COMMON FUND PROJECTS

Papers Presented at a Technical Seminar at the 56th Plenary Meeting of the

INTERNATIONAL COTTON ADVISORY COMMITTEE

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Introduction

At the 56th Plenary Meeting of the ICAC, the meeting of the Committee on Cotton Production Research was held on October 30, 1997. ICAC has sponsored four ongoing projects in twelve countries for financing by the Common Fund for Commodities. In the Technical Seminar, the Committee on Cotton Production Research reviewed the progress of these projects.

The Technical Seminar started with a statement made by Mr. Sietse van der Werff of the Common Fund for Commodities (CFC) on behalf of Mr. Rolf W. Boehnke, Managing Director of the Fund. Mr. van der Werff described the main objectives of the CFC and its collaborative activities with international commodity bodies. Cotton is the most important commodity in the CFC portfolio and accounts for about 15% of the total commitments made by the Fund so far. The four ongoing ICAC/CFC projects, together with the countries involved, the project executing agencies (PEA) and their duration are as follows:

- Environmentally-safe Integrated Pest Management for High Quality Non-sticky Cotton
  Countries: Egypt, Ethiopia, Israel and Zimbabwe
  PEA: The Israeli Cotton Production and Marketing Board Ltd.
  Duration: 4 years Starting date: October 1, 1994
- Integrated Pest Management of the Cotton Boll Weevil in Argentina, Brazil and Paraguay
  Countries: Argentina, Brazil and Paraguay
  PEA: Phytosanitary Institute of Argentina
  Duration: 5 years Starting date: June 1, 1995
- Genome Characterization of Whitefly-transmitted Geminiviruses of Cotton and Development of Virus Resistant Plants through Genetic Engineering and Conventional Breeding
  Countries: Pakistan, UK and USA
  PEA: National Institute for Biotechnology and Genetic Engineering, Pakistan
  Duration: 5 Years Starting date: January 1, 1996
- Improving the Marketability of Cotton Produced in Zones Affected by Stickiness
  Countries: France and Sudan
  PEA: The Sudan Cotton Company Ltd.
  Duration: 3 years Starting date: January 1, 1997

The project "Environmentally-safe integrated pest management for high quality non-sticky cotton" has three main research objectives: Development of environmentally compatible pesticides for controlling whitefly and aphids; development of spray machinery for better coverage; and promotion of biological control, particularly against whitefly. According to Mr. Jonathan Spenser of Israel, on the basis of stability, behavior and toxicity effects, activity duration and phytotoxicity effects on the plant, formulation No. 4 has been found effective in controlling whitefly and aphids. The project has developed an "over the crop drop-tube sprayer" which is capable of throwing insecticides on the lower surface of leaves. Ultraviolet tracers are used to detect insecticides on and under the leaves. At the end of the project, based on the use of safe insecticides, new types of spray machinery and enhanced population of useful insects, recommendations will be prepared for farmers.

In Egypt, the Plant Protection Research Institute is involved in testing oils and development of a manual sprayer intended to throw insecticides on both surfaces of the leaves. Dr. Galal Moawad reported that a 30% emulsion of all oils, i.e. cottonseed oil, castor oil, soybean oil, sunflower oil and safflower oil, did control sucking insects including whitefly, but some oils had more phytotoxic effect on the plant than others. Oils had no effect on larvae and pupae after three days of spraying and, after 14 days of spraying, even the adults were safe from any suffocation effects.

Main research activities are in Israel with some in Egypt. In Ethiopia and Zimbabwe, oil formulations and newly designed spray machinery are to be tested at farmer's field level. Mr. Doug Pascoe of Zimbabwe reported that five oil formulations were tested at three locations in three regions during 1996 and all of them found to be effective against whitefly. Three manual sprayers were tried and a knapsack with tall boom provided better coverage on both sides of leaves at different plant heights.

Dr. Teodoro Stadler provided an introduction of the project "Integrated pest management of the cotton boll weevil in Argentina, Brazil and Paraguay". The project has various working groups covering work on specific aspects. Four papers reviewed the work so far in this project.

Mr. Carlos Lehmacher of Argentina reported on the utilization of a Geographic Information System for controlling boll weevil. The boll weevil was first detected in the area close to the border with Paraguay and, since its discovery in Argentina, joint efforts have been made to contain the pest. But, no program in Argentina can be successful to control boll weevil if the influx from Paraguay is not stopped. Dr. Lehmacher's paper also contained the line of action of various working groups and the role of various cultural and non-chemical measures to control the pest.

Brazil has almost 15 years of experience working with the boll weevil. According to Dr. Walter Jorge dos Santos of Brazil, a high reproduction rate, great mobility and sufficient availability of alternate hosts in the off-cotton season make it difficult to control this pest. The cotton area has significantly dropped in Brazil, but still about 85% of the total area is affected by the boll weevil. In Brazil, efforts have been made to understand the behavior of the boll weevil and to utilize biological control

agents and pheromones in the integrated control package. A technology for mass rearing of *Cataloccus grandis* has been developed and used in Brazil. According to Dr. dos Santos, the Boll Weevil Attract and Control Tube has been found effective for minimizing boll weevil infestation, particularly at early stages.

The boll weevil moved to Paraguay from Brazil about 7 years ago, and now a large part of the cotton producing region is affected. Two papers were presented on the work in Paraguay. Mr. Víctor Gómez described the boll weevil affected areas and population dynamics of the boll weevil in various cotton producing regions in Paraguay. According to Mr. Ivan Gallo, the boll weevil has spreaded in Paraguay at an average speed of 66 km/year. Mr. Gallo's paper contained a number of conclusions made on the basis of work in Paraguay since this pest was recorded in April 1991. Paraguay still has high natural control, and Mr. Gallo stressed the importance of not disturbing this balance, the loss of which would further increase the cost of production and reduce farmer income.

Pakistan produced 2.2 million tons of cotton in 1991/92 and became the third largest cotton producing country in the world after China and the USA. Since that season, cotton production has been severely affected by the leaf curl virus disease (CLCuV). According to Dr. Yusuf Zafar from the Project Executing Agency of the ICAC/CFC project on leaf curl virus, Pakistan is losing about one million tons of cotton every year. The leaf curl virus project aims to characterize geminiviruses responsible for causing the disease and to develop resistant genotypes through genetic engineering and conventional breeding. The work done at the National Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan, has indicated that CLCuV can be transmitted from plant to plant only with the help of a vector, and whitefly is responsible for this activity in Pakistan. According to Dr. Yusuf, a procedure to isolate CLCuV from cotton leaves has been optimized at NIBGE, and the isolated virus particles can be used for the production of polyclonal antisera against CLCuV. The paper also included the three approaches being tried to develop transgenic resistance to geminiviruses, i.e. expression of antisense RNA against complete or fragments of the AC1 gene, over expression of AC1 in transgenic plants and expression of a virus-induced cytotoxin gene in transgenic plants.

The paper from Dr. Judith K. Brown of the University of Arizona, USA, was on the genetic diversity of CLCuV causing geminiviruses and their relationship with the whitefly vector. The paper emphasized the need to understand the geminivirus, as without knowledge a feasible defense against such viruses cannot be developed. She has collected virus isolates from various parts of the world and plans to prepare a global map of geminivirus genotype distribution in cotton. The paper also referred to her work on inoculation of cotton seedlings with infectious virus clones from the virus-affected plants using the biolistic method.

The project "Improvement of the marketability of cotton produced in zones affected by stickiness" began its activities in January 1997. Dr. Ahmed Salih Fadlalla of Sudan described the structure and objectives of the project. The project will develop a commercially feasible method to isolate sticky cotton from non-sticky cotton and will also establish what kind of sticky cotton can be mixed with non-sticky cotton without affecting the spinning process. The High Speed Stickiness Thermodetector (H2SD), developed by the CIRAD-CA of France, will be used to test stickiness. Six such machines have been received in Sudan. The Textile Institute of France is collaborating on spinning aspects.

The Committee on Cotton Production Research considered proposals from the Secretariat on topics for the 1998 Technical Seminar and decided that it will present the conclusions of the World Cotton Research Conference-2 on topics proposed by the Secretariat, including cotton contamination. The proposal from the Secretariat included wild species maintenance and utilization, biotechnology as a tool for gene manipulation, fiber maturity and principles of plant protection.

The Common Fund for Commodities–Cooperation for Development

Rolf W. Boehnke, Managing Director, Common Fund for Commodities
(Presented by Sietse van der Werff, Project Manager)

It is a great honor for the Common Fund for Commodities to address this meeting of the Committee on Cotton Production Research at the 56th Plenary Meeting of the International Cotton Advisory Committee.

The Common Fund is an intergovernmental financial institution which became fully operational only in 1991. The Fund can best be described as a partnership of over one hundred nations, rich and poor, developed and developing, which are joining forces to advance economically commodity-producing, developing countries.

The focus of the Common Fund is on commodities and this has good reasons. As you are aware, many developing and least

developed countries are heavily dependent on commodities which form the backbone of their economies and account for the bulk of their export earnings. The Common Fund, therefore, deals with a core question of development in many regions of the world.

In accordance with the "Agreement Establishing the Common Fund for Commodities," the Fund has three main functions:

Firstly, to contribute, through its so-called First Account, to the financing of international buffer stocks and internationally coordinated national stocks;

Secondly, to finance through its Second Account measures in the field of commodities other than stocking; and

Thirdly, to promote coordination and consultation in the field of commodities.

International buffer stocks have almost disappeared, thus requiring a rethinking with regard to the use of the capital resources of the First Account which were originally earmarked for bufferstocking. This process is presently ongoing in the governing bodies of the Common Fund. An amount of US$12 million has meanwhile been set aside from the net earnings of the Account for projects which would, inter alia,

- promote physical market development
- enhance market infrastructure and support services to facilitate private sector initiatives
- institution strengthening including training at all levels
- enhancement of commodity market risk management and commodity trade financing
- micro policy advice on commodity market development.

The Fund's Executive Board has approved, in July of this year, an amount of approximately US$3.6 million in the form of a grant and US$5.7 million in the form of a loan, to contribute to the financing of a project entitled "Improvement of Cotton Marketing and Trade Systems in Eastern and Southern Africa." The project's design and formulation are being finalized and it is envisaged that this three-year project will be operational in early 1998. The project will focus on the establishment of transparent and efficient marketing systems within the framework of open and liberalized markets. It aims to provide better insight in the functioning of the international market, and improved access thereto, for small scale producers, traders and exporters. Key elements of the project will be the development of a system of warehouse receipts which will serve as collateral for obtaining credit, development of efficient market/production information mechanisms and the establishment of quality control and assurance systems. The project will be located in Tanzania and Uganda, and be implemented by the Eastern and Southern African Trade and Development Bank. Its results and experiences will be widely shared among the member countries of the Fund and the International Cotton Advisory Committee.

The larger part of the Common Fund's activities is being financed from the so-called Second Account. In the short time since 1991 that the Second Account of the Fund has been operational, nearly sixty commodity development projects have been approved which benefit over 100 countries. This portfolio implies a total financial commitment of some US$130 million, of which approximately half is financed by the Common Fund. The finance has been mostly in the form of grants. Loan financing of projects will follow in the coming years.

The projects financed by the Second Account are commodity development measures, aimed at improving the structural conditions in markets and aimed at enhancing the long-term competitiveness and prospects of particular commodities. Such measures include:

- research and development
- productivity improvement
- marketing
- vertical diversification

The Fund concentrates on low cost, high impact projects which have the potential of becoming self sustainable, involving, whenever possible, the private sector.

Projects have to be submitted to the Common Fund through a designated International Commodity Body such as the International Cotton Advisory Committee in the case of cotton. The Fund's collaboration with the 23 International Commodity Bodies contributes to a focus on areas where projects can be most effective. This collaboration also ensures that target beneficiaries fully play their role in project design and execution. The Fund prefers to support projects which provide solutions to general problems of the commodity concerned or which involve a number of commodity producing developing countries, in particular the least developed among them. Single country based projects are the exception and should be pilot projects with replicable results for other developing countries. These "national based" projects should preferably be financed by loans which will need to be guaranteed by the government of the country concerned, or by an entity acceptable to the Common Fund.

With regard to project formulation and preparation, these fall within the responsibilities of the International Commodity Bodies. Any project submitted to the Fund for financing should fit in the context of an overall commodity development strategy of the International Commodity Body. The Common Fund welcomes the dialogue with the International Commodity Bodies at an early stage of project formulation. The International Commodity Body acts as Supervisory Body for its projects, while the actual project execution is performed by a specialized institution with expertise in the field concerned (the Project Executing Agency).

The International Cotton Advisory Committee has been, and continues to be, an active player in the field of cooperation with the Common Fund. The present project portfolio of the Second Account is a clear reflection of this. The four ongoing projects (excluding the one that has meanwhile been completed), have a Common Fund budget of approximately US$7.7 million, representing some 15% of the Fund's total commitments. Cotton is therewith the most important commodity in the Fund's project portfolio. In spite of this already large share, we would be pleased to further strengthen the collaboration between the International Cotton Advisory Committee and the Common Fund for Commodities. The initiative taken by the ICAC to devote part of the proceedings of the 56th Plenary Meeting to review the progress made in our common projects is seen by the Fund as a sign supportive to the efforts to further the collaboration between the two organizations.

The successful continuation of the close working relations between the International Cotton Advisory Committee, the Common Fund and the Project Executing Agencies of the four cotton projects will no doubt have marked results on strengthening the position of cotton as a commodity of great importance to both producers and consumers, in developing and developed countries alike.

Integrated Pest Management for Whitefly in Cotton

Jonathan Spenser, The Israeli Cotton Production and Marketing Board Ltd., Israel

This project, named "Integrated Pest Management for Cotton" is an international joint effort involving Egypt, Ethiopia, Israel and Zimbabwe, aimed to develop integrated methods for the control of stickiness-causing insects, whitefly and cotton aphids. The project was initiated and is supervised by the ICAC as an ICB and substantially supported by the Common Fund for Commodities (CFC).

The CFC approved the project in 1994 for a research period of four years. The project is now in its third year and managed by a committee at the Israel Cotton Production and Marketing Board Ltd., designated by the Fund as the Project Execution Agency (PEA).

The specific objectives include the production of high quality non-sticky cotton; improvement in profitability for both raw cotton producers and processors; and reduction in damage to the environment.

The project is developing new target oriented environmentally compatible pesticide formulations and their application methods; promoting biological pest control; developing guidelines for economic use of these methods; and disseminating the project findings to staff of participating and other countries.

Annual reports are available for 1995 and 1996. Annual work plans and budgets are made available before the onset of trials each year. Two external consultants have been engaged in the evaluation of various components of the project, and an additional mid-term evaluation mission nominated by the ICB and the Fund presented a report in August 1997.

Activities and Project Status

Research, trials and development are in progress in Israel and Egypt. Field trials and development have been underway from the second project year in Zimbabwe and Ethiopia.

Activities in Israel are a coordinated effort of four different and independent research groups of three distinct research organizations. The entities involved are the Israel Ministry of Agriculture (IMA), the Agricultural Research Organization (ARO); the Chemistry and Engineering Dept.; Tel Aviv University (TAU); and Sivan/Granot Research Dept. in conjunction with the IMA Extension Service. In Egypt, the Plant Protection Research Institute (PPRI) is leading the project. The Commercial Cotton Growers Association (CCGA) is leading the project in Zimbabwe and the Institute of Agricultural Research (IAR) is running the project in Ethiopia. Monitoring of research activities and coordination is followed on a regular basis by the project management at the PEA in Israel.

