rainfall. After reaching their peak in November, i.e. after the rainy season ended, the pest populations started to decline. This decline coincided with the drying up of the surrounding vegetation and lower mean temperatures (i.e. < 25°C) as the dry season progressed. The decline of pest populations was delayed in insecticide treated plots. When warmer temperatures (i.e. > 25°C) resumed in March, the onset of rainfall allowed little increase of *B. tabaci* populations. The suppression of parasitism most likely contributed to a higher increase of pest populations in insecticide treated plots compared to controls, and the reduction of the biomass of adjacent host plants during the dry season contributed to the overall reduction of pest populations in both control and treated plots.

In general % parasitism followed the abundance of the pest in both treated and untreated plots, except in March and April where parasitism increased up to 53.7 and 88.7% respectively in insecticide treated and control plots while *B. tabaci* populations decreased (fig. 3). Bellows and Arakawa (1988) and McAuslane *et al.* (1994) also reported an increase in % parasitism with the rise of *B. tabaci* populations respectively in cotton, and in peanut. The increase of parasitism while *B. tabaci* populations were decreasing coincided with the end of lower temperatures. The duration of nymphal development of *B. tabaci* averages respectively 34.7 and 16.4-days at temperatures of 20 and 30°C (Butler *et al.*, 1983). Given that the means of lower temperatures approximated 24°C, we can deduce that the increase of parasitism while the whitefly population was decreasing was due to the fact that *B. tabaci* nymphs were exposed for longer periods to the parasite populations during the lower temperature period. There were significant interactions (P = 0.05) between % parasitism and rates of pest populations, indicating that the increase of the pest densities was slowed down by parasitism. A

significant (P = 0.05) and positive curvilinear relationship was observed where % parasitism on a linear scale rose to a plateau with logarithmic increases in host density from September to December in the control plots (R^2 = 0.41, fig. 4A). This relationship was not significant in insecticide treated plots (R^2 = 0.07, fig. 4B) indicating that the insecticides being applied were suppressing parasitoids in these plots. The relationship changed to a linear inverse type from January to August and was stronger in treated plots (R^2 = 0.67, fig. 4D) than in control plots (R^2 = 0.28, fig. 4C). This reversal was due to the fact that parasitoids exceeded the extinguishing pest nymphal population. Parasitism levels had two distinct phases i.e., 1) from September to December increasing host density was associated with increasing parasitism level, and 2) from January to July the declining *B. tabaci* populations provided an excess of parasitoids for the succeeding pest generations.

The % parasitism in insecticide treated plots (fig.3C) was significantly lower than that in control plots (Fig.3B) from October to March and parasitism due to *Encarsia* spp. was lower than that due to *Eretmocerus* spp. particularly in insecticide treated plots (fig. 3B&C). The suppression of parasites instead of *B. tabaci* populations in insecticide treated plots supports the findings of Otoidobiga *et al.* (2003) who reported that some populations of *B. tabaci* had developed resistance to the insecticides being used while the parasites were more sensitive. The low levels of *Encarsia* spp. in insecticide treated plots suggest that *Encarsia* spp. were more sensitive than *Eretmocerus* spp. to the insecticides used. Our results support those of Eveleens (1983) who considered that *B. tabaci* has been indirectly raised to pest status by insecticide use that resulted in the depletion of parasitoids and predators.

4. 4. 2. Augmentative release of *Eretmocerus* spp.

On February 2, before parasitoid releases, a mean density of 1.47/leaf of *B. tabaci* nymphs was recorded per cage. On March 9, pest density had risen to 39.4 nymphs/leaf in control cages. In the

cages where parasitoids were released, pest populations first increased to 12- 20.2 nymphs/leaf by February 23 before collapsing to 0.6-1.8 nymphs/leaf by March 9 (table 14). When first or second instar nymphs of *B. tabaci* are parasitized, they continue to feed, grow, and develop until the red eye nymph stage. When third or fourth instar nymphs are parasitized, they stop development (Jones and Greenberg, 1998). Foster and Kelly (1978) also observed that densities of *Trialeurodes vaporiarium* Westwood red eye nymphs on greenhouse tomatoes typically increased three-fold before declining following the release of *Encarsia formosa* Gahans. The fact that parasitized first and second instar nymphs continued to develop until red eye nymph stage and that parasitized third and fourth red eye nymphs stopped their development produced an accumulation of *B. tabaci* red eye nymphs, and resulted in the increase of red eye nymph densities.

The initial rate of parasitism averaged 7.5% in all cages on February 2. On February 16 parasitism was 9.8% in the controls and 26.8-60.1% in cages where augmentative releases were made. There were significant differences in the rates of parasitism between treatments. For example on February 23, 22.8% and 43.4-74.3% of parasitism were recorded respectively in the control and in all other treatments. A faster increase of parasitism was observed in the cages where higher numbers of parasitoids were released. For example 58.6 and 89.3% of parasitism were recorded on March 2 in 1 and height-parasitoids/plant release rates respectively, compared to 32.4% in the controls. While 72.8-98% of nymphs were parasitized on March 9 in all cages where *Eretmocerus* spp. had been released, only 44.30% of nymphs were parasitized in the controls. The experiment was ended because the highly parasitized pest populations collapsed (table 14). A Significant linear inverse relationship (R^2 = 0.45) between *B. tabaci* nymphs and % parasitism was observed in the cages (fig. 5). There were significant interactions (P = 0.05) between % parasitism and rates of pest populations, indicating that the increase of the pest populations was slowed down by parasitism. These results are consistent with

those observed in the field experiment when declining *B. tabaci* populations provided an excess of parasitoids for the succeeding pest generations. Our results support those of Sanderson and Ferrentino (1994) who observed that weekly releases of ten adults *Eretmocerus* nr. *californicus* per plant, into individually caged plants reduced *B. tabaci* population densities from an initial average of 13 nymphs/plant to an average of 1.4 nymphs/plant in a two week period. Similarly Minkenberg *et al.* (1994) and Simmons and Minkenberg (1994) obtained average peak parasitism rates of 63% in field cage studies with *Eretmocerus* sp. nr. *californicus*, with corresponding reductions in whitefly numbers in the cages. Our findings indicate that an augmentation of two *Eretmocerus* /plant efficiently suppressed the pest in a four week period and that the parasitoid species could be used in augmentative release to establish reproducing populations.

4. 5. Conclusion

The seasonal abundance of *B. tabaci* and its parasitoids has been described for the first time in Burkina Faso. Our study also demonstrated, for the first time, the effect of parasitism on the population dynamics of *B. tabaci* and the relationship between the pest and its parasitoids after the cotton-growing season. *Bemisia tabaci* populations usually decline when mature cotton plants desiccate in the field. Declining pest populations provide an excess of parasitoids for the succeeding pest generations, and higher levels of parasitism combine with the drying up of surrounding hosts to decimate *B. tabaci* populations. Following pest decimation, parasitism levels decrease, and a new cycle begins. When augmentative release of *Eretmocerus* spp. was tested, higher release rates hastened the suppression of pest populations indicating that the species used are promising candidates for the biological control of *B. tabaci*.

4. 6. Acknowledgements

This research was supported by the Institut de l'Environnement et des Recherches Agricoles (INERA), and the Société des Fibres Textiles (Sofitex) du Burkina Faso. We are indebted to Konaté Gnissa and Dakuo Déhou, respectively Head of the Department of Crop Production of INERA and Adjunct Director of the Direction du Développement de la Production cotonnière (DDPC) of Sofitex for facilitating research activities. We are grateful to Bambara Issa, Dakuo Patrice, Traoré Thomas and Sinon El Adj Adama for technical assistance. We thank Gregory Zolnerowich of Kansas State University for identifying *Eretmocerus* species.

4. 7. References

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Table 14. Impact of augmentative release rates of *Eretmocerus* sp. on *B. tabaci* nymph densities in field cages following release on 2 February 2001.

Trts	16 February		23 February		2 March		9 March	
Ers./plt	Ny/leaf	Mean % parasitism	Ny/leaf	Mean % parasitism	Ny/leaf	Mean % parasitism	Ny/leaf	Mean % parasitism
0	14.80a*	9.80c	26.40a	23.00d	28.90a	32.40d	39.40a	44.30c
1	13.60ab	26.80b	20.20ab	43.40c	5.30b	58.60c	1.80b	72.80b
2	9.00bc	54.15a	18.00b	56.30b	4.50b	73.50b	1.40b	92.86a
4	6.4.00c	54.07a	17.20bc	67.60ab	4.70b	78.60ab	0.80c	95.72a
8	6.60c	60.10a	12.00c	74.30a	2.70d	89.30a	0.60cd	98.28a

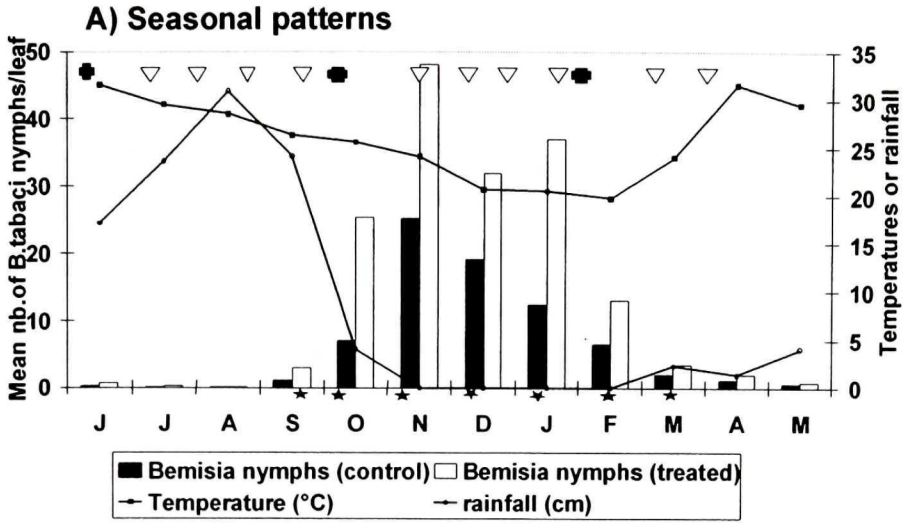
*Within a column, means followed by the same letters are not significantly different (Fisher's LSD test, $P \leq 0.05$). (Trts = treatments; *Ers*/plt = *Eretmocerus*/plant; Ny/leaf = Nymphs/leaf).

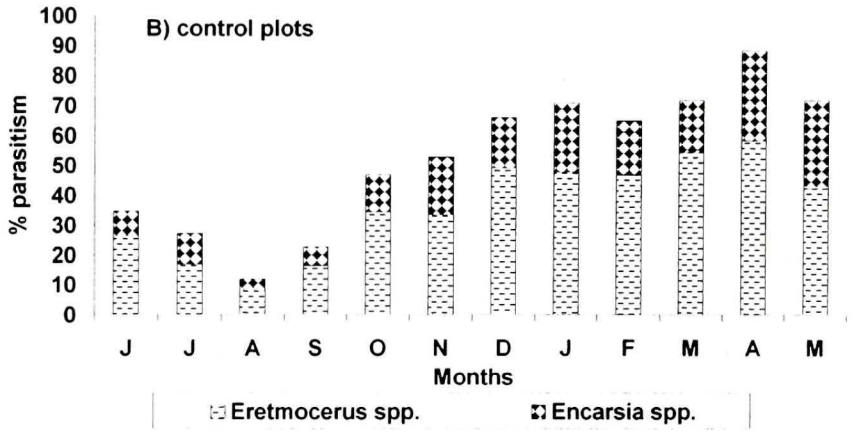
Legends of figures.

Fig. 3: A) Seasonal patterns of rainfall, temperatures, and *B. tabaci* populations in continuously grown cotton; B) % parasitism in control plots; C) % parasitism in insecticide treated plots. Black crosses indicate the dates of sowing, white triangles, the dates of insecticide sprays. Black stars (Fig. A) and open circles indicate significant differences between control and insecticide treated plots (Fig. 1B vs C) ($P<0.05$, MANOVA repeated measures).

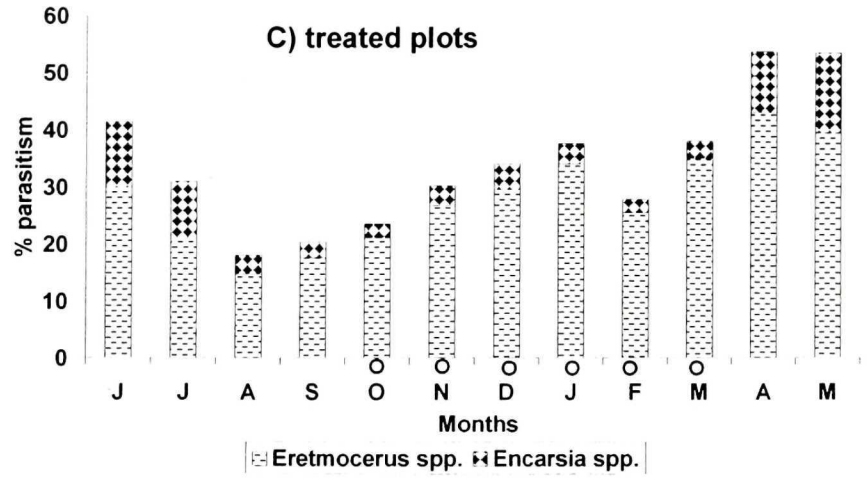
Fig. 4: Relationship between *B. tabaci* nymphs' densities and % parasitism in: A) untreated and, B) treated plots from September to December. C) Untreated and, D) treated plots from January to August.

Fig. 5: Relationship between *B. tabaci* nymph densities and parasitism by *Eretmocerus* spp. in field cages from January to February.