Development of Target Oriented, Environmentally Compatible Pesticide Formulations

Activities at present are divided between research of new or modified formulations and up-scaling studies of the best candidates. Parameters under study are stability, behavioral and toxicological activity on various life stages, activity duration, phytotoxicity tendencies and foliar residue characteristics. Formulations which passed lab trial criteria were cleared for field trials. One such formulation, designated as "No. 4" has been found effective against whitefly under field conditions. In addition, in Egypt, mineral oils are under assessment for efficacy against whitefly.

Design of Criteria for a Cotton Sprayer and Design of a New Sprayer

The development of an "over the crop drop-tube sprayer" has continued. Last summer experiments showed that the one-row prototype of this sprayer is capable of creating vortex streams from the ground upward, causing an even and dense coverage of all the lower plant parts. A six-row sprayer was designed and

constructed in recent months and is now under preliminary tests in 120 cm high cotton fields. The present tests are aimed at determining the final geometry of the drop-tube air duct and the nozzle location. Most of the experiments involve spraying of an ultra-violet tracer in the field and droplet counting on leaves and other plant parts in the laboratory.

A manual operated sprayer for "low toxicity" materials is under development; a final design of the components has been performed. Rotary atomizers (Micron-Mini-ULVA) were chosen and tested for droplet size and spray cloud geometry at different positioning. Two different models for relatively large (ULP/100) and for small (MUL/100) droplets were chosen. The sprayer design permits installing alternatively each of the models. An air carrier duct is also under construction. The manual operated sprayer was completed and tested in September 1997.

Biological Control Methods

Natural enemies of cotton are very often responsible for the prevention of outbreaks and avoidance of damage. Therefore, it is important to establish the appropriate natural enemies in the field, encourage their activities and protect them from hazardous effects of insecticides. Activities include surveys concerning the presence, effectiveness and means of conserving and utilizing natural enemies in the cotton agro-ecosystems; evaluation of toxic effects of new and established insecticides upon natural enemies; employment of additional natural enemies to control pests; transfer and augmentation of existing fauna of beneficial organisms; and integration of the use of natural enemies with other methods according to thresholds.

Establishment of Appropriate Economic Thresholds for the New Methods

The concept followed considers that the pests in question tend to exhibit exponential growth. The controlling strategy and the thresholds that underlie it, therefore, must intervene to prevent massive build-up. In order to develop a strategy based on cost/benefit reasoning, the influence of pest levels during selected crop growth periods on quantity and quality is under investigation. In addition, precise scouting is under development to estimate natural enemy effects on threshold related decisions to optimally utilize natural enemies and other novel pest control approaches. The ultimate goal is to develop usable action guidelines for growers.

Knowledge Dissemination

Regular annual Project Coordinating Committee meetings take place. These include project and component leaders and additional staff. Three training programs (2 in Israel and 1 in Egypt) including staff from all participating countries took place during 1997. Additional training programs and workshops for participating and other countries are planned for the concluding year of the project, including comprehensive reports and summaries of the findings and applicable guideline manuals for end users.

Field Trials

Extensive field trials are underway in all participating countries. In Israel, field trials include novel material screening for phytotoxicity and control efficacy, prototype sprayer efficiency, and prototype material replicated trials. Additional sets of trials include biological control in the greenhouse and in the field. Threshold development trials are underway separately. In Egypt field trials include manual backpack sprayer trials, mineral oils control in addition to novel material and natural enemy experimentation. Replicated field trials in Zimbabwe were conducted at three locations on novel materials, biological control and spray methods. In addition, an independent evaluation of various hand held sprayers was carried out. In Ethiopia, novel material screening, biological control and aphid economic threshold level studies have been undertaken.

Integrated Management of Cotton Sucking Insect Pests in Egypt

Galal M. Moawad, Plant Protection Research Institute, Egypt

Introduction

Bemisia spp. is a major worldwide pest of cotton, vegetables and ornamental plants. The introduction and excessive use of pesticides in Egypt led to resistance of *Bemisia* spp. to organochlorines, organophosphates, carbamates and pyrethroids (Henneberry, 1993). However, these pests are known to be attacked by three genera of hymenoptera, (López-Avila, 1986 and Viggiani and Evans, 1992) as well as many predators representing eight arthropod orders (Nordlund and Legaspi, 1995).

Environmental pollution has occurred in the Egyptian agroecosystem for centuries, but it became a significant problem the last few decades. This is mainly due to the excessive use of pesticides. In the Middle East, Africa and India pesticide usage reduced the role of natural parasitoids in controlling *Bemisia* spp. The percentage of parasitism rarely exceeds 70 to 80% (Hafez et al, 1978, 1979a & 1979b, Abdelrahman 1986, Shalaby et al, 1990, Abdel-Gawaad et al, 1990). However, during the last few years the Egyptian government changed its

policy and restricted the use of many pesticides whose residues in the environment appear to constitute hazard to man, domestic animals and wildlife.

Objectives

The present work was carried out to investigate the following points:

- The effect of plant oils, as environmental friendly compounds, in controlling these insects and their action on natural parasites and predators as well as phytotoxic action on the cotton plant.
- Evaluation of the bioinsecticide "naturalis – L" against whitefly in cotton fields.
- The role of natural enemies (parasites, predators and mites) in the cotton field as an important component of integrated pest management against whitefly and cotton aphids.

Material and Methods

Screening the Efficacy of Plant Oils

Six samples of plant oils, received from Dr. Veierov were used in this investigation. They are as follows:

1 - Cottonseed oil emulsion 30%
2 - Castor oil emulsion 30%
3 - Stabilized cotton seed oil emulsion 30%
4 - Soybean oil emulsion 30%
5 - Sunflower oil emulsion 30%
6 - Safflower oil emulsion 30%

Experiments were carried out to evaluate the phytotoxity actions of these compounds on the cotton plant.

Phytotoxic Effect

These experiments were carried out at Sakha Research Station on cotton at 2 true leaves stage. Each oil emulsion was tested on 4 cotton rows. In addition, one treatment was sprayed with water, using a knapsack sprayer, and another one unsprayed as a control. The first application started on May 1 and replicated every two weeks until the end of July 1996.

Observations were carried out twice a week to estimate phytotoxity actions. These include records of scorching leaves, color change of the leaves and growth retardation. Also, at harvesting, plant characters (height, leaves and branches, vegetative weight, dry matter, number of small and large bolls, number of bolls, and lint and seed) were also included.

Control of Cotton Whitefly and Aphids

Experiments were carried out in a cotton field at Damietta Governorate infested with the cotton whitefly and aphids. The experimental area had 8 plots, 1-kerate (175 m^2) each. The knapsack sprayer was used to apply the previous mentioned 6 plant oils. In addition one plot was sprayed with water only as a control and another untreated control (check) plot was also maintained. Before treatment, a number of leaves from each plot were sampled and examined in the laboratory for whitefly (immatures) and aphid infestations. The same number of leaves was examined weekly from each treatment.

Evaluation of the Bioinsecticide "Naturalis-L"

Small plot experiments were conducted in cotton fields in Zayan area (Damietta Governorate) in late August 1996. Each plot was about 175 m^2. All experimental plots received normal agricultural practices during the cotton growing period and no pesticides were used. Three applications of Naturalis-L were carried out every 5 days. The action of this biological product is based upon the naturally occurring fungal organism (*Beauvaria bassiana* 1.6 7% 2.3 x 10.7 conidia per milliliter of product 98.33%). Two rates, 400 and 600 ml. per feddan (4200 m^2) were applied using a conventional motor alternate of 600 litters of water per feddan. Each treatment was replicated four times including untreated control. Pre and post-treatment whitefly adults, larvae and pupae were counted after the 5^{th}, 10^{th} and 15^{th} days of spraying. In each replicate, adults were counted early morning on ten leaves located on the main stem of the plant. Ten leaves at each of the three levels of the cotton plant (upper, middle and lower) were picked up from each treatment. Each group was put in a paper bag and transferred to the laboratory for examination. Data were adjusted by Henderson and Tilton's (1955) equation for natural changes in population density occurring in the untreated plots.

Natural Enemies

The aim of this work is to investigate the role of natural enemies associated with *Bemisia* spp. and aphids in reducing the rate of infestation. The effect of pesticides on these natural enemies in cotton fields was also investigated.

This work was conducted at two experimental research stations, Sakha (Kafr El-Sheikh Governorate) and Malawi (El-Minia Governorate) during the 1995 and 1996 seasons. Two feddans were chosen at each station and planted with Giza 86 in late March. One feddan was left without pesticidal treatment and the other treated with recommended pesticides. At Sakha station, cotton was sprayed four times against cutworm, cotton leafworm and bollworm on April 20, July 12, August 21 and September 3 by Kafromone (methamedophos), Catabron (profenofos + chlorfluazuron), Delphos (chlorpyrifos + Hexafumuron) and Larven (thiodicarb), respectively. At Malawi station, cotton was sprayed five times against the same insect species on April 15, July 13 and 29; August 17 and September 9 with Kafromone, Delphos, Kendo (lambada cyhalothrin), Larven (thiodicarb) and Seven (carbaryl) respectively.

Normal cultural practices, regularly used for cotton fields were applied according to the instruction laid by the Ministry of Agriculture. Samples were taken weekly from June to early October in each Governorate. At Sakha station, during 1995, treated cotton was sprayed seven times against the aforementioned pests on April 4, July 1 and 6, August 1, 8 and 21 and September 6 with Hostathion (triazophos), Dennate (methomyl + diflubenzuron), Catabron, Kendo, Catabron, Koracron (profenofos) and Kindo respectively. The studied factors were as follows:

The Role of Parasites on Whitefly

Ten plants were chosen randomly. Three leaves, located on the main stem of the plant, (node number 5, 6 and 7) were selected. The terminal node represented node number one. Each leaf of the same node was put in a paper bag and directly taken to the laboratory for careful examination with the aid of a stereomicroscope. Third instars and pupae were counted and put on wetted filter paper in petri-dishes till the emergence of parasitoid adults. Parasitoids were identified, counted and the percentage of parasitism by each parasitoid species and the total percentage of parasitism were estimated. Phytoseiid mites were also recorded.

Population Fluctuation of Aphid (*Aphis gossypii* Glover) and its Parasitoids

Twenty plants were chosen at random. Three leaves were taken from each plant representing the three levels of the cotton plant. Each level comprised twenty-one leaves. The number of winged and wingless aphids were counted and recorded directly in the field. The leaves of the same level were put in a paper bag and directly taken to the laboratory. Mummied aphids were kept in glass jars till the possible emergence of parasitoid species for identification and percentages of parasitism were calculated.

Seasonal Abundance of Predators

Five locations were chosen at random in one feddan area, each represented by five adjacent plants located in a one-meter row. The number of each predator species was counted and recorded directly in the field.

Results and Discussion

Phytotoxicity of Plant Oils

Results indicated that, in general, no significant color change, scorching and growth retardation were observed. However, a slow rate of color change was observed on cotton leaves after spraying with cottonseed oil emulsion and stabilized cotton seed oil. These changes increased continuously, especially after the second spray. Also, on August 20, observation showed that scorching damage appeared on cotton leaves, flowers, bolls and new branches of cotton plants sprayed with cottonseed oil and stabilized cottonseed oil. Light damage of scorching was also observed with sunflower oil, castor oil and soybean treatments.

The damage occurred on the treated cotton plants in July 1996 may be due to the emulsifier agent or the concentration of active material of these formulations. Moreover, phytotoxity may also be due to weather factors during this period (temperatures, humidity, light, solar radiation, etc.).

However, it is possible to improve the performance of these formulations by introducing another emulsifier, which may reduce or prevent the phytotoxity effect and increase sensitivity of the insect to these compounds.

Effectiveness of Plant Oils

Zayan Experiment

Tables 1-3 show the efficiency of the tested plant oils on different stages of *Bemisia tabaci*. Data in Table 1 indicate that the number of whitefly adults decreased in all treatments with reduction percentages ranging between 17.6 - 53.3% after three days of spraying. Reduction in whitefly adults also increased after 7 days of spraying with stabilized cottonseed oil (96.2%), castor oil (61.8%) and soybean oil (51.8%). Moreover, reduction in adults after 14 days of treatment with the last two oils was 63.8% and 68.3%, respectively. The reduction in whitefly adults may be due to the effect of these oils as a repellent and/or by changing the leaf surface of cotton plants for feeding and egg laying.

The reduction in *Bemisia tabaci* eggs on cotton plants three days after plant oil treatments was 77.1 and 71.1% with stabilized cottonseed oil and sunflower oil respectively (Table 2). Reduction after 7 days was 71.6 and 92.8% in both treatments. After two weeks the reduction of eggs was 88.8%, 79.4%, 77.6% and 61.2% with cottonseed oil, castor oil, sunflower oil and stabilized cotton seed oil treatments, respectively.

Data in table 3 show the reduction in *Bemisia tabaci* larvae and pupae after treatment by plant oil emulsions. No reduction occurred on *Bemisia tabaci* larvae and pupae after three days of application for all tested oil formulations. Reductions detected on day 7 were 85.6%, 65.4%, 63.9% and 51.2% for safflower, sunflower, cottonseed oil and stabilized cottonseed oil, respectively. Effectiveness of plant oils did not extend against *Bemisia tabaci* larvae and pupae until 14 days after application except with stabilized cottonseed oil, which gave a reduction of 48.8% (Table 1).

Belkass Experiment

Tables 4-6 show the effectiveness of plant oil emulsions on *Bemisia tabaci* stages on cotton plants at Belkass. The number of whitefly adults was less in Belkass than in Zayan area. However, reductions were 19.4%, 64% and 38.7% after 3 days with stabilized cottonseed oil, soybean oil and sunflower oil, respectively. The percentage of reduction after 14 days was 75.6, 22.3 and 43.7% for the three plant oils, respectively (Table 4).

Table 1. Effectiveness of Plant Oil Emulsions on *B. tabaci* Adults on Cotton Plant in Zayan Village, Damietta Governorate, 1996 Season

Treaments	Pre-treatment	Post treatment (in days)					
		3		7		14	
	No.	No.	Red. %	No.	Red. %	No.	Red. %
Cottonseed oil	947	1020	17.6	865	41.7	588	0
Castor oil	2013	1230	53.3	1203	61.8	345	63.8
Stabilized cottonseed oil	1281	1150	31.4	77	96.2	765	0
Soybean oil	1412	1240	32.9	1065	51.8	212	68.3
Sunflower oil	1522	1260	36.7	1119	53	660	8.4
Safflower oil	945	950	23.1	1002	32.3	995	0
Water spray	1172	1520	0.8	923	49.7	496	10.6
Untreated	1124	1470	0	1760	0	532	0

Table 2. Effectiveness of Plant Oils Emulsions on *B. tabaci* Eggs on Cotton Plant in Zayan Village, Damietta Governorate, 1996 Season

Treaments	Pre-treatment	Post treatment (in days)					
		3		7		14	
	No.	No.	Red. %	No.	Red. %	No.	Red. %
Cottonseed oil	2288	3626	21.6	2925	13.8	366	88.8
Castor oil	2285	3504	24.2	2168	36	672	79.4
Stabilized cottonseed oil	2397	1112	77.1	7730		1325	61.2
Soybean oil	2685	2866	47.2	2461	38.2	1624	57.6
Sunflower oil	10696	6260	71.1	4500	71.6	1095	92.8
Safflower oil	2428	5171		2424	32.7	775	77.6
Water spray	1714	2542	26.7	1858	26.9	1318	46.2
Untreated	1343	2716		1992		1918	

Table 3. Effectiveness of Plant Oils Emulsions on *B. tabaci* Larvae and Pupae on Cotton Plant in Zayan Village, Damietta Governorate, 1996 Season

Treaments	Pre-treatment	Post treatment (in days)					
		3		7		14	
	No.	No.	Red. %	No.	Red. %	No.	Red. %
Cottonseed oil	1990	1679		1174	63.9	2250	19.5
Castor oil	1271	1418		1991	4.1	1941	
Stabilized cottonseed oil	2012	2327		1603	51.2	1448	48.8
Soybean oil	1594	1725		1672	35.7	3372	
Sunflower oil	1804	1600		1020	65.4	3296	
Safflower oil	2082	1737		489	85.6	3200	
Water spray	1936	1006	19.2	1659	47.5	2872	
Untreated	1944	1250		3175		2732	

Table 5 also summarizes the reduction percentages of *B. tabaci* eggs after application. Reduction was detected on day 3 with castor oil and stabilized cottonseed oil applications. Reduction in the number of whitefly eggs was more than 80% for cottonseed oil, stabilized cottonseed oil and castor oil, but decreased on day 14 after application (62.5% and 55.1% for cottonseed oil and stabilized cottonseed oil respectively).

Table 6 shows the reduction in *B. tabaci* larvae and pupae on cotton plants in Belkass after plant oil applications. Reduction after 3 days of spray was 29.2-80.5%. No reduction was estimated after 7 and 14 days in castor oil and sunflower oil treatments. However, reduction was 54.1 and 61.9% with cottonseed oil at 7 and 14 days. The highest reduction (80.5%) in the number of *B. tabaci* larvae and pupae was recorded in stabilized cottonseed treatment after three days, which may be due to the effect on newly hatched larvae and/or first and second instars. These instars are more highly sensitive to plant oil treatment than pupae

Efficiency of Plant Oils against Aphid

Table 7 shows the relative reduction in *Aphis gossypii* after exposure to plant oil on cotton plants. Reduction in aphid numbers to 99.3%, 98.5%, 95.2% and 96.9% was obtained 3 days after spray by castor oils, soybean oil, cottonseed oil and safflower, respectively. However, satisfactory reduction was caused by sunflower (63.9%) and stabilized cottonseed oil (66.89%). All plant oil treatments reduced aphid numbers after 7, 10 and 15 days. It is obvious that the reductions which occurred in the aphid population are not attributed only to toxic effects of plant oils, but also to the decline in the aphid population on cotton plants during this period (July) as a result of high temperatures. Thus, several experiments should be carried out in future years to clarify this observation and to explain the cause of phytotoxity and to improve the efficiency of these compounds.

Evaluation of Naturalis - L against Whitefly

Adults

Table 8 summarizes the reduction in whitefly adult numbers on cotton plants after "Naturalis - L" application at two rates after 5,10 and 15 days of spraying. Reduction percentages after 5 days, were 35.33 and 62.59% at the rates of 400 and 600 ml./feddan (fed. = 4200 sq. meters) respectively. The reductions of

Table 4. Effectiveness of Plant Oils Emulsions on *B. tabaci A* dults on Cotton Plant in Belkass Area, Dakahlia Governorate, 1996 Season

Treaments	Pre-treatment	Post treatment (in days)					
		3		7		14	
	No.	No.	Red. %	No.	Red. %	No.	Red. %
Cottonseed oil	230	345		212		290	
Castor oil	332	237		142		305	
Stabilized cottonseed oil	450	255	19.4	92		89	75.6
Soybean oil	522	132	64	120		329	22.3
Sunflower oil	515	222	38.7	221		235	43.7
Safflower oil	330	396		137		495	
Water spray	840	435	26.3	53		336	50.7
Untreated	555	390		73		540	

Table 5. Effectiveness of Plant Oils Emulsion on *B. tabaci* Eggs on Cotton Plant in Belkass Area, Dakahlia Governorate, 1996 Season

Treaments	Pre-treatment	Post treatment (in days)					
		3		7		14	
	No.	No.	Red. %	No.	Red. %	No.	Red. %
Castor oil	3033	927	55.3	349	80.7	1484	
Stabilized cottonseed oil	5341	675	81.5	608	81	1051	55.1
Soybean oil	2022	1542		1961		1499	
Sunflower oil	1868	1401		770	31	1213	
Safflower oil	1614	561	49.2	442	54.2	668	5.5
Water spray	2303	2730		543	60.5	1575	
Untreated	2890	1977		1727		1266	

Table 6. Effectiveness of Plant Oils Emulsions on *B. tabaci* Larvae and Pupae on Cotton Plant in Belkass Area, Dakahlia Governorate, 1996 Season

Treaments	Pre-treatment	Post treatment (in days)					
		3		7		14	
	No.	No.	Red. %	No.	Red. %	No.	Red. %
Cottonseed oil	1155	1209	29.2	340	54.1	558	61.9
Castor oil	656	579	40.3	499		1883	
Stabilized cottonseed oil	2555	738	80.5	376	77	1615	50.1
Soybean oil	1192	714	59.5	267	65	1362	9.8
Sunflower oil	572	861		1075	0	2306	
Safflower oil	805	756	36.4	937	0	522	48.8
Water spray	704	1401		433	4	2325	
Untreated	966	1428		619	0	1224	

whitefly adults increased to 50.11 and 64.71% after 10 and 15 days respectively after the second and third spray at the rate of 400 ml./fed. However, reductions at the rate of 600 ml./fed. were 57.54% and 55.96% after 5 and 10 days respectively.

Larvae and Pupae

The results in table 9 show the efficiency of Naturalis - L against whitefly larvae and pupae at three levels on cotton plants in August 1996. The two rates (400 and 600 ml./fed) caused satisfactory reduction in *B. tabaci* larvae and pupae in comparison with untreated plants, but the higher rate gave more effective control. On day 15 after treatment, reduction percentages at the higher rate were 81.4%, 76.94% and 80.94% at upper, middle and lower levels of the cotton plant respectively. However, the low rate reduction percentages were 76.03%, 67.65% and 74.01% for the three levels respectively. Results indicated that satisfactory control of whitefly was obtained through 15 days after treatment by Naturalis - L. The obtained results may help to enhance rapid control of this pest necessary under field conditions, using Naturalis - L alone or in combination with other insecticides.

Natural Enemies

Whitefly

Parasites

Two hymenopterous species *Encarsia lutea* (Masi) and *Eretmocerus mundus* Mercet (Fam. Aphelinidae) were identified as parasitoids on *B. tabaci* in the two Governorates. (Fig. 1 and 2). The percentages of parasitism ranged from zero to 46.3% and zero to 45.2% for *E. lutea* and *E. mundus* respectively, at Sakha, and from zero to 32.6% and zero to 60.7% for the two parasitoids respectively at El-Minia. Parasitism percentages on cotton plants treated with insecticides to control cotton leafworm and bollworm at two locations (Sakha and El-Minia) were high during July and decreased gradually until harvest time. The highest parasitism by *E. lutea* occurring during July, early August and end of September was 32.4%, 32%, and 46.2% respectively, at Sakha, and 32.6%, 30.2% and 30% respectively, at El-Minia. The highest parasitism by *E. mundus* occurring in early July, late July and late September was 43.8%, 45.2% and 38.5% at Sakha (Fig 1) and, on July 8, late July and mid-August 30.6%, 46.2% and 60.7% at El-Minia. Parasitism by *E. lutea* and *E. mundus* together on *B. tabaci* nymphs and pupae increased gradually on cotton plants and continued through the season until harvest time. The natural mortality inflicted by both parasitoids on *B.*

Table 7. Efficiency of Plant Oils against *Aphis gossypii* Infesting Cotton

Investigation time	Untreated (ckeck) No.	Spray with water		Castor oil		Soybean oil		Stab. cottonseed oil		Sunflower oil		Cottonseed oil		Safflower oil	
		No.	Red%	No.	Red.%	No.	Red%	No.	Red%	No.	Red%	No.	Red%	No.	Red%
Pre-treatment	300	310		1156		1055		310		350		988		1021	
Post-treatment															
3 days	38	53		1	99.3	2	98.5	13	66.89	16	63.9	6	95.2	4	96.9
7 days	44	35	23.02	12	92.92	15	90.3	17	62.09	0	100	5	96.54	12	91.98
10 days	8	7	15.32	4	87.7	0	100	0	100	0	100	0	100	0	100
15 days	94	12	87.65	65	82.05	0	100	0	100	0	100	5	98.38	0	100

Table 8. Effectiveness of Bioinsecticide Naturalis-L against Whitefly Adults on Cotton Plants, 1996 Season

Time of investigation	Whitefly adult				Untreated (check)
	400ml./fed.		600ml./fed.		
	No.	Red. %	No.	Red. %	
Pre-treatment	281		244		505
Post-treatment					
5 days	213	35.33	107	62.59	592
10 days	161	50.11	119	57.53	580
15 days	108	64.71	118	55.96	550

Table 9. Effectiveness of Bioinsecticide Naturalis against Whitefly Larvae and Pupae on Cotton Plants, 1996 Season

Level of plant	Time of investigation	*B. tabaci* Larvae and pupae				Untreated (check)
		400 ml./fed.		600 ml./fed.		
		No.	Red. %	No.	Red. %	
Upper	Pre-treatment	459		539		364
	Post-treatment					
	5 days	346	64.08	334	64.08	597
	10 days	228	67.07	112	86.22	522
	15 days	185	76.03	171	81.14	582
Middle	Pre-treatment	381		508		551
	Post-treatment					
	5 days	238	33.68	266	44.41	519
	10 days	218	40.28	193	60.35	528
	15 days	121	67.65	115	76.94	451
Lower	Pre-treatment	539		466		382
	Post-treatment					
	5 days	421	32.03	312	41.74	439
	10 days	152	79.24	111	81.46	519
	15 days	183	74.01	116	80.94	499
Whole of plant as an average	Pre-treatment	1379		1513		1279
	Post-treatment					
	5 days	1005	40.06	912	50.42	1555
	10 days	598	64.65	416	77.8	1569
	15 days	489	72.03	402	79.64	1662

tabaci, pronounced during July, August and September, was 43.8%, 51.5%, and 84.6% at Sakha; and 48.8%, 53.1% and 60% at El-Minia during the 1995 cotton season. These factors should be taken into account when planning control management of *B. tabaci* on cotton plants.

In the 1996 cotton season, the populations of the third and fourth instars were higher on treated cotton than on untreated at the two Governorates. They reached a maximum of 7.9 individuals per leaf at the beginning of the third week of August at Kafr El-Sheikh and 6.6 at El-Minia in the 3rd week of July (Figs 3 & 4) respectively. The percentage of parasitism reached 100% on untreated cotton plants in the late season when *B. tabaci* population was very low at Kafr El-Sheikh. These results were in agreement with those obtained by Joyce (1955), who found that the percentage of parasitism by *Eretmocerus* spp. reached 100% on *B. tabaci* pupae in Sudan. Gameel (1969) also mentioned that *E. lutea* and *E. mundus* caused 100% parasitism on *B. tabaci* pupae in the same country. On the other hand, the percentage of parasitism on treated cotton plants reached 73.8%. Hafez et al (1978 and 1979a), Abdel-Rahman (1986) and Sundaramurthy (1992) mentioned that pesticides reduced the role of parasitoids and the percentage of parasitism rarely exceeds 70 to 80%. Price and Schuster (1991) reported that pesticides significantly reduced the survival of several *Encarsia* species attacking *Bemisia* on poinsettia in a greenhouse. At El-Minia, the percentage of parasitism was ap-

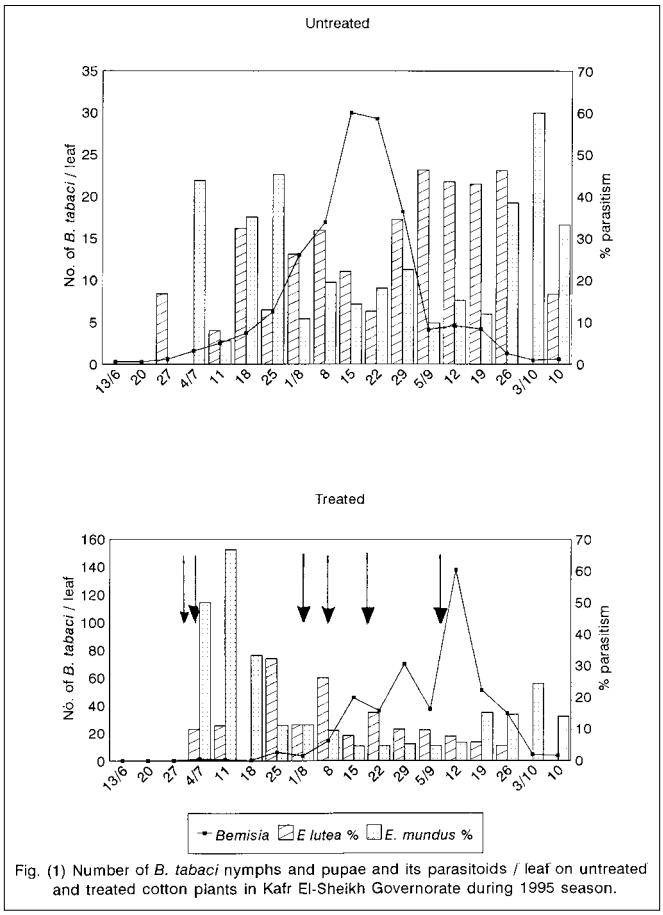

Fig. (1) Number of *B. tabaci* nymphs and pupae and its parasitoids / leaf on untreated and treated cotton plants in Kafr El-Sheikh Governorate during 1995 season.

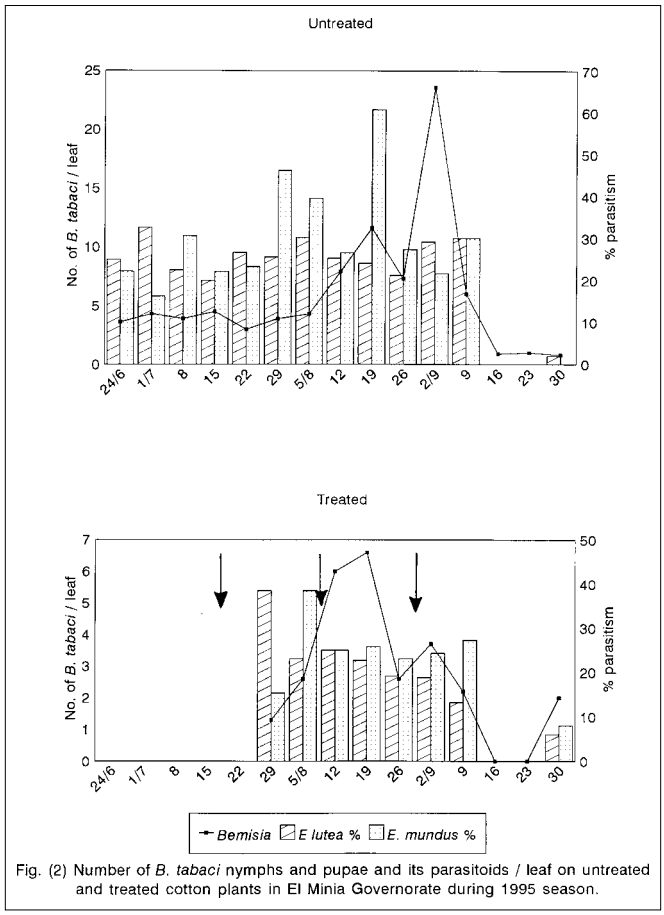

Fig. (2) Number of *B. tabaci* nymphs and pupae and its parasitoids / leaf on untreated and treated cotton plants in El Minia Governorate during 1995 season.

proximately equal on treated and untreated cotton plants. Gerling (1967), Bellows and Arakawal (1988) and Kapadia et al (1992) have reported that sometimes parasitism is not significantly affected by application of pesticides.