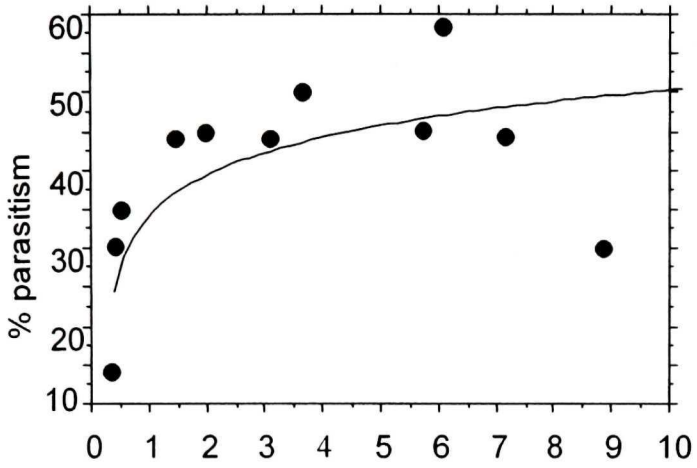


Parasitism in control plots

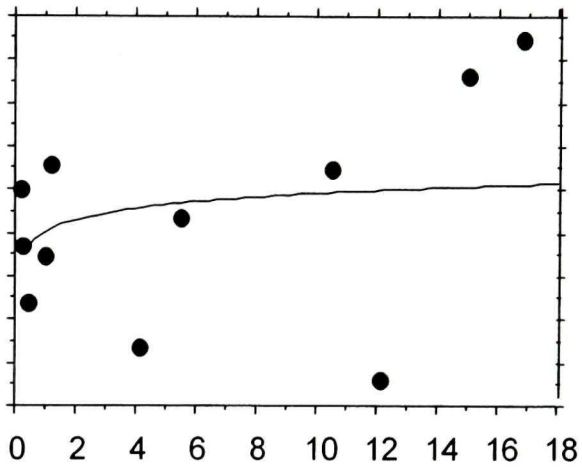


Parasitism in treated plots

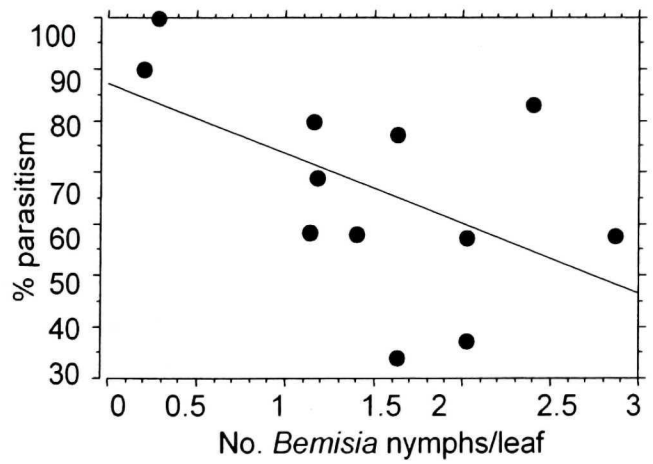
A) Control: $Y = 35.098 + 6.417 * \ln(X)$; $R^2 = 0.41$



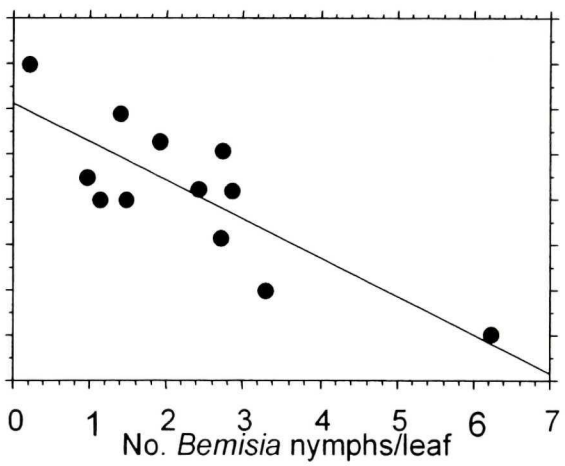
B) Treated: $Y = 30.172 + 1.927 * \ln(X)$; $R^2 = 0.07$



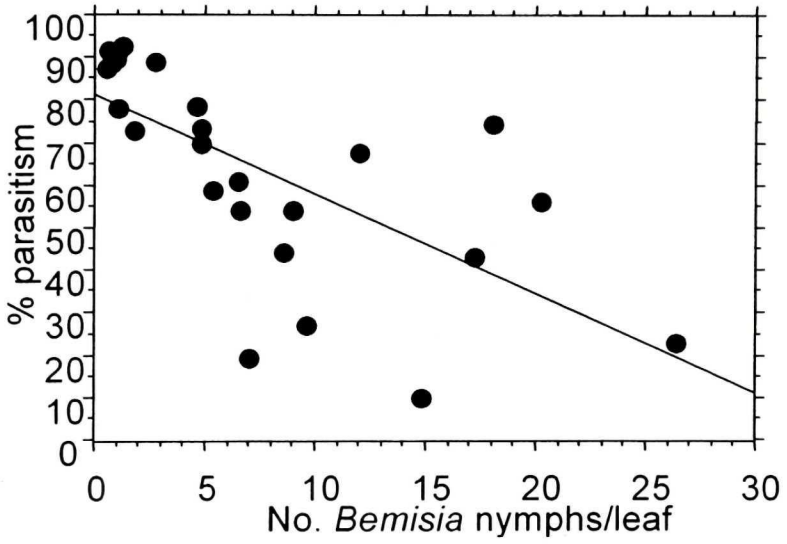
C) Control: $Y = 87.199 - 13.658 * X$; $R^2 = 0.28$



D) Treated: $Y = 81.393 - 8.549 * X$; $R^2 = 0.67$



$Y = 81.2 - 2.34 * X; R^2 = 0.45$



Preface to chapter 5.

A number of adverse side effects (e.g. toxicity to non target organisms, high prices, environmental contamination) are associated with the exclusive use of synthetic insecticides to control pests. It is desirable that locally obtainable products and socioeconomically sustainable plant protection be sought in developing countries to alleviate the financial burden of smallholding peasant-farmers. Among these is the use of natural botanical insecticide extracts. Botanical insecticides are naturally occurring insect toxins extracted from plants. The most well known are azadirachtin from the neem tree *Azadirachta indica* L., pyrethrins from *Chrysanthemum cinerariaefolium* L., and rotenone from the stems and roots of certain tropical plants, such as the jewel Vine or flame tree (*Derris* spp.), lacepod (*Lonchocarpus* spp.), or hoary pea (*Tephrosia* spp.). The modes of action of botanical insecticides often differ from thoses of synthetic insecticides. They might be sound alternatives to the management of *B. tabaci* because they do not persist in the environment, may have low mammalian toxicity, and resistance has not yet been reported against them.

In chapter 5, we investigated the biological activities of three plants not previously reported against *B. tabaci.* All three plants are abundant in Burkina Faso and can be easily cultivated in many parts of the world. Chapter 5 will be submitted to Environmental Entomology.

Chapter 5.

Biological Activities of the Aqueous Extracts of the Seeds of *Ricinus communis* L., *Jatropha curcas* L. (Euphorbiaceae), and *Datura innoxia* Mill. (Solanaceae) on *Bemisia tabaci* (Homoptera: Aleyrodidae).

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To be submitted to: Environ. Entomol.

5. 1. Abstract.

The ovicidal, adulticidal, larvicidal, repellent, and oviposition suppression activities of the aqueous extracts of the seeds of *Jatropha curcas* L, *Ricinus communis* L. and. *Datura innoxia* were investigated against *Bemisia tabaci* Gennadus on cotton using foliar spray. No ovicidal activities were observed but the extracts caused moderate to high mortality respectively of adults and nymphs. In addition *J. curcas* L and *R. communis* L. extracts significantly reduced adult abundance and oviposition on cotton foliage in the field and laboratory. The extracts represent promising tools for the control of *B. tabaci*.

Key words: *Jatropha curcas* L, *Ricinus communis* L., *Datura innoxia* Miller., *Bemisia tabaci* Gennadius, biological activities, foliar spray, cotton.

5. 2. Introduction.

The whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) causes severe crop losses amounting to hundred of millions of dollars annually worldwide (Gerling and Mayer 1996). Damage is due to feeding and deposition of honeydew (Schuster *et al.* 1996), physiological disorders (Yokomi *et al.* 1990), and transmission of geminiviruses (Bedford *et al.* 1994). Insecticide applications are the main control tactics against this pest (Horowitz and Ishaaya 1996). However insecticide usage may induce resistance in *B. tabaci* populations (Palumbo *et al.* 2001, Otoidobiga *et al.*, 2003), and compromise the effectiveness of natural enemies (Naranjo, 2001).

Among alternatives, botanical insecticides may typically have some modes of actions different from those of synthetic insecticides. They might be a sound alternative to the management of *B. tabaci* because they do not persist in the environment, may have relatively low mammalian toxicity, and resistance has not yet been reported against them. The physic or purging nut, *Jatropha curcas* L. (Euphorbiaceae) has insecticidal activity against a number of insect species including the european corn borer *(Ostrinia nubilalis* Hübner) (Lepidoptera: pyralidae) (Czapla and Johnston 1990), and the cotton bollworm (*Heliothis armiger*a Hubner) (Solsoloy 1995). Similarly the insecticidal activity of the castor bean plant, *Ricinus communis* L. has been reported against the green peach aphid, *Myzus persicae* Susler (Olaifa *et al.* 1991).

J. curcas kernels contain 43-59% of oil. Two strains have been reported i.e. one with toxic seeds and the other with nontoxic seeds (Kingsbury, 1964). The toxic strain contains 0.87-3.32 mg/g of phorbol esters; the nontoxic does not (Makkar *et al.* 1997). Four phorbol esters have been identified in *J. curcas* seeds i.e. 4β-phorbol, 4α-phorbol 12,13 didecanoate, 4β-phorbol 12-myristate 13 acetate and 4β-phorbol 13-decanoate (Liu *et al.* (1997). The kernels of a strain collected in Burkina Faso have been estimated at 1.71mg/g of phorbol ester (Makkar *et al.* 1997). Phorbol esters exert insect toxicity by

ingestion (Sauerwein, *et al.* 1993), and molluscicidal activities by contact (Sporer *et al.* 1995 Liu *et al.* 1997). They stimulate protein kinase C, are involved in signal transduction and developmental processes of most cells and tissues, and hence may induce a variety of biological effects over a wide range of organisms (Adolf *et al.*, 1984, Hirota *et al.* 1988). Saponins, lectins (curcins), phytates and protease (trypsin) inhibitors, are present in J. *curcas* and may contribute to its toxic/antinutritional activity (Makkar *et al.* 1997).

R. communis kernels contain 45-51% oil and 2-3% toxic substances, principally ricin. Ricin is made of two polypeptide chains, an A chain and a B chain, which are joined by a disulfide bond. The B chain binds to target cell surface receptors and the toxin-receptor complex is taken into the target cell. In the cell, the A chain exerts endonuclease activity that inhibits protein synthesis (Wiley and Oeltmann, 1991). Ricin exerts insect toxicity (Olaifa *et al.* 1991) and acts via ingestion and inhalation routes. *R. communis* also contains saponins, lectins (curcins), phytates, protease (trypsin) inhibitors that may contribute to their toxic or antinutritional activity.

J. curcas and *R. communis* are known for their uses in Traditional Medicine as cathartic purgatives, and to treat skin ailments, dropsy, gout, paralysis, and rheumatism (Duke and Wain 1981). In addition to their potential use in crop and health protection, both *J. curcas* and *R. communis* can be used as biofuels and as live hedges (Heller, 1996).

The thorn apple or Jimson weed, *D. innoxia* produces tropane alkaloids such as atropine (dl-hyoscyamine) and scopolamine (Adrian *et al.* 1990, Koichiro *et al.* 1991). Atropine (dl-hyoscyamine), scopolamine, and their synthetic derivatives are important anticholinergic agents. They are used clinically in ophthalmology (mydriasis, cycloplegy), gastrointestinal tract diseases (peptic, ulcer, spasmolytic), anesthesia, dentistry and, diseases of circulatory system (treatment of cardiac infraction). They are also used in the prevention of motion sickness (scopolamine) and as antidotes in the treatment

of poisoning by organophosphorous compounds. Moreover, atropine and scopolamine are used as model compounds in pharmacological research (Ceyhan *et al.* 2001).

Because hundred of adult *B. tabaci* were found dead on *D. innoxia* Miller plants in the field (Otoidobiga, personnal observation), the seeds of this plant, and those of *J. curcas* and *R.* communis were collected at Bobo-Dioulasso, Burkina Faso, and bioassayed against *B. tabaci.* Our objectives were to determine the biological activities of *J. curcas, R. communis,* and *D. innoxia* seed extracts against *B. tabaci* as measured by adult mortality, repellency, ovipositional responses and egg and larval mortality on foliar treated cotton in laboratory and field tests.

5. 3. Materials and Methods

5. 3. 1. Laboratory Bioassays

Cotton (*Gossypium hirsitum* L. cultivar Stam 52) was grown individually in 15-cm plastic pots. When the plants were in the six-to seven true leaf stages, they were trimmed, leaving only the fifth true leaf just before the bioassay. Live *B. tabaci* specimen were aspirated using Eppendorf® pipette tips (101-1000 µl) cut to a diameter of ca. 2 mm and mounted on Tygon plastic tubes. One of the openings of the plastic tubes to be mounted on the large opening of the pipette tips was covered with a thin nylon mesh to retain whiteflies in the pipette tips. *J. curcas, R. communis* and *D. innoxia* seeds were collected and hulled in Bobo-Dioulasso, (11°10'N, 4°19'W) Burkina Faso, West Africa. The day before treatments, the seeds were ground in water using a commercial blender and diluted to a 7.5% (w/v) aqueous solution. The solutions were held in ice coolers and filtrated 12 hours before leaves were sprayed. All the experiments were repeated three times. Data were subjected to ANOVA (Statview, SAS Institute Inc., 1992-1998) and the means separated by Newman-Keuls test.

5. 3. 1. 1. Egg mortality

Trimmed plants were first exposed for a 24-h oviposition period in the plot where swarms of whiteflies gathered on cotton. Before treatment eggs were removed on test leaves, leaving only 50 eggs per leaf to be treated. Controls were treated with water. After treatment the plants were allowed to air dry. They were next placed in a growth chamber maintained at 26.7 ± 2°C, 30-40% RH, and with a 12:12 (L: D) photoperiod. Egg hatching was determined on day 8 after oviposition.