Figure 3 shows that the percentage of parasitism by *Eretmocerus mundus* surpassed *Encarisa lutea* in all inspections except two, when the population of whitefly declined on untreated cotton plants at Kafr El-Sheikh. The highest percentage of parasitism reached 100% in late season when the population was very low (0.07 pupae/leaf) for the first parasite and 58% in mid September for the second one. Figure 3 also shows that *E. lutea* was significantly affected by pesticides while *E. mundus* was not.

On the other hand, the percentage of parasitism by *E. lutea* was higher than *E. mundus* at El-Minia (Fig. 4). The percentage of parasitism was 51.7% in late July and 34.93% at the end of August by *E. lutea* and *E. mundus,* respectively. The two parasites were not affected by pesticides (Gerling 1967; Bellows and Arakawal 1988 and Kapadia et al 1992).

Predator Mite

Figure 5 shows the population dynamics of *B. tabaci* and the predator mite *Amblyseius swirskii* (Athias and Henriot) on treated and untreated cotton fields at Kafr El-Sheikh during the 1995 season. Data revealed that mite individuals began to appear the first week of August and increased gradually to reach the maximum number in late season on untreated cotton plants. After two weeks from the appearance of mites, the population of whitefly began to decline. The opposite was true for the number of whitefly on treated cotton plants. The population of whitefly was high until the end of the season with very few numbersof mites appearing in late season. Extensive application of pesticides during the season against the abnormal infestation by cotton leaf worm significantly reduced the populations of predator mites. On the other hand, in the 1996 season (Fig. 6), mite populations began to appear in the third week of July and increased gradually until early September and then declined gradually until the end of the season. However, in treated cotton plants mite numbers were very low except in late season, when the population of mites increased compared with untreated cotton plants. This result indicated that the last spray by Larven (thiodicarb) had no effect on the mite population. Generally, pesticides significantly reduced the population of predator mites during the two seasons. The results were similar to those obtained by Swirski et al (1967), El-Badry (1967), Gameel (1971) and Meyerdirk and Codriet (1985). They found that predator mites effectively reduced *Bemisia* population in cotton and vegetable fields.

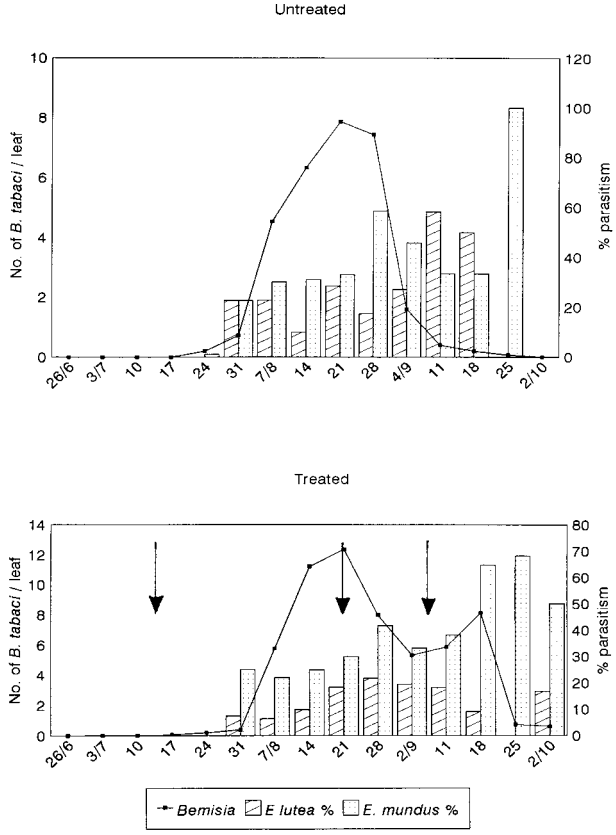

Fig. (3) Number of *Bemisia tabaci* nymphs and pupae and its parasitoids on untreated and treated cotton plants in Kafr El-Sheikh Governorate during 1996 season, (Arrows indicate dates of insecticide treatment)

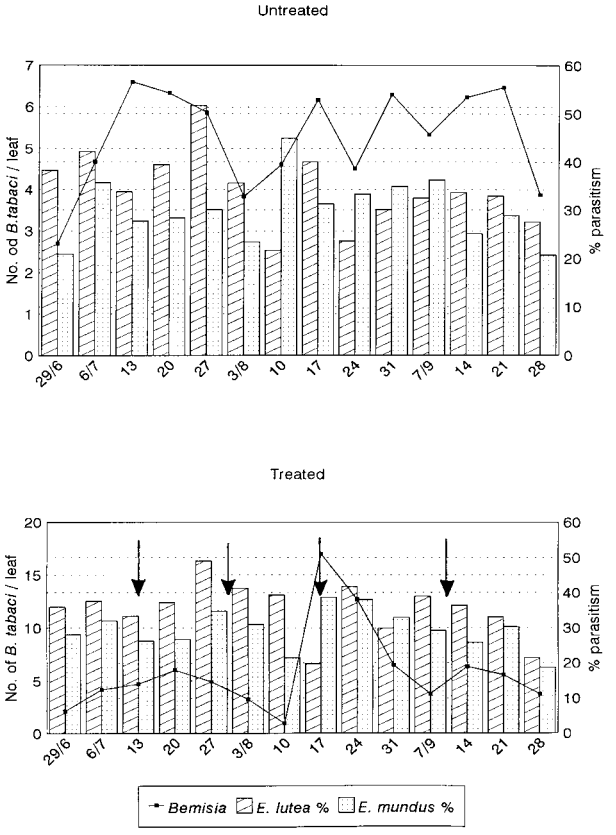

Fig. (4) Number of *Bemisia tabaci* nymphs and pupae and its parasitoids on untreated and treated cotton plants in El- Minia Governorate during 1996 season.(Arrows indicate dates of insecticide treatments).

Other Predators

Figures 7 and 8 show predators associated with the whitefly on treated and untreated cotton plants at two Governorates. Generally, the total number of all predator species at El-Minia surpassed those of the same predator species at Kafr El-Sheikh. The predators recorded in the two Governorates were as follows:

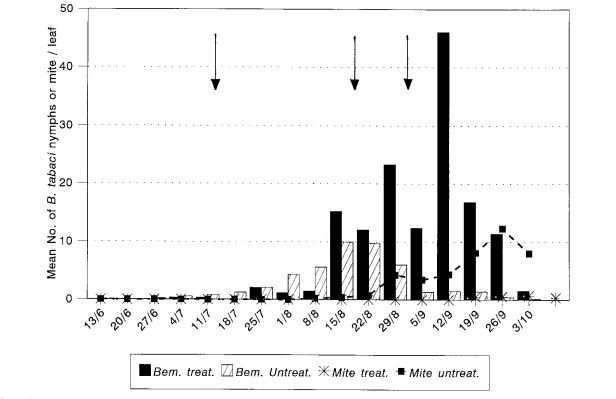

Fig. (5) Population levels of *B. tabaci* and the predacious mite, *A. swirskii* in treated and untreated cotton fields, Kafr El-sheikh, 1995 (Arrows indicate dates of insecticide treatments.)

Order: Coleoptera; Family: Coccinellidae

- **Lady bird (*Coccinella undecimpunctata* L., *C. septempunctata* L. and *Cydonia vicina nilotica*)**

These predators appeared in few scattered numbers on treated and untreated cotton plants at Kafr El-Sheikh, while at El-Minia they were abundant. The highest number of these predators (16.6 individuals/five plants) occurred in mid-July on untreated cotton plants when the population of whitefly reached a maximum (Fig. 8). The entomologist of CIBC (1983) in Pakistan and Abdel-Shalaby et al (1990) in Egypt mentioned that *Coccinella septempunctata* L. attacked nymphs of *B. tabaci*. Kapadia and Puri (1989) stated that the coccinellid *Serangium parcesetosum* is an important predator of *Bemisia* spp. in India.

- ***Scymnus syriacus* (Marshall) and *S. punctillum* (Weise)**

As shown in Figure 7, *Scymnus* spp. began to appear in late July and reached a maximum in August, with the highest number of whiteflies at Kafr El-Sheikh. At El-Minia, this predator occurred all season and the highest number was recorded in the first week of July and in September (Fig. 8). In India, Rahman (1940) reported that *Scymnus* spp. attacked *B. tabaci* nymphs. In Egypt, Hafez et al (1978/1979a) and in Israel Gerling (1986) recorded that *Scymnus syriacus* was associated with *B. tabaci*.

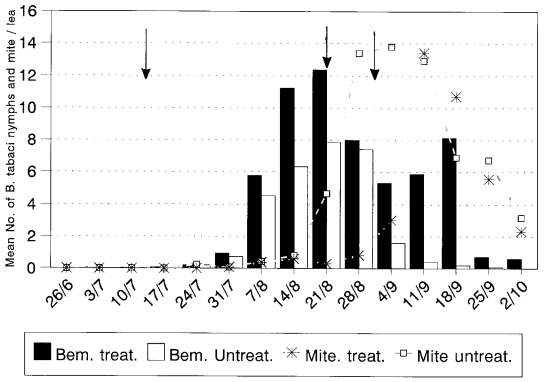

Fig. (6) Population fluctuation of B. tabaci and its predacious mite, A. swirskii in treated and untreated cotton fields, Kafr El-Sheikh 1996. (Arrows indicate dates of insecticide treatments).

Order: Neuroptera; Family: Chrysopidae

- ***Chrysopa carnea* (Stephens)**

This predator was recorded at the two Governorates and appeared in the third week of August, with the highest number during the second week of September at Kafr El-Sheikh (Fig. 7). At El-Minia, this predator was more evident and reached a maximum in the third week of August (Fig. 8). In Pakistan, Afzal and Khan (1978) reported that larval period of *C. carnea* consumed on average 510.8 pupae of *B. tabaci* and 487.2 individuals of *A. gossypii*. In Egypt, El-Helaly et al (1971), Hafez et al (1978/79a) and Abdel-Gawaad et al (1990) reported that the larvae of *C. carnea* feed on nymphs of *B. tabaci*. Gerling (1986 and 1990), Kapadia and Puri (1989) and Henneberry et al (1993 and 1994) revealed that ten lacewing species feed on immature *Bemisia* spp., including such well-known species and commercially available species as *Chrysoperla carnea* (Stephens) and *C. rufilabris* (Burmeister).

Order: Hemiptera; Family: Anthocoridae (*Orius albidipennis* [Reuter] and *O. lavegatus*)

- ***Orius* spp.**

It was recorded during all inspections and the highest number appeared during the third week of August with the highest population of whitefly at Kafr El-Sheikh (Fig. 7 and Table 7-b). This predator reached a maximum in the third week of August at El-Minia, with the highest population of whitefly (Fig. 8 and Table 8-b). In Egypt, Hafez et al (1978 and 1979a) reported that *Orius* spp. attacked nymphs of *B. tabaci*. Abdel-Rahman et al (1986) mentioned that *Orius albidipennis* (Reuter) preyed on *B. tabaci* nymphs. Gerling (1995) found that the population of *Orius* spp. is related to the age of cotton plants.

Order: Araneae; Family: Theridiidae (*Steatoda* spp.), Fam. Thomisidae (*Thomisus citrinellus*), Fam. Corinnidae (*Castianeira antinorii*), Fam. Lycosidae (*Paradosa* spp.), Fam. Linyphiidae (*Erigone* spp.), Fam. Gnaphosidae (*Zelotes* spp.), Fam. Clubionidae (*Chircanthum* spp.) and Fam. Salticidae (*Neatha oculata*)

As shown in Figures 7 and 8, the number of spiders was recorded during the season before the appearance of the whitefly

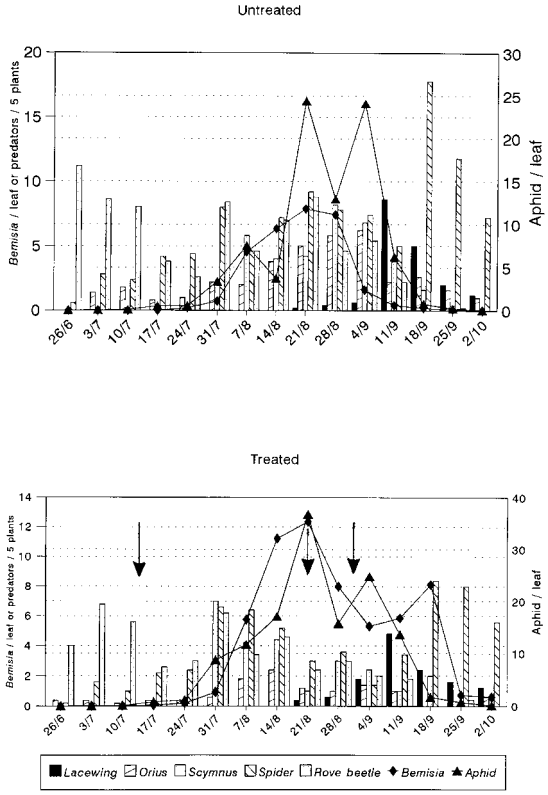

Fig. (7) Population dynamics of *B. tabaci*, *A. gossypii* and their predators in treated and untreated cotton fields Kafr El-Sheikh 1996. (Arrows indicate dates of insecticide treatments).

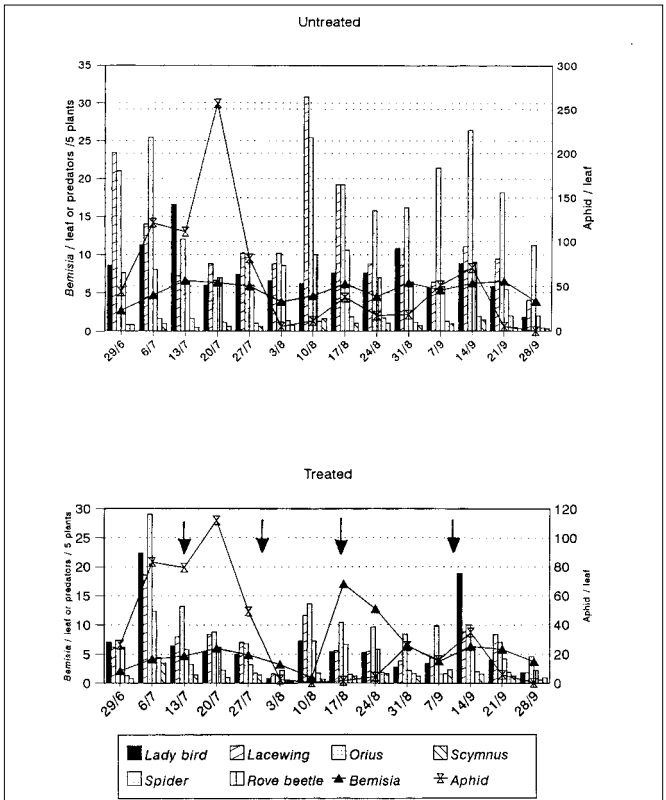

Fig. (8) Population fluctuation of *B. tabaci*, *A. gossypii* and their predators in treated and untreated cotton fields, El-Minia, 1996 (Arrows indicate dates of insecticide treatments)

population. The highest number was recorded in late season, especially in mid September, in the two Governorates.

From the aforementioned data we can conclude that spider populations exceeded all other predator species, followed by *Orius* spp., *Scymnus* spp. and lacewing at Kafr El-Sheikh. Spiders outnumbered all other predator species followed by lacewing, *Orius* spp., lady bird and *Scymnus* spp. at El. Minia. Pesticides affected the population of predator species especially *Orius* spp. at the two Governorates. Breene et al (1993 and 1994) and Provencher and Riechart (1994) reported that spiders are known to cause considerable mortality to *Bemisia* spp. adults.

Aphid

Parasitoids

No parasites were recorded on cotton aphids during this investigation at the two Governorates.

Predators

Aphid populations appeared the first week of July and increased gradually to a maximum in the third week of August until early September, then declined in the second half of September, at Kafr El-Sheikh (Fig. 9). On the other hand, they reached a maximum during July at El-Minia. At Kafr El-Sheikh, the population of aphids was lower in untreated cotton plants than in treated ones and the opposite was true at El-Minia. All predator species were recorded (Figs. 7 and 8) and explained with whitefly except rove beetle, *Paederus alfierii* Koch (Family: Staphylinidae; Order: Coleoptera). This predator was represented by high numbers in the two Governorates. The highest number was recorded in late June and early July at Kafr El-Sheikh (Fig. 9), while at El-Minia in the third week of August (Fig. 10). All the aforementioned predators are aphidophagous in their habits. The total number of predator species was higher on untreated cotton plants than on treated ones. Hafez et al (1977) reported that the number of predators in cotton fields treated during the previous season with Nuvacron ULV was considerably lower than the number in other fields previously treated with insecticides including Nuvacron in normal volume. Also El-Heneidy et al (1987) reported that predators were about two-fold in untreated cotton fields compared with treated ones.

Summary

Results showed that there are significant differences between characters of cotton plants in check and treatment plots. After treatment, the phytotoxity damage on cotton plants occurred in oils in July (cottonseed oil, stabilized cottonseed oils, soybean oil and sunflower oil).

After three days of spraying, the number of whitefly adults decreased (17.6 - 53.3%) in all treatments. After 7 days of treatment by cottonseed oils, castor oil and soybean oil adults were reduced by 96.2%, 61.8% and 51.8% respectively. These reductions were 63.8 and 68.3% for castor oil and soybean oil after 14 days respectively. As for larvae and pupae of *B. tabaci*, no reduction occurred after 3 days of spray by plant oils. However, reductions were detected after 7 days of treatment by soybean, sunflower oil, cottonseed oil and stabilized cottonseed oil (85.6%, 65.4%, 63,9% and 51.2%, respectively). Efficacy did not extend to day 14 after treatments against *B. tabaci* larvae and pupae. In Belkass experiments, results obtained had the same trend of Zayan results. Stabilized cottonseed oil and cottonseed oil at 30% concentration of active material gave a high reduction of *B. tabaci* adults and eggs. Castor oil and sunflower reduced whitefly larvae and pupae on cotton plants after 7 days of treatment at 30% concentration. Results obtained from phytotoxity tests emphasized the injury effect on cotton plants after plant oil treatments.

It could be concluded that plant oils with no phytotoxicity effect could be used first to control *B. tabaci* with other pesticides or separately at low levels of whitefly infestation. All plant oil treatments reduced aphid numbers after 7, 10 and 15 days in comparison with check and water treated plants. It is obvious that the reduction in aphid populations is not attributed to the toxic effect of plant oils only, but also to the natural decline of aphid populations on cotton plants during July as a result of high temperatures. Thus, we need to make several experiments in future years to study the effects of phytotoxity and improve the efficiency of plant oils against these insect pests.

Two hymenopetrous species, *Encarsia lutea* (masi) and *Eretmoserus mundus* mercet (Fam. Aphelinidae), were identified as parasitoids on *Bemisia tabaci* (genn) at the two Governorates. Pesticides reduced the percentage of parasitism on treated cotton plants at Kafr El-Sheikh, while at El-Minia they did not. Parasitoids on aphids were nil during the present investigation. The highest number of third instar and pupae of *B. tabaci* was on August 21 at Kafr El-Sheikh, while at El-Maina on September 21. The population of aphids reached its maximum in the third week of August at Kafr El-Sheikh while at El-Minia during July.

The most common predators surveyed during this study were

- *Amblyseius swirskii* at Kafer El – Sheikh. The population of *A. swirskii* in the 1995 season began to appear in the first week of August and increased gradually until the end of the season. Whereas in the 1996 season, mites began to appear at the beginning of the third week of July and increased gradually until mid September. Pesticides reduced significantly the population of predator mites during the two seasons.
- Lady birds (*Coccinella undecimpunctata*, *C. septempunctata* and *Cydonia vicinia*) appeared in scattered numbers on treated and untreated cotton plants at Kafer- El- Sheikh. At El-Minia, they were present in significant numbers, the highest number (16.6 individuals per five plants) occurred in mid July.

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ICAC/CFC Whitefly Project Activities in Zimbabwe

Doug R. Pascoe, Commercial Cotton Growers' Association, Zimbabwe

Zimbabwe joined the project in Project Year II (PYII), following a series of visits to Zimbabwe from personnel from Israel. Due to Southern Hemisphere location of Zimbabwe, trials were only established mid to late November 1996 at three different bio-climatic regions of Zimbabwe. Several novel crop oils were supplied by Israel and tested principally against whiteflies at the three sites, and their effects on aphids and predators were also evaluated. Each site had five crop oils tested.

Two of the sites had high populations of whiteflies recorded. All the five crop oils tested seemed to have some control on whitefly nymphs as shown by the results, but further work on these "novel" oils needs to be done to see their trend over a number of seasons.

The team also evaluated "hand held" sprayers on spray coverage on cotton. The objective being to assess spray coverage on the upper and lower leaf surfaces of cotton achieved by different hand-held sprayers. The second objective was to compare the different hand-held sprayers in terms of spray coverage at recommended calibration parameters. Three sprayer types were used.

- PJ16 knapsack with hand lance
- PJ16 knapsack with tail boom
- Micronspinning disc sprayer ULVA+

It was evident from both methods that the knapsack fitted with tail boom achieved better coverage on both surfaces of leaves at all levels of the plant. However, the ULVA+ and the knapsack with a hand lance would be suitable for a small and less dense crop, where pests are found mostly on the top leaves and upper surfaces of the leaf and when a systematic pesticide is applied. The knapsack with tail boom will be most ideal for pests that are found under leaves and where a "contact" pesticide is used. However, the results show that ULVA+ has several advantages particularly for small-scale growers in that less water is needed due to the very low volume. A wider swath of 4 rows can be covered and there is less distance to cover per hectare when using ULVA+ than when using a knapsack.

The program for the 1997/98 season (PY4) will be in 4 parts:

1) Field trials for whitefly only
2) Screening of oils for aphids
3) Quantifying spray cover using locally available equipment
4) Identifying and evaluating whitefly predators

The objective is to evaluate six of the more promising oils (as determined by Israel) against whitefly at three different bioclimatic zone sites. A number of different oils (max. 20-30) will be screened against aphids in the field.

With regard to the whitefly predators, the objective is to identify the predators of whitefly present in Zimbabwean cotton fields and attempt to evaluate the effect they have on whitefly populations in an unsprayed situation. If possible, the effect of oils and traditional insecticides on predators will also be studied.

The qualification of spray cover will involve evaluation of the recovery of spray droplets, on both upper and lower surfaces, from spraying equipment locally available in Zimbabwe.

Equipment to be tested:

1) Cannon type sprayer
2) Air assisted boom sprayer
3) Knapsack and tail boom with hollow core nozzles
4) Knapsack and tail boom with flat fan nozzles
5) Knapsack and lance
6) Hand held ULV sprayer

Integrated Pest Management of the Cotton Boll Weevil in Argentina, Brazil and Paraguay

Teodoro Stadler, Project Director, Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), Argentina

Project Description:

Project Sponsoring Agency:
International Cotton Advisory Committee (ICAC)

Project Granting Agency:
Common Fund for Commodities (CFC)

Project Execution Agency:
Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA)

Project Supervisory Body: ICAC

Location of the Project:
Argentina, Brazil and Paraguay

Total Project Cost: US$8,213,170

CFC Commitment: SDR 1,360,329 (approx. U$S1,971,280)

Counterpart Contribution (US$):

Argentina	2,326,290
Brazil	2,564,960
Paraguay	1,334,600
ICAC	16,040
TOTAL	6,241,890

Project Starting Date: June 1995

Project Scheduled Completion Date: June 2000

Main Participating Institutions:

- SENASA (ex IASCAV): Servicio Nacional de Sanidad y Calidad Agroalimentaria, Paseo Colon 367 (1063) Buenos Aires, Argentina. Tel:54-1-3425856, Fax: 54-1-3427699
- INTA, IMYZA-Castelar: Instituto Nacional de Tecnología Agropecuaria - Instituto de Microbiología y Zoología Agrícola, cc 25 (1712) Castelar, prov., Buenos Aires, Argentina. Tel: 54-1-481-4320
- INTA, EEA-Sáenz Peña: Instituto Nacional de Tecnología Agropecuaria, EEA-Sáenz Peña C.c. 164 (3700) Chaco, Argentina. Tel: 54-732-21781 Int.109
- CONICET: Consejo Nacional de Investigaciones Científicas y Técnicas, Av. Rivadavia 1917 (1011), Buenos Aires, Argentina. Tel/Fax: 54-1-953-7230/39
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- IBONE: Instituto de Botánica del Nordeste, C.c. 128 (3400) Corrientes, Argentina. Tel/Fax: 54-783-67324
- EMBRAPA: Empresa Brasileira de Pesquisa Agropecuaria, Rua Osvaldo Cruz 1143 Campina Grande - PB Caixa Postal 174 CtP 58107.720, Brazil. Tel: 55-83-3222197
- IAPAR: Instituto Agonomico Parana, Rod. Celso García Cid Km 375 Caixa Postal 481 CtP 86.001.970, Londrina – PR, Brazil. Tel. 55-43-3261525 Int. 2276, Fax: 55-43-3267868
- INSTITUTO BIOLÓGICO: Estacao Exp. de Campinas Caixa Postal 70 Campinas-SP, Brazil. Fax: 55-192-518705
- MAG: Ministerio de Agricultura y Ganadería-Dirección de Investigación Agrícola, Pte. Franco y Ayolas-Ed. AyFRA Piso 2°, Asunción, Paraguay. Tel: 595-21-447304
- DIA: Dirección de Investigación Agrícola, Ministerio de Agricultura y Ganadería, Asunción, Paraguay. Tel/Fax: 595-21-449305 Int. 227
- IAN: Instituto Agronómico Nacional, Caacupé Ruta 2-Km. 48.5, Tel/Fax: 595-511-2255, Caacupé, Paraguay.

Overview of Project Activities

Many relevant actions were executed since the project started, most importantly in the framework of the organization of the activities of each participating country. The Project Executing Agency (PEA) has been encouraged by different country representatives to centralize administration of the project by managing separately the participating institutes and working groups in the three countries. This new working scheme caused substantial changes in the general organizational schedule of the project; however, the changes introduced were quite positive due to the active participation now shown by Brazil, as well as the initiatives undertaken recently by some working groups in Argentina.

Efforts in the scientific and technical cooperation area have been focused on consolidating recent experience and initiatives to strengthen the efficiency and effectiveness of the project. Two key activities now provide a more strategic orientation to co-operation between countries:

- Entomopathogenic fungi as biological control agents for the boll weevil
- Insecticide resistance monitoring

These initiatives resulted from the interaction of working groups from Argentina and Paraguay, (IMYZA-INTA, CONICET and IAN-MAG) as an outcome of the promotion of joint activities between institutes and countries undertaken by the PEA. A new and unifying goal for the PEA was to establish “joint research activities” between scientists from different countries. This goal has required a significant emphasis on planning and assessment, which dominated coordination and execution activities during the year.

Over the last two years, the PEA has gained valuable experience in organizing different activities of the project and understanding the particular situation in each country. One lesson learned is the need to identify clear objectives and develop verifiable indicators of project performance. Such indicators have now been established for each activity and component objectives have been reviewed in view of the concern and skills of each working group in different countries.

A review of the status of the project indicated that a more extensive system of coordination and accountability would be necessary to help different countries achieve the aim of this project. Two new management elements were signaled out as being essential for making the project function effectively: A

clearly identifiable focal point for activities; and a firm commitment to time-limited objectives.

Efforts were increased to systematically plan technical activities. The concept of thematic or sectional planning is being elaborated in boll weevil IPM research, which has achieved significant results in most participating countries in 1996 owing to effective integration of components. Work also began on identifying capabilities such as biological control, pesticide management and evaluation of trap and kill devices. Work also continues on identifying capabilities in different countries for certain skills, such as GIS or boll weevil geographic varieties. This effort is tied to both the PEA coordination activities and resource mobilization efforts. The feasibility of boll weevil IPM, along with the capacity of a particular working group to develop a technology are important benchmarks for planning technical cooperation activities between participating countries.

During the last two years the PEA performed its activities within the framework of a number of well-defined programs which were designed to meet the following objectives:

- Maintain and foster research excellence in the framework of the project.
- Assist institutes and researchers to attain the necessary research infrastructure needed for successful completion of their individual projects.
- Promote, develop and coordinate multidisciplinary and multiinstitutional cooperation in research activities.
- Establish, reinforce and exploit international scientific contact and collaboration.
- Evaluate reports and programs of participants and their outputs
- Optimally utilize all available funds to realize the above mentioned objectives.

The optimal use of available resources underlies the PEA´s activities, supported by the ongoing evaluation of the performance and outputs of all participants benefiting from this project. The objectives of the ongoing research were formulated and approved focusing on the measurement of the performance on an annual basis.

Acknowledgments

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Special acknowledgment is due to Mr. Sietse van der Werff (CFC), Dr. Lawrence Shaw and Mr. Carlos Valderrama (ICAC) for their continuous and effective assistance and understanding of the expected as well as unexpected difficulties which have appeared during execution in the last two years of the project.

Argentinean Research in the Context of the Project "Integrated Management Program of the Cotton Boll Weevil in Argentina, Brazil and Paraguay"

Carlos Lehmacher, Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), Argentina

Introduction

Since 1983, SENASA has had a national program for the prevention and eradication of the boll weevil. In coordination with the provincial governments, INTEL and the private sector, it has been carrying out efforts for the surveillance, trapping, eradication and quarantine control of *Anthonomus grandis*, and training and information dissemination about the pest.