5. 3. 1. 2. Adult mortality

The upper and lower surfaces of leaves were sprayed to runoff with aqueous extracts and allowed to air dry. Controls were sprayed with water. We used a leaf-cage technique adapted from Kishaba *et al.* (1976) to keep whiteflies on the treated leaves. The cages had four ventilation holes of ca. 10 mm diameter and six other ventilation holes of ca. 2 mm diameter respectively perforated on the upper and the lower parts of Falcon® 1008 Easy Grip® 35 X 10 mm plastic petri dishes. The holes on the upper side were covered with a thin nylon screen (3 mesh/mm) to prevent the escape of insects. The two sides were assembled with aluminum clips using Araldite® glue. The edges of the two components of the leaf-cages were lined with a thin layer of sponge that kept cotton leaves undamaged during the experiment. The cages were clipped on the lower side of leaves, the leaves closing the ventilation holes of the upper side of the cages. A small opening made on the side of the cages was closed with a cork after whiteflies introduction.

In the laboratory, 40 whiteflies were released per leaf-cage through the small opening. Each treatment was replicated six times. The leaf cages were incubated for 24 hours at 25°C and then were chilled for ca. 3 min in a refrigerator. The whiteflies were emptied in a petri dish and were examined for

movements under stereomicroscope. Immobile and shriveled whiteflies were considered dead, while living whiteflies recovered quickly from chilling and were active.

5. 3. 1. 3. Nymph mortality

Unsprayed plants containing eggs were placed in a separate growth chamber under the same conditions described for egg mortality tests. The leaves were examined every second day. When the majority of nymphs on leaves were at a given stage, all immature stages were removed from test leaves, leaving only 50 individuals of the desired stage per leaf just before treatment. The average length of time after oviposition to obtain the first, second, third and fourth instar was 7, 10, 13, and 16 days respectively. Leaves containing nymphs were sprayed to runoff with the solutions, and nymph mortality was determined 48h after treatment. Controls consisted of 50 individuals of the desired stage per leaf treated with water.

5. 3. 1. 4. Multiple choices repellency.

To verify if whiteflies avoided to settle on the cotton plants treated with *J. curcas*, *R. communis* or *D. innoxia* seed extracts, six plants treated with each extract and six plants treated with water (control plants) were randomly placed in the plot where swarms of whiteflies gathered on cotton leaves. Whiteflies that settled on the treated leaves were counted every day at 18h00 to assess their abundance on the different treatments. Initially i.e. on day zero of the treatments, on average 1.27 adults/leaf settled on the most effective extract, *J. curcas*. Counts were ceased on day three because the average number of whiteflies exceeded 10 adults per leaf on this extract.

5. 3. 1. 5. No choice repellency

Three plants were placed in each of six replicate metal frame cages (1x1x1m) covered with 3-meshes/mm nylon screens just before the release of adults. Two hundred and fifty adults were aspirated from a cotton plot and released in each cage through a 10-cm diameter opening. The opening was closed with a nylon screen (3 meshes/mm) after whitefly release. Settled adults were counted every day at 18h00 to assess their abundance on the different treatments. Tests were stopped on day 3 because less than 0.5 adult/leaf were found on leaves treated with *J. curcas* extract.

5. 3. 1. 6. Multiple choices ovipositional tests

Three plants of each of the four treatments were randomly placed in each of six cages (1 x 1 x 1m) covered with 3-meshes/mm nylon screens just before the release of adults. Six hundred unsexed adults were aspirated from a cotton plot and released in each cage through a 10-cm diameter opening and allowed a 24-h ovipositional period. The opening was closed with a nylon screen (3 meshes/mm) after whitefly release. After the ovipositional period, the plants were removed from the cages and the adults discarded from the plants. Using a stereomicroscope, eggs were counted on 5 leaf disks of ca. 1cm^2 randomly cut between the main veins of test leaves where most *B. tabaci* eggs are laid (von Arx *et al.*, 1984).

5. 3. 1. 7. No choice ovipositional tests.

The experimental procedures were similar to those described in the multi choice ovipositional tests except that six plants of the same treatment were placed in each of six replicate metal frame cages for each of the four treatments, making a total of 24 cages used in this experiment.

5. 3. 1. 8. Field experiment.

A local cotton cultivar Stam 52 was sown on October 15, 2001 at Bobo-Dioulasso, in a randomized complete block design with four replicates. Each experimental unit (i.e. treatment x replicate) was 0.01 ha, irrigated and 1.5m apart. Treatments were done on day 45 after sowing when the plants had 5 to 6 true leaves and all the developmental stage of *B. tabaci* were present. The experimental units were sprayed with 500l/ha of each seed extract using a Shogun□ back-portable sprayer. The application was made so that both upper and lower sides of leaves were sprayed to runoff. Control plots were sprayed with 500l/ha of water. After treatment, whitefly adults and nymphs were counted on 30 leaves/plot every second day to monitor the effectiveness of the seed extracts.

5. 4. Results

5. 4. 1. Laboratory Bioassays

5. 4. 1. 1. Egg, adult and, nymph mortality

No ovicidal effects were observed as compared to the control. Aqueous extracts of *J. curcas, R. communis,* and *D. innoxia* had insecticidal activities against *B. tabaci* adults and nymphs (Table 15). The lethal activity of leaf residues against adults varied from 12 to 33.5% depending on the extract used, compared to 2.34% in the control. The lethal activity against nymphs was much higher and varied depending on the extract and the nymph stage treated. For example the mean mortalities caused by *J. curcas* were » 85.5% against all nymph stages, while those caused by *R. communis* and *D. innoxia* extracts were high against L1 (respectively 96 and 78%), moderate against L2 and L3 (respectively 54.5 -

69.5%), and marginal against L4 (respectively 13 - 15%) compared to 2.5-9% of natural mortality in the control. In general the insecticidal activity of *J.curcas* extracts against *B. tabaci* was higher than those of *R. communis* and *D. innoxia*.

5. 4. 1. 2. Multiple and no choice repellency

There were significantly fewer adults that settled on *J. curcas, R. communis* and *D. innoxia* seed extracts compared to the control in choice and no choice tests (Table 16). In choice tests, significantly fewer adults settled on *J. curcas* treated leaves (1.27 to 21 adults/leaf) than on both *R. communis* (16.5 to 55.6 adults/leaf) and *D. innoxia* treated leaves (33.1 to 142.2 adults/leaf), and fewer adults settled on *R. communis* treated leaves than on leaves treated with *D. innoxia* from day 0 to day 3 after treatment. In no choice tests, significantly fewer adults settled on leaves treated with both *J. curcas* and *R. communis* extracts (0.5 to 6.7 adults /leaf) compared to those treated with *D.innoxia* extract (7.7 to 18.8adults /leaf) from day 0 to day 3 after treatment. No significant differences between numbers adults settled on *J. curcas* treated leaves (0.5 to 3 adults/leaf) compared to those settled on *R. communis* extracts (2 to 6.7 adults/leaf). However, the numbers of adults settled on *J. curcas* were consistently lower than those settled on *R. communis* extracts from day 0 to day 3.

5. 4. 1. 3. Multiple and no choice oviposition.