Research carried out by Argentinean scientists in the framework of the integrated management of the boll weevil in Argentina, Brazil and Paraguay Project is being coordinated with the activities of the Argentinean National Program, the history of which is as follows:

1983 Training of Plant Health Services technicians.
Information exchange with neighboring countries.
Conferences sponsored by IICA and FAO.

1984 SAGPyA establishes the Inter-institutional Technical Committee (CTI) to schedule and coordinate prevention measures.
Trap installation begins in the borders with Brazil and Paraguay.

1987 FAO's TCP 6653 is carried out.

1991 CTI's Technical Secretariat develops a Monitoring and Training Program.

1992 The installation of a national trap network begins.
Training is intensified.Two international technical assistance projects are developed with INTA and a binational one with Paraguay.
FAO Program on Integrated Pest Management (IPM). ICAC Program.

1993 The Program for the Eradication and Control of the

boll weevil is extended to the country as a whole.
Operation of the trap and monitoring system.
Training of provincial supervisors for monitoring activities.
The dissemination program begins.The FAO TCP 2261 begins.
The Program is signed with Paraguay.

1994 The ICAC project is implemented. Joint activities in Paraguay.

1995 Extension and intensification of the monitoring network.

1996 The national and trinational programs' activities continue.
The Agreement with Paraguay is signed.

The Problem

Because of its high reproduction rate, small number of natural enemies and the high number of insecticide sprayings required for its control, the boll weevil is the most destructive cotton pest in the continent. If the boll weevil extends its range to new production areas, it would become a serious threat to our country's cotton sector.

Objectives

- Prevent its spread to pest-free production areas through the implementation of quarantine and monitoring systems.
- Eradicate infestation foci through cultural and chemical control of cotton crops and related facilities.
- Train producers and technicians and disseminate information on monitoring and control techniques.
- Organize area control committees made up by producers and other active groups in cotton-region municipalities.

Program Areas

The program is being developed throughout the country's cotton growing areas, covering approximately 1 million hectares. The early detection of the pest is crucial for its effective prevention and control. To that end, a trapping and monitoring system has been installed with special emphasis on high-risk areas. Crop monitoring through visual inspections is also being carried out. At present, 16,667 traps have been installed throughout the country.

Technical Cooperation Between Paraguay and Argentina

The cost of this three-year program, US$1,759,509, will be covered by both countries with the support of international organizations.

The program was signed in December 1996. Its objectives are:

- To implement the second stage of the Bilateral Technical Cooperation Program between the governments of Paraguay and Argentina for the control of the boll weevil.
- Strengthen mutual cooperation between the plant health services of both countries in order to develop common control strategies as well as consistent phytosanitary standards aimed at improving the health of the cotton crop and its byproducts.

During the first stage of the program's implementation, joint efforts will be carried out in the Department of Ñeembucú (Paraguay) and, later on, in the Misiones and Itapúa Departments, both in Paraguay.

The joint actions to be carried out in Ñeembucú are the following:

- Pest monitoring and setting up traps.
- Eradication of infestation foci.
- Enforcement of effective technical standards and legal procedures.
- Organizing producers into local committees against the boll weevil
- Continuous training of producers and technicians in pest prevention and control measures.
- Mass information on the dangers posed by the pest.

PROSAP- Boll Weevil

As of this year, Argentina will operate under the boll weevil PROSAP Project. The executing agency of the project is SENASA; it will last 5 years and will cost US$21,369,568. The Inter-American Development Bank (IDB) will finance 48% of the project cost; the rest will be covered by local counterpart funding obtained from the $2/ton tariff charged on raw cotton produced domestically.

Thus, as of this year, the National Program will fund part of its activities with PROSAP Project resources; they will be used to enhance present activities and add new ones.

The project's general objective is to eradicate the pest in Formosa within five years and carry out joint activities with Paraguay in order to decrease the presence of this insect in Argentina.

The specific objectives include:

- Strengthening trapping and monitoring activities.
- Reduce, on a yearly basis, the boll weevil population in infested areas.
- Validate modern boll weevil control techniques.
- Update phytosanitary legislation.
- Organize area control committees as a tool for the involvement of all producers.

- Human resource training and scientific-technical exchange with other countries as part of pest eradication programs.

Characteristics of Argentinean Cotton Area

Agricultural Development

The production systems in the Northeast (NEA) and Santiago del Estero regions are of a dual nature: The agricultural methods used in the area are modern and dynamic and involve medium and large producers. As well there are small producers with few productive resources, family labor, a high illiteracy rate and weak marketing of their production. These rural production sectors have very little access to technology.

Statistical information on the assistance given to the cotton sector, provided by participating provinces, reveals that during the 1991/92 season, out of the 37,000 cotton producers in the country, around 43% were small producers whose lots were smaller than 10 hectares with a raw cotton yield of approximately 1,000 kg/hectare.

If the boll weevil extends its range to the country's producing areas, cotton growing would not be a viable activity for a large percentage of producers since they would be unable to cover pest control costs.

Stratification in Northeast Area (NEA)

NEA covers 75% of the country's cotton growing area. Comparing the Corrientes province with Santa Fe, one finds that in Corrientes 97.1% of the farms are under 10 hectares, whereas in Santa Fe only 16% are that size. In the province of Formosa, 48% of producers have less than 10 hectares, but in its Departments of Pilagás and Pilcomayo, the area infested by the boll weevil, 88% are small farms.

Production and Yield

Fiber production has increased substantially from 100,000 tons during the 1986/87 season to 425,000 tons in 1995/96.

Production Data

The average cultivation area between 1990/91 and 1996/97 was 689,930 hectares/year and the production of raw cotton during that period was 903,911 tons/year. The average yield of rainfed seed-cotton was 1,500 kg/year and of irrigated cotton 1,800 to 2,000 kg/hectare.

At present there are 11 cotton producing provinces. The provinces with the greatest production are the NEA and Santiago del Estero, which account for 96% of the production. In the NEA, the largest producer is Chaco with 63% of the country's total production.

Present Distribution of the Pest in Argentina

Misiones Province

Since 1993, when the boll weevil was first detected in the border post of Puente Tancredo Neves, very close to Iguazú National Park, the pest has rapidly appeared in the area north of Misiones province, more specifically in Andresito, Libertad, Puerto Bossetti, Wanda and San Pedro. Infestation was limited to non-cotton growing areas. According to the Misiones provincial government, in 1996, three years after its entry, the cotton crop in the province was destroyed, and it was decided that due to the risk, no further cotton would be grown.

The pest continued to plague Misiones province, especially in its western region, due especially to the insects coming from the cotton producing area of Paraguay close to Misiones province. Thus, joint activities were carried out in Paraguay in 1993 and 1994, and towards the end of last year, a binational Paraguay-Argentina program agreement was concluded for the eradication of the boll weevil.

Formosa Province

In June 1994 the first simultaneous catches of *Anthonomus grandis* were made at Puente San Ignacio de Loyola, on the border with Paraguay, and in the Department of Pilcomayo, and later on in the Department of Pilagás.

The number of traps was immediately increased and included crop monitoring. As part of the binational program, technical personnel that had been working in Paraguay were assigned to the affected areas, and equipment and operational funds for intensive monitoring were increased.

The trap network in the province increased from 183 to 1,630. Later on, catches were made in Herradura, in the southeast region of the province, near the border of the province of Chaco. Thus, the pest was eradicated in the area, with no further boll weevil incidence.

The number of traps laid down up to now is 7,053. At present, catches are limited to the Departments of Pilagás and Pilcomayo. In these Departments, as of July 1997, foci covered 84 hectares, specifically in El Espinillo, Buena Vista, Palma Sola and Naineck.

Corrientes Province

In April 1996, the pest was detected in Corrientes. At present, it is not in the cultivation area. Since it appeared, the number of traps has increased, and Boll Weevil Attract and Kill Tubes (TMP) have been installed. At present, there are 1,805 traps and 800 TMP in the province. The area where the boll weevil is present is in the Northeast Region of the province.

Surveillance and Control Actions

Surveillance in the border areas and field experiments are carried out for early detection of the pest, as well as immediate eradication and monitoring of cotton imports and shipments from infested areas.

Lines of Action

- Trapping and monitoring
- Foci control: Application of insecticides; stubble destruction; and external and internal quarantine measures
- Establishment of Area Control Committees
- Adaptation of the legal framework
- External and internal quarantine measures

Control of Infestation Foci

Foci control is carried out through the followingactivities:

- Chemical treatment of affected lots
- Destruction of crop waste
- Attraction and control treatments

Treatment with Chemical Insecticides

The customary practice in the infested area of Formosa is the installation of Boll Weevil Attract and Kill Tubes (TMP) which are replenished every 45 days. Towards the end of the 1996/97 season, an area was circumscribed by placing TMPS. In all lots where the pest had been detected and crop waste was destroyed, the density was 3 TMP/hectare; the density was 1.5 TMP/hectare for lots where there was no detection; and 2 TMP/hectare for lots where the boll weevil had been detected the previous year. The Program has also installed TMP in Misiones and Corrientes, on federal and provincial routes.

Crop Control

In tropical and subtropical climates a cotton-free period is required. An important method to control cotton pests in crops is by ensuring that the fields are completely cotton-free for 4 to 5 months a year. The objective is to prevent pest reproduction during the non-productive season.

Quarantine Control

There is a phytosanitary control system in place for monitoring entry into the country, and a system of internal phytosanitary barriers.

The first system is used in ports and border posts to prevent the pest from being transported into the country. To that end, and under present rules, land points of entry of cotton and its byproducts are Paso de los Libres (Corrientes), Posadas (Misiones) and Clorinda (Formosa), the latter being used only part-time. The incoming cotton lots and transports are chemically treated: the insects are eliminated at the border itself (the entry of unginned raw cotton is prohibited so as to ensure that *Anthonomus grandis* does not spread to pest-free provinces).

This year a resolution was adopted whereby all empty and loaded cargo transportation coming into the country from Brazil or Paraguay, through any of the eligible ports or border posts, must be externally sprayed. Implementation will begin in September 1997.

Four quarantine posts have been set up, both as plant health barriers and in order to circumscribe parts of Formosa: Puesto Ramona, Laguna Gallo, Monte Lindo and Cañada 12.

Raw cotton and cotton fiber and seeds cannot leave these posts without prior fumigation. The fumigation posts in Formosa are in Laguna Blanca, Riacho He He, El Colorado and Puerto Velaz. The fumigation of merchandise is carried out in these posts by registered private companies licensed by SENASA and supervised by technicians authorized by the Program.

In Formosa, there are two authorized posts in which a fumigation certificate is required in order to transport cotton, of any type, to the province of El Chaco, Puerto Velaz/Lucio Mansilla and Puerto Libertad/El Colorado.

Transfer of Technology

Lines of Action

- Training of trainers (official and private technicians) in the prevention and eradication of the boll weevil.
- Training, through control committees, of regional technicians and producers in the prevention and eradication of the boll weevil.
- Training producers' children to recognize pest insects.
- Transfer of technology through demonstration events for producers, and tours of affected areas.
- Dissemination through mass media of manuals as well as technical and information publications, to support training activities.

Trinational Project Research in Argentina

Design and Implementation of a Geographic Information System on the Boll Weevil

Working Group: SENASA

Geographic Information Systems (GIS) are high-technology tools designed to collect, manage, handle, analyze, model and visualize geographically referenced data. With these systems, a number of analytic processes are carried out to generate the "information" required for the decision-making process.

One of the fields in which GIS has had the greatest conceptual and operational development is in natural resource management. Resource management entails the use of large amounts of data which increase every year as a result of advanced tech-

nologies such as satellite images. In addition, the phenomena to be analyzed depend on multiple variables (climate, physiography, biology, crops, etc.) connected by complex processes which, in general, are of a highly spatial and temporal nature. GIS easily adapts to different types of analysis used to understand these processes, especially during monitoring, evaluation and planning stages.

In the specific case of pest control and monitoring, since GIS can interrelate numerous variables, it is ideal for defining pest development in terms of environmental characteristics, crop use and soil management. Up to now, SENASA has developed and implemented GIS as a pilot project and as a tool for data management within the program to control the Mediterranean fruit fly in the province of Mendoza (presently being implemented). An evaluation is being carried out of the requirements and its potential use in programs aimed at controlling citrus canker, horn-tail and coddling worm; the system is presently being implemented for the boll weevil. The technicians coordinating the latter program consider GIS to be an essential short-term tool.

We believe that the inclusion of GIS in the trinational program will benefit the three countries as follows:

- It will speed up the updating of trapping data.
- It will serve as a data collection and filing standard to transfer information to third parties.
- Data quality will improve.
- It will allow for better utilization of data provided by third parties.
- Redundancy in data collection will be reduced or eliminated.
- The ability to analyze and summarize data will improve, both at the higher decision-making levelsand at the intermediate and technical decision-making levels.
- Data presentation and its dissemination to third parties (producers, international control agencies, financial organizations, etc.) will improve substantially.

Objectives

The purpose of the project is to develop GIS as a tool to support various activities of the boll weevil program. The capabilities offered by these systems will be used to plan the program's monitoring activities, its operation, research on the pest's characteristics, dissemination of program results to third parties and to control present and future health measures.

Applications

Regarding planning of the monitoring activities, GIS will be used to locate cotton fields through an updated cadastre, identifying host sites, the strategic distribution and location of traps, defining the access to sampling sites, determining routes for trap control, fast identification of the best trapping areas, etc.

Regarding the operation of the monitoring program, GIS will be used to store information, classify and analyze sampling data, prepare detailed maps at different scales to be used in offices and in the field, identify infested areas, determine the spatial and temporal distribution of the pest and monitor its evolution.

Regarding dissemination, through GIS, highly visual graphs and maps will be prepared to help disseminate the program's results among the public and to the pertinent decision-making levels.

Regarding controlling activities, through the system information will be obtained on compliance with measures adopted to destroy stubble and concentrate cultivation.

The system will be used in the provinces of Formosa, Chaco, Corrientes, Entre Rios, Santa Fe, and Santiago del Estero, under the coordination of IASCAV.

The main results obtained during this working period were

- Standardization of the information generated by boll weevil monitoring in Argentina.
- Standardization of forms used to take down data.
- Standardization of information collection and storage.
- Setting up of a single data base.
- Development of basic Department maps, at a scaleof 1:500,000.
- Drawing up of a map of over 3000 sites populated by more than 500 inhabitants.
- Hardware installation.
- Training in the use of software.

During the next period we expect to remodify the area's traps covered by the project and install the data management programs for Chaco and Formosa.

Insecticide Susceptibility and Resistance in *Anthonomus grandis* Populations

Working Group: LPE-CONICET

It is a well recognized fact that *A. grandis* populations are difficult to control through their natural enemies, so that the application of insecticides and crop control are, at this point, the main vehicles for pest control.

It is likely that the intense and constant application of insecticides for the control of *A. grandis* would eventually lead to the development of resistance. In order to identify its causes, a careful study must be made of the biochemical mechanisms that cause resistance and their degree of selectivity. On the basis of this information, operational factors such as the type of insecticide, its dosage and mode of application, could be selected in order to develop a strategy to delay the development of resistance and extend the useful life of insecticides presently on the market.

In order to be successful in a boll weevil eradication and control program, one must first be aware of the mechanisms that lead to insecticide resistance. We must also define the role played in this area by ecophysiological factors, since they directly affect the magnitude and speed with which resistance develops; combined in different permutations, they can produce, promote or delay the development of resistance.

Toxicological information obtained in Central and North America on *A. grandis* is quite complete, while information on the insecticide susceptibility of *A. grandis* in Paraguay and northeast Argentina is virtually nonexistent. Consequently, any extrapolation made from assays carried out on exotic strains of *A. grandis* must be taken with caution. Also, biographical data on toxicologic and biologic parameters and variables related to pest control must be carefully reviewed on the basis of bioassays and biochemical studies of local strains. These studies cover three basic topics:

1. Pest susceptibility to various insecticides.
2. Resistance to organophosphorus compounds, carbamates and pyretroids.
3. In case resistance is detected, its biochemical causes must be determined.

From the bibliographic study carried out at the beginning of the project, a general view was obtained on *A. grandis*' susceptibility to insecticides during the last thirty years at an international level. The conclusions reached were

- LD50 obtained through assays with azine-methyl-phosphate is not significantly different.
- LD50 obtained in assays with methyl parathion, on different strains and under different authors, showed a gradual and significant increase of tolerance between 1962 and 1993.
- LD50 obtained with permethrin shows significant differences; however, information is inadequate to determine if the phenomenon is a resistance to pyrethroids.

Studies on insecticide susceptibility and on resistance development in local strains of *A. grandis* began with laboratory bioassays carried out jointly in Paraguay by researchers of an Argentina-Paraguay working group. An evaluation was carried out on the effectiveness of various active substances (β-cypermethrin, deltamethrin, β-cyfulthrin, permethrin, methamidophos and methyl-azinophos) on a local population of *A. grandis*. With these results, foundations were laid for the development of a future resistance monitoring system.

On the basis of this information, the Argentinean working group will develop, if possible in cooperation with Paraguayan researchers, a resistance monitoring kit whereby plant health authorities or outreach personnel will carry out a rapid and simple evaluation of the effectiveness of chemical control in given regions. Studies on insecticide susceptibility will lead to the selection of an alternative product in case changes are detected in the tolerance to products presently being used.

The working group carrying out these insecticide studies, which range from basic research to technological innovation, is also testing natural products as alternative tools to man-made pesticides; in addition, these products are consistent with integrated cotton pest management programs.

We should emphasize that the ultimate objective of insecticide management is the rational use of these products for pest control purposes and the replacement of man-made products, as early as possible, by bio-rational insecticides within the framework of an integrated control program. Due to the increase in their effectiveness, the rational use of insecticides will lead to their partial quantitative reduction. This, in turn, will lead to a decrease in water, atmosphere and water-table pollution, and protect the pest's natural enemies. The latter will have a multiplying effect since it will reduce the use of chemicals.

Other working groups that have begun doing research but that have not yet submitted their results:

Population Dynamics and Dispersion

Working Group: INTA - Saenz Peña

Objectives

- To find out about pest dynamics in the Argentinean cotton systems.
- To determine the predominant sources of boll weevil reproduction that infest cotton crops every year.
- To determine the direction of boll weevil displacement/movement.
- To relate the dynamics with the environmental conditions.
- To determine the seasonality of movement.

As a result of the work carried out, researchers have acquired experience in handling sampling instruments and in the use of pheromone and photocleptor traps placed in stubble.

Review of South American Species of *Hibiscus* Secc. *Furcaria*, Alternative Host Plants of *Anthonomus grandis*

Working Group: Northeast Botanical Institute-CONICET

The Ciefuegosia genus is cotton's closest native relative. Its observation became relevant when the boll weevil was detected in the northeast region of the country. The last review of this genus was carried out in 1969, and the latest sampling shows that this species' distribution data, as well as its environment, must be updated since it has been shown to host and promote multiplication of the boll weevil.

Pollen Feeding of the Boll Weevil

Working Group: Universidad del Nordeste

By identifying the pollen content in *A. grandis*' digestive tract, one can infer the plant species it visits and selects in the presence/absence of cotton crops. On the basis of these studies one can also establish if this species truly transposes a diapause period in subtropical environments. The information obtained from these studies may contribute greatly to the design of pest control strategies.

Molecular Studies of *Anthonomus grandis* Populations in Argentina, Brazil and Paraguay

Working Group: UNLP-FCEyN (UBA) - CONICET

In spite of the many papers published on the taxonomy, biology and control of *A. grandis*, there are few molecular studies on this species. These studies are an essential tool to estimate some relevant parameters for pest control, such as the genetic flow between populations, migration patterns, plant-insect interrelationship patterns, as well as the genetic bases of its susceptibility to insects.

Objectives

- The analysis of DNA markers and their polymorphic and/or polytypical variations, in order to molecularly define the pest population in the three countries.
- Integration of molecular data with other evidence in order to answer questions in the areas of geography, migration, population structure, etc.
- Molecularly define *A. grandis* lines susceptible or resistant to given insecticides.
- Develop studies on the genetic incompatibility of *A. grandis* with species close to it in order to produce genetically based sterility.

Microbial Control of the Boll Weevil

Working group: IMYZA - INTA Castelar

For some years, Brazil and the United States have been successfully testing some *Beauveria bassiana* fungus strains. Through these fungi, mortality values of 50-100% were obtained under controlled conditions. In addition, pathogenicity tests of *A. grandis* with *Metarhizium anisopliae* have also shown very satisfactory results. This encouraging information has led researchers to carry out a detailed assessment of these mechanisms' potential as a pest control tool.

The objective of this research is to select native or alien strains and analyze them with IMYZA's mycotic material in order to establish the pest's pathogenicity.

The virulence and aggressiveness of the selected strains will be determined through controlled studies, and an analysis will be carried out of the various substrates for mass production of the selected strain. The final stage will be a field evaluation of the entomopathogen as well as registration of the product as experimental.

With this research and technical development, we expect to minimize the impact of the boll weevil by causing natural mortality not present up to now in the cotton agro-ecosystem. We also hope to have available a pest control alternative whereby biological inputs can be used together with other control options.

Transfer of Technology and Information to Farmers and Outreach Workers

Working group: INTA - Saenz Peña

Objectives

- To improve knowledge about the boll weevil, its impact on production, and the appropriate control methods that will lead to a low-cost cotton production that is environmentally and socially acceptable under various crop conditions.
- Make the community aware and instruct it on the dangers posed by the boll weevil, in order to prevent its progress.
- Obtain commitment of the various relevant sectors to the Boll Weevil Prevention and Eradication Program.

Activities To Be Developed

- Technical/practical meetings on stubble destruction and fallow land techniques.
- Prepare a follow-up of demonstration plots.
- Courses for insect recognition.
- Educational tours.
- Informational activities.
- Coordination and follow-up meetings.
- Information dissemination and teaching materials.
- Training

Conclusions

Argentina has been able to benefit from the early detection of the pest through its monitoring network and through immediate control efforts, focusing its attention on a limited area.

Foci control strategies aimed at pest eradication consist of

- Cultural methods
- Chemical methods
- Permanent quarantine activities

The formal integration of producers since 1996, through a tariff of $2/ton of raw cotton produced in Argentina, and the involvement of the private cotton sector (organized in various groups), will contribute to the program's continuity.

Eradication in Argentina's border area depends on joint ventures with Paraguay. To that effect, an agreement was signed with Paraguay for coordinated actions as part of a Binational Program.

Argentina and Paraguay should coordinate the planting and stubble destruction dates, which will also benefit Brazil and Paraguay in their joint activities. To that end, the three countries' regulations should be harmonized.

We should emphasize that the opportunity to exchange research information as a result of the trinational program will likely extend pest control activities carried out through joint action plans.

Development and Validation of Strategies for Integrated Management of the Boll Weevil, (*Anthonomus grandis*, Boh., 1843) in Brazil

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Summary

The boll weevil, *Anthonomus grandis* Boh., 1843, was first found in Brazil in 1983. At that time, approximately 3,500,000 ha were cultivated with cotton. During the last 14 years, the insect colonized different productive regions and is now found in close to 85% of planted areas. Over the years, this has resulted in a distinct reduction of planted area, resulting in 995,000 ha left in 1996. The boll weevil has clearly affected the social and financial problems of cotton cultivation, leading to the elimination of millions of small producers from this activity. Currently, Brazil is one of the main importing nations of cotton in the world, with about 55% of consumption imported in 1996, or approximately 500,000 tons of cotton fiber. The insect showed good biological adaptation with reproduction rates and significant survival rates in relation to the Brazilian tropical and subtropical conditions. For a rational existence with the boll weevil and always taking into consideration financial and ecological aspects, Brazil is developing or adjusting a number of different approaches within the principle of integrated pest management. Among these methods, the following are under study and evaluation: species control, natural biological control, short-duration varieties, the use of pheromone, population monitoring, and the management of insecticides. Starting from the premise that only one isolated control technique is never agriculturally sustainable, the integrated pest management of the Cotton Boll Weevil Project, in Argentina, Brazil, and Paraguay will lead to the development and evaluation of all control methods that could regulate boll weevil populations along with other cotton pests for the implementation of ecologically balanced cotton cultivation.

Introduction

The boll weevil appeared as a dividing phenomenon of cotton production periods in Brazil. By adding to the social and financial problems during the decade of the 1980s, the pest exerted significant influence on the departure of a large part of producers from cotton cultivation activities, especially those with low technical levels. As a result, the area cultivated with cotton gradually decreased to levels of approximately 70%. Currently, Brazil imports about 60% of its consumption. The boll weevil is considered to be an important pest because of its high rate of reproduction, great mobility in the agricultural system, and the occurrence of multiple generations (Bradley & Philips, 1978). All countries that were affected by the boll weevil suffered serious social and economic problems in addition to difficulties in controlling the entire pest environment because of the imbalance caused by excessive use of insecticides. For example, the control of *Heliothis* spp. has become a problem in almost all cotton producing regions in the Americas where the boll weevil is present. This is because the intensive use of insecticides increased resistance and the mortality rate of natural enemies. The fight against the boll weevil increased the amount of insecticides used in the Brazilian cotton belt by about 35%. As a coinciding factor, since 1995, we have observed resistance in *Alabama argillacea* species to pyrethrin insecticides, possibly because of the increased use of these products in cotton cultivation. There are few means of controlling the boll weevil population and its increase depends on weather conditions, food availability and biological processes inherent to the species.

As a result of the physiological features of the insect and plant diversity, the species develops certain characteristics that permit survival between crops at sufficiently elevated levels to begin infestation in subsequent crops. The ecosystem of cotton cultivation areas in Brazil offers adequate conditions to assure adult survival. The availability of pollen from different vegetation complement food reserves that the species requires naturally and enables it to survive in a cotton plantation. Cotton cultivation is migrating and undergoing changes in Brazil. In general, this activity is being reduced in the traditional states (Sao Paulo and Parana) and shifting to central Brazil. In this

region, cotton cultivation is being implemented with different technological standards, such as the large size of plantations, crop rotation, machine-power cultivators, and even direct planting. A large part of these regions consists of the Brazilian open pasture land, with its own biological diversity and more defined weather conditions. Temperatures are high almost throughout the year and rainfall occurs mainly between the months of November and April. The boll weevil is appearing gradually in these new ecosystems and developing differentiated behavior, appropriate to this open pasture land.

Biology and Behavior

The cotton boll weevil *Anthonomus grandis* (Coleoptera, Curculionidae) is a pest of great financial impact because of its force of destruction and reproduction capabilities. Levels of infestation increase rapidly and losses can reach 100% of production if control measures are not adequate. The boll weevil is an insect of sexual reproduction. Females deposit their eggs inside flower petals, placing an average of 150 eggs per bud. After three to four days, the buds turn yellow and open. They then fall to the ground, containing larvae of the developing species that become the new adults. The life cycle from egg to adult is complete in about 17.5 to 19.41 days. The cotton boll, the hibiscus flower, and banana fruit, available to the adults, provide a longevity of 97.68, 71.73, and 85.48 days respectively (Gabriel et al, 1986). Adults feed on flower buds and when these are not available, or under pressure because of increased population, cotton bolls are also attacked. The adults that develop in cotton bolls are generally better prepared to survive between crops. Toward the end of planting, the adult boll weevils migrate to areas with permanent vegetation (shrubs, grasslands, etc.) close to cultivated areas. The adults remain in these shelters with a reduced physiological metabolism, feeding sporadically on pollen from different kinds of vegetation. Under these conditions, many survive until the next crop. In the presence of hosts like cotton plant stumps there may be reproduction between crops. The longer the period between crop planting, the more likely it is that the mortality rates of adult pests remain high because of pathological insect activity, predators, parasites and, above all, the drought. When strong frost occurs, followed by long periods of drought, there is a definite reduction in the pests' population growth for the next crop. Newly emerging cotton plants are an attraction to boll weevil that have survived between crops. The attack begins from the field edge through damage to the green parts of the plant, such as leaves, stem, and top of the trunk. The adult boll weevil generally remains on the edges, waiting for the development of flower buds, that appear after 35 days in most Brazilian cotton varieties. The species moves very little during the initial phase of infestation but at the peak flowering, from 70 to 80 days, there is strong population pressure. Under these conditions, behavioral processes are developed that are known as migration and dispersion. First, the plantation is affected and subsequently the neighboring one; gradually and with differing speed the pest spreads to other plantations close or farther removed from the initial cluster. In addition to the cotton plant, Brazil has certain plants that host the boll weevil, as described by Lukefahr et al (1986), such as the "algodão-do-Pará" (cotton from the State of Pará) (Thespesia populnea), "algodão-bravo" (wild cotton) or "algodão-do-campo" (field cotton) (*Cienfugosia affinis, C. glabrifolia*, *C. drummondii*, *C. heterophyla*, *Hibiscus heterophyla* and *Hibiscus pernambucensis)*. The presence of natural enemies (parasites and predators), that attack boll weevil larva has been observed mainly during the cotton development and harvesting phase in different cotton cultivation regions of Brazil. Environmental conditions, such as the high temperatures that generally occur in northeastern Brazil, lead to significant boll weevil larva mortality, resulting from the drying flower buds in contact with the hot ground.

Strategies for Integrated Management

Destruction of Stumps

The destruction of cotton plant stumps, right after harvest, is a determining factor for economical control of the boll weevil. During the period between crops, there should not be any old, stagnating, or rebudding cotton plants because these favor pest survival and reproduction. The stumps can be destroyed with a scythe, by cutting as low as possible, resulting in destruction of the stem. After this, the area should be checked again to manually or chemically (herbicides) remove any remaining stumps. When weather conditions favor regrowth, additional leveling or plowing is required. Cotton should not be planted near areas where stumps were not destroyed properly or late because under these conditions the level of boll weevils is so high that control becomes impossible.

Early Preparation of the Land

Land preparation operations (aeration and gradation) stimulate the process of migration of adult boll weevils to permanent shelters (shrubs and grassland) found at the borders of areas to be cultivated. To reach the dislocation of the pest, however, it is necessary that this operation takes place at least 40 days before planting the crop.

Early Varieties

Early varieties and acceleration of plant maturity are desirable features for a safer survival from the boll weevil. Most of the varieties cultivated in Brazil currently, such as Deltapine A-90, IAC-22, ITA-90, ITA-96, IAPAR-71, can be considered as medium to short cycle.