There was significantly fewer eggs laid on leaves treated with *J. curcas* and *R. communis* seed extracts than in the control in choice tests (Table 16). Significantly fewer eggs were laid on *J. curcas* (0.03 to 0.26/eggs cm^2) than on those treated with *R. communis* and *D. innoxia* (1.60 to 5.04/eggs cm^2). No significant differences in the number of eggs laid were observed between *R. communis* and *D. innoxia* treated leaves in both multi choices and no choice tests. In choice tests no significant difference

was also observed between the control and *D. innoxia*, while in the no choice tests as much as 1.8 times more eggs were laid on leaves treated with *D. innoxia* seed extracts (5.04/eggs cm^2) compared to the control (2.80/eggs cm^2) and no significant differences were observed between *R. communis* and the control.

5. 4. 1. 4. Field experiment.

An average of 54 adults/leaf was counted before treatment. On day 0, 12h after treatment, the mean number of adult increased to 84/leaf in the control and dramatically declined in the plots treated with the seed extracts (Table 17). The magnitude of the decline depended on the extract used. For example, the lowest mean number of adults (3.8 adults/leaf) was observed on *J. curcas* treated plots, followed by *R. communis* (14.3 adults/leaf), and *D. innoxia* treated leaves (44.17 adults/leaf) treated plots. On days 2, 4 and 6, adult populations increased in all plots, but there were significantly fewer adults in *J. curcas* and *R. communis* treated plots than in the control and *D. innoxia* treated plots.

Nymph populations declined progressively to 0.1 and 1.1 nymph/leaf on day 6, respectively in the plots treated with *J. curcas* and *R. communis* seed extracts. Significant differences were observed between treatments on day 2. *J. curcas* and *R. communis* treated plots significantly had significantly fewer nymphs than the control and D. *innoxia* treated plots.

5. 5. Discussion

Our observations of the insecticidal activities of *J. curcas* agree with those reported by several authors for many other insect species. For examples Heal and Rogers (1950) observed that *J. curcas* seed extract was lethal against the American cockroach *(Periplaneta Americana* L.), the German cockroach *(Blattella germanica* L.), and the large Milkweed Bug (*Oncopeltus fasciatus* Dallas). The

extract was also lethal against the tobacco hornworm (*Manduca Sexta*) L. (Sauerwein *et al.* 1993), the Pink Stalk Borer (*Sesamia calamistis* Hampson) and the African maize stem borer (*Busseola fusca*) (Mengual 1994), and against the Cotton Bollworm (*Helicoverpa armigera* Hubner), the cotton aphid (*Aphis gossypii* Glover), the pink Bollworm (*Pectinophora gossypiella* Saunders), the Eggplant fruit and shoot borer (*Empoasca biguttula* Shiraki), *Callosobruchus chinensis* L., and the maize weevil (*Sitophilus zeamais* L.) (Solsoloy, 1995). The fact that *B. tabaci* adult were less abundant on the leaves treated with the extract also suggests that the compounds may repel them.

To our knowledge only Olaifa *et al.* (1991) have previously reported the inseticidal activity of ricin the active ingredient of *R. communis* against the green peach aphid, *Myzus persicae* Susler. In contrast little is known about the spectrum of the insecticidal activity of *D. innoxia.* The mortality observed when adults were confined on treated leaves indicates that toxicity may results from inhalation or ingestion.

The reduction in the number of eggs laid on both *R. communis* and *J. curcas* treated leaves compared to the control is a consequence of the reduced abundance of adults. Similarly, fewer eggs were laid on leaves treated with *J. curcas* than on those treated with *R. communis* because fewer adults settled on the former. In contrast *D. innoxia* stimulated egg laying, resulting in 80% more eggs laid compared to the control. Insecticide-stimulated reproduction, termed hormoligosis, is the result of the target organism's reaction to stress (Luckey 1968). Hormologosis improves the target organism's ability to cope with environmental changes by means of new and better mechanisms developed in response to stress. Several workers in different countries have observed that hormoligosis through chemicals accelerates *B. tabaci* population growth in the field. For example in Israel, Koren *et al.* (1983) observed an increase of *B. tabaci* nymphs in plots treated with pirimiphos methyl or pyrethroids when compared with those treated with mecarbam, and David and Jesudasan (1986) reported acceleration of *B. tabaci*

populations on cotton sprayed repeatedly with cypermethrin, deltamethrin, and phosalone. In India, Satpute and Subramaniam (1983) observed an outbreak of *B. tabaci* following a ULV application of Phosalone on rain-fed cotton. Dittrich (1987) considers hormoligosis as a driving force behind pest outbreaks.

The toxicity of *J. curcas*, *R. communis* and *D. innoxia* seed extracts against *B. tabaci* differed from that observed using neem-seed extract. Neem-seed extract reduced egg hatch by 29% in 6 days and killed 90% of nymphs by day 20 after application (Coudriet *et al.* 1985) while *J. curcas*, *R. communis* and *D. innoxia* had no ovicidal activity but killed nymphs in 2 days. The repellency of the seed extracts may be caused by their oil components (Veierov, 1996), and is similar to those reported for neem-seed extracts (Coudriet *et al.*, 1985).

During the experiment whitefly adults were migrating from senescing neighboring fields (see Byrne and Blackmer, 1996) into the experimental field and this migration may explain the high number recorded on day 4 and 6 after treatment. The re-infestation of adults in the plots treated with the extracts also suggests that the repellency and killing activities of the extracts are of short duration. *J. curcas* and *R. communis* were efficacious at controlling *B. tabaci* nymphs in the field while *D. innoxia* performed poorly. The important reduction of nymph populations on leaves treated with *J. curcas* and *R. communis* extracts indicates that most of the nymphs treated died. The evaluation of other formulations based on vegetable oils that can act both as repellent and as physical poisons for the control of *Bemisia* in the field and in greenhouses has been conducted by several authors, e.g. Butler and Henneberry (1991), and Munthali (1994).

Our field experiment results were consistent with our laboratory and cage experiment results regarding the potency of *J. curcas*, *R. communis* and *D. innoxia* against *B. tabaci* in respect to adult abundance and nymphs mortality. Many synthetic insecticides achieve insufficient control of *B. tabaci*

partly because they exert only adulticidal activity (Horowitz and Ishaaya, 1996). *J. curcas*, *R. communis* and *D. innoxia* seeds can be processed by farmers and mixed with such synthetic insecticides to produce formulations active against both adults and nymphs and improve *B. tabaci* control at a reasonable cost.

5. 6. Conclusion

To our knowledge, this is the first report of the biological activities of the seed extracts of *J. curcas*, *R. communis*, and *D. innoxia* against *B. tabaci*. *J. curcas*, *R. communis* seed aqueous extracts caused *B. tabaci* adult and larval mortality, and reduced adult abundance and oviposition on cotton foliage. *D. innoxia* caused *B. tabaci* adult and larval mortality in the laboratory but was not effective in the field. The extracts represent promising tools for the control of *B. tabaci*. Supplemental studies should be conducted to verify if the extracts have additional modes of action such as systemic activity, to determine their toxicity to mammals and beneficial insects, and to facilitate their integration in IPM programs.

5. 7. Acknowledgements

This research was supported by the Institut de l'Environnement et des Recherches Agricoles (INERA) of Burkina Faso. We are grateful to Georges Kambou for commenting the protocol, and to Issa Bambara and Tibregma Ouedraogo for technical assistance.

5. 8. References

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Table 15. Mortality of adult and nymph *B. tabaci* following spraying of *J. curcas, R. communis,* and *D. innoxia* seed aqueous extracts.

Stage treated (No. individuals treated/extract)	Extracts (7.5%w/v)	Mean % mortality (SEM)*
Adult (480)	*J. curcas*	32a (2.37)
	R. communis	12b (1.30)
	D. innoxia	33.5a (1.41)
	Control (water)	2.34c (0.64)
L1 (200)	*J. curcas*	100a (0.00)
	R. communis	96a (1.83)
	D. innoxia	78b (10.31)
	Control (water)	9c (3.70)
L2 (200)	*J. curcas*	85.50a (11.84)
	D. innoxia	69.50a (2.50)
	R. communis	68.5a (14.77)
	Control (water)	2.5b (1.50)
L3 (200)	*J. curcas*	97a (2.40)
	R. communis	65b (3.42)
	D. innoxia	54.5b (4.35)
	Control (water)	9a (3.00)
L4 (200)	*J. curcas*	89a (3.50)
	R. communis	15b (5.07)
	D. innoxia	13b (3.32)
	Control (water)	8c (1.83)

* Within a column and a stage means followed by the same letter are not significantly different ($P < 0.05$; Newman-Keuls multiple range test).

Table 16. Repellant effects of *D. innoxia*, *J. curcas*, and *R. communis* aqueous seed extracts (7.5% w/v) on *B. tabaci* adult abundance and egg laying (after 24-h egg laying period) on cotton leaves following treatment in A) multi choice tests, and B) No choice tests.

Treatments	Mean no. adults/leaf on day after treatment				Mean no. Eggs/cm^2
	Day 0	Day 1	Day 2	Day 3	After 24h
A)Multi-choice tests					
J. curcas	1.27d	1.95d	7.00d	21.00 d	0.26c
R. communis	16.50c	38.22c	34.33c	55.60c	1.60bc
D. innoxia	33.10b	75.10b	88.50b	142.20b	2.40ab
Control (water)	90.30a*	147.70a	173.30a	175.70a	3.00a
B) No choice tests					
J. curcas	3.00c	2.50c	1.30c	0.50c	0.03c
R. communis	6.70c	4.80c	3.50c	2.00c	2.10b
D. innoxia	18.80b	13.20b	10.70b	7.70b	5.04a
Control (water)	41.50a	37.50a	34.2a	28.20a	2.80b

* Within a column and a day means followed by the same letter are not significantly different ($P < 0.05$; Newman-Keuls multiple range test).

Table 17. Mean numbers of adults (per leaf) or nymphs (per cm^2) on leaves treated with *J. curcas*, *R. communis* and *D. innoxia* aqueous extracts (7.5% w/v) in the field.

Treatments	Day 0		Day 2		Day 4		Day6	
	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
J. curcas	3.8cd	4.4a	7.3c	0.3b	12d	0.5c	20c	0.1c
R. communis	14.3c	4.1a	16.3c	1.5c	37.8c	1.9b	58b	1.1b
D. innoxia	44.17	3.8a	64.25b	3.9a	73.1b	4.8a	115.5a	3.7a
Control (water)	84a*	3.2a	101.5a	5.1a	103.8a	5.8a	106.8a	3.9a

* Within a column and a day means followed by the same letter are not significantly different ($P < 0.05$; Newman-Keuls multiple range test).

Chapter 6

General conclusion

Landlocked in the middle of West Africa, Burkina Faso Faso has a high population density, few natural resources, and a fragile soil. About 90% of the population is engaged in (mainly subsistence) agriculture, which is highly vulnerable to variations in rainfall. Its GDP per capita is 1,040US$ per year, one of the lowest in the world. Cotton is its main commercial crop, representing 30% of its exports (The World Fact Book, 2002).

The sudden outbreak of *B. tabaci* populations in cotton fields of the country in 1998 should be viewed in this context of extreme poverty that exacerbates the dilemma of ressource allocation among the many priorities that wait for solutions. In these circumstances, funds for agricultural reseach are allocated to emergency issues. This explains why although the *B. tabaci* problem was looming across the world and even in vegetable crops in the country, in 1998 only one investigation by Konaté *et al.* (1995) was available on the subject in Burkina Faso. Konaté *et al.* (1995) warned that increased vegetable viral diseases transmitted by *B. tabaci* were being observed across the country. Because vegetable production is less structured than cotton production, no assessment of economic losses was made, so that *B. tabaci* was regarded as a minor pest.

Consequently, when the pest crisis ignited, causing extensive economic losses to the Burkinabe cotton industry, no entomological information was locally available to deal with the problem. How such dramatic changes in the status of the pest have occurred and, how to deal with it immediately to limit crop losses and protect the next growing-season crops become central concerns. Politicians, peasant farmers, laymen, and goverment technocrats blamed entomologists for failing to foresee the developing problem.

It appeared that although temporary solution i.e. buying novel chemistries could be easly done, reseaching the actual causes of this pest crisis was the ultimate way to engineer long lasting solutions. My Ph.D. studies were undertaken under these melodramatic circumstances to diagnose the crisis and propose reliable solutions.

Specific conclusions have been drawn in each chapter of this thesis. There were four main areas of study. **I)** Status of insecticide resistance against conventional insecticides and baseline susceptibilities of the pest to the recently introduced novel insecticides (Chapters 2 and 3), **II)** Impact of insecticides on parasitoids (Chapters 2 and 3), **III)** Relative abundances and population dynamics of the pest and its parasitoids (Chapter 4), and **IV)** Development of locally obtainable and socioeconomically sustainable products (Chapter 5). These are the major findings reported in each area:

I. Status of insecticide resistance against conventional insecticides and baseline susceptibilities of the pest to the recently introduced novel insecticides.

The status of insecticide resistance against conventional insecticides was studied in farmers' fields. It was found that *B. tabaci* has developed resistance to numerous conventional insecticides. Many insecticides could no longer provide economic and environmentaly friendly controls of the pest because their LC50s' exceeded several times the recommended field doses. Fortunately, several new classes of insecticide chemistry have been developed recently that effectively control *B. tabaci*, and more importantly, provide producers with a diversity of chemistries with which to battle resistance. The newly introduced active ingredients pyriproxyfen, acetamiprid, and diafenthiuron are effective at controlling the pest. A resistance management program should be implemented to track the susceptibilities of whitefly populations to these insecticides. If a decrease in susceptibility is observed, a shift to compounds with different modes of actions should be initiated to mitigate selection pressure.

II. Impact of insecticides on parasitoids

The impact of insecticides on parasitoids has been investigated in the field and in laboratory. It has been observed that although most conventional insecticides severely affected parasitism, some parasitoids, particularly species of the genus *Eretmocerus* develop some degrees of tolerance to insecticides, that allow greater survival of these species in insecticide treated fields. The species have been sent to Dr. Gregory Zolnerowich (Kansas State University) and colleagues for identification. The novel insecticides pyriproxyfen, acetamiprid, and diafenthiuron, little affected parasitism compared to controls. Given the effectiveness of these insectides against *B. tabaci*, this observation may be explained by the facts that 1) parasitoids had smaller whitefly populations to parasitize; 2) because these materials did not significantly disrupt parasitism, they allowed greater development of parasitoids populations.

III. Relative abundance and population dynamics of the pest and its parasitoids

The abundance of *B. tabaci* in the field is affected by temperature, rainfall, surrounding alternate host biomasses and parasitism. During lower temperatures the nymphal population of the pest is exposed to a longer period of parasitization, so that when warmer temperatures resume, parasitism was at its highest levels and decisively contributed to the collapse of the pest populations.

IV. Development of locally obtainable and socioeconomically sustainable products

It has been shown that *J. curcas*, *R. communis* and *D. innoxia* seed aqueous extracts cause *B. tabaci* adult and larval mortality, and reduce adult abundance and oviposition on cotton foliage. These plants are adapted to marginal areas with poor soil and low rainfall in many tropical and subtropical areas of the world, frequently appearing spontaneously. They are resistant to diseases, are not browsed by livestook, and do not compete with conventional food or feed crops for land and water. In addition

to their potential use in crop and health protection, they can be used as biofuels and as live hedges (Heller, 1996). The aqueous extracts represent promising tools for the control of *B. tabaci*.

The insecticide program I have developed for use in Burkina Faso is drawn from both the Israeli and Arizona strategies (Horowitz *et al.* 1996, Ellsworth and Martinez-Carrillo, 2001). In essence, we divided the cotton-growing season into three periods during which insecticides of specific classes could be used at specified thresholds of the cotton pest complex. The introduction of thresholds for every insecticide use is critical, for it is aimed at avoiding unnecessary applications of chemical that may lead to disruption of natural enemy activities. In particular, the control of *B. tabaci* has been entirely under the authority of SOFITEX, the Burkinabe cotton company. Starting early August to late November every year, scouts of the cotton company that I have trained, are deployed in all cotton-growing areas to monitor and treat the pest populations when the threshold (Naranjo and Flint, 1994) is reached. During this period each of the novel insecticides, pyriproxifen, acetamiprid and diafenthiuron should be used only once in a given field. This mandatory rotational scheme allow insecticides with different modes of action to be applied only once during the pest populations' outbreak. The program restricts the use of insecticide during the growing season to delay the onset of resistance and, sustain the long-term efficacy of insecticides by optimizing their use. In addition, limiting and optimizing chemical use conserve natural enemy populations that aid in suppressing *B. tabaci* and other importance cotton pests.

My work has contributed to temporarily solving the *B. tabaci* crisis in Burkina Faso. Cotton production, which was severely reduced in 1998, 1999 and, 2000 has increased again to a higher level (Fig. 6). One major concern of the program is the high cost of novel insecticides compared to mixtures of pyrethroids and organophosphates. Since one single treatment with these insecticides provides better control of the pest than several treatments with mixtures, peasants find them more economical in

terms of saved labor and money. However, long-term management of *B. tabaci* is difficult to sustain. Sustainable management of the pest is largely dependent on the availability of a diversity of insecticide chemistries and, on maintaining of their efficacy by preventing resistance building in the pest population. This difficulty is compounded by the fact that growers in other industries (e.g. tomato and vegetable growers) are now using the novel insecticides. Control in the vegetable industry is difficult because it is less structured than the cotton one. Ultimately a long lasting solution depends on better coordinating efforts between the vegetable and cotton producing industries.

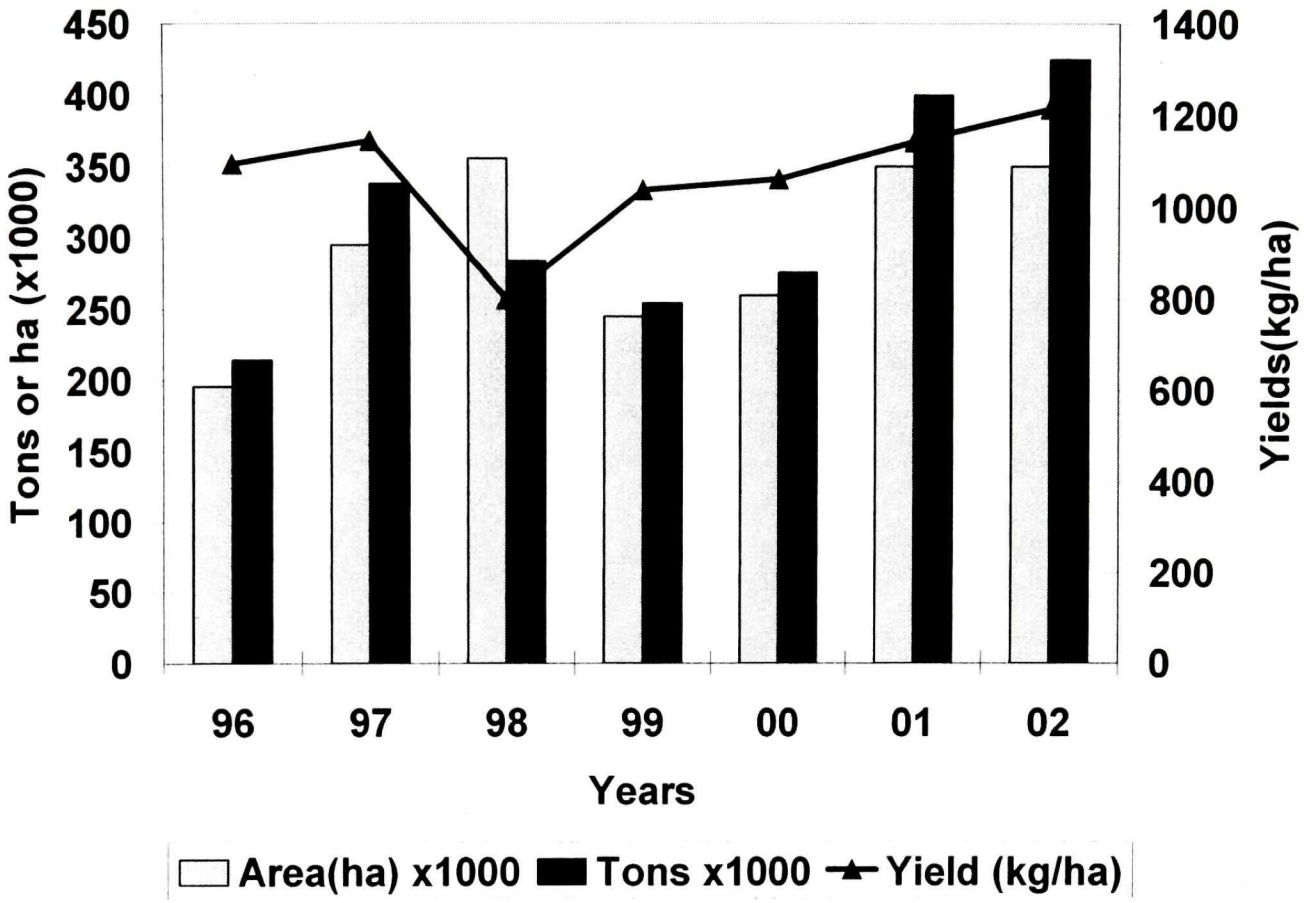

Fig.6. Seed cotton production in Burkina Faso from 1996 to 2002 (Source, Sofitex, Annual Report 2002).

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