Seeding Season

Sowing of the cotton crop should take place during the time recommended for each region. Neighboring areas should be

planted at the same time, if possible, always avoiding anticipation or delays. Late planting is subject to heavy infestation, making boll weevil control financially unviable.

Trap Cropping

Trap cropping takes place in small strips (100 to 500 m^2) near borders and close to shrubs and grassland, as well as near rivers. Trap cropping should take place before sowing for the purpose of attracting boll weevils that survived between crops and that are sheltered in shrubs and kill them with insecticides. Adult boll weevils are easily attracted to the first emerging cotton plants appearing in the area to be cultivated. The trap plants should be at least five days older than those of the cultivated area. Trap plants should be placed at the borders of planted areas for intercepting the pests' migration routes. Seeds used for this purpose should be treated with fungicides and insecticides. After 10 days, when you notice the presence of adult weevils or affected buds, the trap plants should be sprayed, especially starting from the emergence of blossom formation. However, insecticide should be applied every five days with weekly pickup of the buds for as long as necessary. There should be a strip separating the trap cultivation areas and the rest of the plantation. This separating area should be about four meters wide and kept without any vegetation. This area facilitates operations on the trap cultivation and delays the entrance of the boll weevil and other pests into the crop area. Implementation of trap cropping reduces the initial boll weevil population considerably, delaying and therefore reducing the number of applications on the total area and consequently reducing control costs (Figure 1). These conditions enable improved regulating activities of the cotton pests' natural enemies by minimizing damage and environmental problems. The trap cropping should place preference on varieties resistant to viruses, such as ITA-96. Spraying trap plants should be with phosphorous insecticides or acid-based products that activate on contact, such as "endosulfan" or "phosmet."

Collecting of Buds from the Ground

The quick dropping of flower buds, until the plants reach 85 days, is generally caused by the boll weevil. These fallen flower buds contain larva and pupa that develop into new adult boll weevils. Collection of infested buds is indispensable in the areas of trap planting and is also recommended at the borders where the boll weevil enters the plantation. In these areas, flower buds should be picked and destroyed on a weekly basis, thereby reducing the pests' population growth. A full collection is recommended for small rectangular plantations of up to 10 ha or those that contain various shelter locations for the boll weevil. Pickup should take place in at least two phases, the first between 55 and 60 days and then again between 75 and 80 days. Operational aspects of this collection should vary from 1.5 to 2.0 ha/day/man. Collection is a complementary control measure of the boll weevil. It can reduce the population but does not preclude the application of insecticides (Figure 2).

Control of Borders

In areas that are known to be infested, sequential spraying should take place at the borders where the insects enter, beginning at the "pinhead square" stage. Fallen buds that carry larva must be picked up. Applications should contain only phosphorous and acid-based insecticides, never pyrethrin based, until 90 days.

Growth Regulators and Defoliants

The advent of new varieties, increased density in planting and cultivation geared to machine-power harvesting has made the use of growth regulators indispensable on many plantations. The use of these products must be timed and begin at 30-40 days, with 1/4 or 1/3 of total amounts, reapplied every 10 days, taking into consideration plant growth and weather conditions and to increase early harvesting. Growth regulators result in more uniform growth of the plant and facilitate penetration of insecticide inside the vegetation.

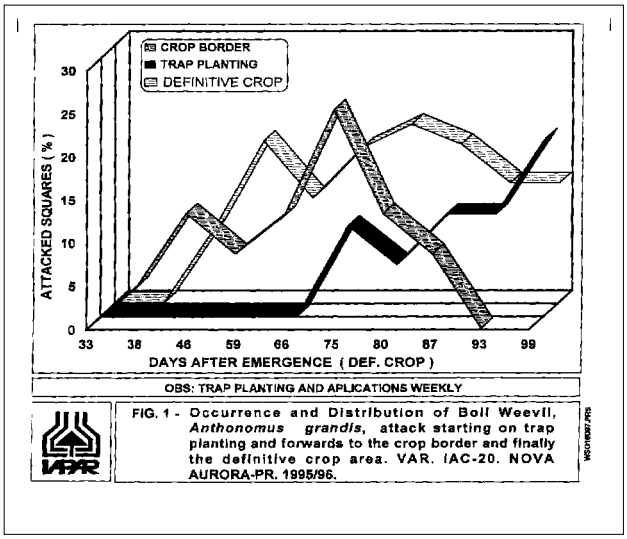
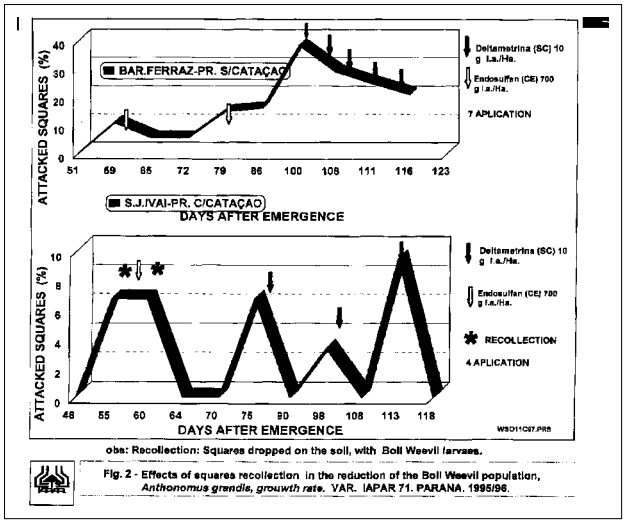


FIG. 1 - Occurrence and Distribution of Boll Weevil, *Anthonomus grandis*, attack starting on trap planting and forwards to the crop border and finally the definitive crop area. VAR. IAC-20. NOVA AURORA-PR. 1995/96.



obs: Recollection: Squares dropped on the soil, with Boll Weevil larvaes.

Fig. 2 - Effects of squares recollection in the reduction of the Boll Weevil population, *Anthonomus grandis, growuth rate.* VAR. IAPAR 71. PARANA. 1995/96.

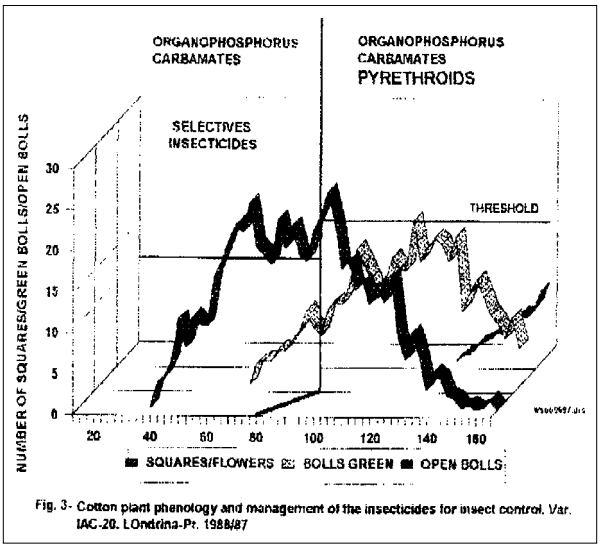


Fig. 3- Cotton plant phenology and management of the insecticides for insect control. Var. IAC-20. LOndrina-Pr. 1988/87

Defoliants result in rapid leaf fall and favor quicker ripening of the cotton bolls. The application of defoliants temporarily stops flowering, affecting population growth of the boll weevil at the end of the cultivation cycle, thereby reducing population density of the pest for the next crop.

Samples

Field inspections should be performed at a minimum on a weekly basis. Buds should be inspected at two-thirds of maximum development and observation should include extremities of plants to see if any adults are present, especially at feeding locations or those that harbor eggs. A person should walk in a zigzag through different areas inspecting 50 buds per area (± 10 ha.) Sampling should separate data obtained at the borders and/or trap cropping, from those that represent the rest of the crop. Findings of 10% of damaged buds at 80 days and 15% after this period can determine the decisions for pest control (Figure 3).

Crop Rotation

Crop rotation is always desirable in crop management because it makes agricultural operations more sustainable. It is especially important in the direct planting system as a means to minimize risks resulting from the boll weevil and other cotton plant pests.

Stump Trap Plants

Small strips of 200 m^2 of abundantly green trap plants should be maintained at the edges of cultivated areas to reduce the boll weevil population between crops. These should be sprayed every five days, for a 20-day period, and then destroyed.

Biological Control

Ramalho et al (1996) indicated the presence of thirteen parasite species and ten predators of the boll weevil in Brazil. In Brazil, research on natural enemies of the boll weevil is being conducted by the "Unidade de Controle Biológico (UCB)" (Biological Control Unit) of the "Centro Nacional de Pesquisa de Algodão - EMBRAPA" (National Center for Cotton Research) in Campina Grande, State of Paraíba. The main parasites affecting boll weevil larvae are *Bracon vulgaris* and *Catolaccus grandis*. Some adjustments were made in the current *B. vulgaris* development methodology that resulted in higher operational efficiency. Results of studies, conducted by the UCB, about the effect of temperature on the development of *B. vulgaris* have shown that the egg stage presents resistance to temperature variations, larvae are sensitive to temperature variations; the parasite is more resistant to high temperatures than the boll weevil; the boll weevil, when feeding on cotton bolls, is more vulnerable to the *B. vulgaris*, and high temperatures play an important role in determining the number of *B. vulgaris* generations (Ramalho et al, 1997). UCB is also working with the *Catolaccus grandis* parasite, for which it has developed a mass production method. UCB is also assessing the use of alternative hosts for the development of the parasite to arrive at cost reductions. UCB has already applied *C. grandis* in the field with very promising preliminary results.

Chemical Control

The only time during the boll weevil's life cycle, when it is susceptible to insecticides, is during adulthood. Applications should be based on sample data and on infection intensity as well as risks, which should all determine the frequency of spraying. Until 80 days, we recommend the use of phosphorus and acid-based insecticides (endosulfan, malathion, methyl parathion and phosmet.) Full development of the beneficial fauna (parasites and predators) occurs in cotton plantations between 40 and 80 days. Consequently, to preserve natural enemies and keep their actions viable as complementary population regulators of the range of pests affecting cotton plantations, selective insecticides must be applied, such as endosulfan, which among other products mentioned above, has been shown to control the boll weevil consistently. Pyrethrin also offers adequate control of the boll weevil. The most efficient ones are betacyfluthrin (SC) deltamethrin (SC and UBV) and zetacyfluthrin (EW), that offer an extended period of control because of their formulations (Santos, 1997.) To manage insecticides for the control of the boll weevil and other pests we recommend the use of more selective products until the 80th day after budding of the plants and the application of pyrethrin after this period (Figure 3).

Pheromone - Monitoring and Control

The pheromone "grandlure" can be used as trap for population monitoring. This is an efficient way to attract and detect adult boll weevils at the beginning and end of the cultivation periods. The "grandlure" has grown in recognition as a method to suppress boll weevil populations through a device such as the Boll Weevil Attract and Control Tube (BWACT) or "Tubo Mata

Picudo" (TMB) (Boll Weevil Killer Tube) in Brazil. TMB tubes are highly efficient instruments for the initial reduction in boll weevil populations. Preferably, the TMB should be installed one week before seeding and at the edges of entrance borders for adult boll weevils. The tubes should be 40 meters apart and should be protected from dust that reduces their efficiency. The tubes can remain in place for more than 40 days attracting and killing adult boll weevils and can even substitute trap cropping and trap stump cultivation. Results obtained with the TMB in Brazil have shown good results in controlling surviving boll weevils between crops, with significant reductions in the number of sprays and consequently reducing the cost of pest control (Santos, 1996.) The "grandlure" can also be used in a formulation with a greasy consistency that contains 0.8% of pheromone and 6.4% of cypermetrin. Santos & Hofer (1996) while working with this mixture made three applications on plants (in the form of drops) during the period from 24 to 40 days after bud formation. They observed a significant reduction in the population growth rate of the boll weevil, with good control of the pest.

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Boll Weevil Population Dynamics in Paraguay

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In Paraguay, cotton cultivation is an important occupation in terms of available manpower, because it is done almost entirely by hand. It also creates indirect employment involving considerable movement of capital. The Ministerio de Agricultura y Ganaderia - MAG (Ministry of Agriculture and Livestock) along with international cooperation, is undertaking research to improve production because of the great social and economic importance of this crop. Included in recent progress is the dissemination of new varieties, which reflect improvement in production, as well as quality. Problems with production emerged over the years and, among them, pests were and are considered as being compromising, and overall the boll weevil is mentioned as a major problem. The appearance of the boll weevil in Brazil (1983) (Braga et al, 1983) was an admonition for Paraguay. Pheromone lures were installed along the border with Brazil, covering the main access routes, cotton storage centers, as well as cotton clearing installations all over the country. The main objective was early detection of the boll weevil. The first boll weevils, that infested cotton plantations at the border with Brazil, were found in the pheromone lures in April 1991 (Marengo & Whitcomb, 1991).

During the initial harvests, the boll weevil was not detected by cotton producers although it continued to spread in the NE-SW direction (66 Km/year, Feb. 1996) (Gomez, 1996) carried by prevailing winds, infesting new crops in new areas.

Currently, this pest covers a large part of the cotton producing regions (Figure 1) with exception of the Southern area, bordering with Argentina and the Western Region, that is not yet in-

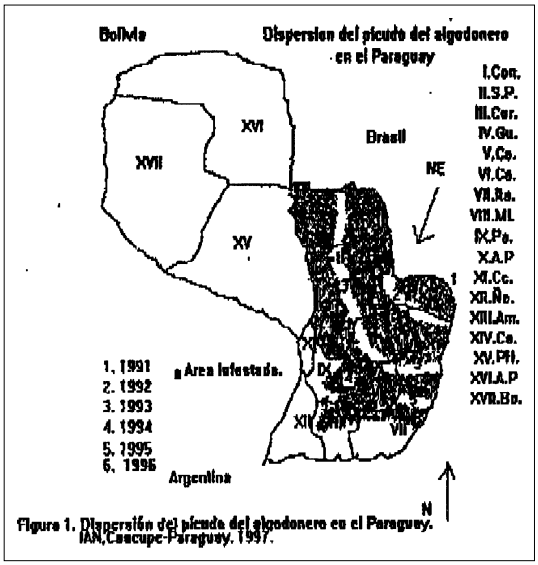

Figure 1. Dispersión del picudo del algodonero en el Paraguay. IAN,Caacupe-Paraguay. 1997.

fested. The Southern region is covered by an agreement with Argentina to establish an area to contain the boll weevil, avoiding its spreading to Argentine territory.

Since 1995, the boll weevil population dynamic in various areas of the country has indicated a permanent activity of the insect during the period between crops. On cotton plantations, the appearance of the boll weevil was somewhat delayed so that the intensity of damage was still relatively low. Nevertheless, during the 1994/95 crop year, a loss of 30% of production occurred (especially affecting late planting) in crops of areas with higher infestation, the equivalent of about 34.5 million dollars. This is without considering the environmental impact caused by the increase in the frequency of insecticide applications. The behavior of the pest mentioned above has lead to the raising of different hypotheses (natural control, period of adaptation to the weather, among others). Meanwhile, the boll weevil currently needs to be seen as a key pest to this crop and strategies for control have to be found within a context of economic sustainability and in accordance with environmental aspects.

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Incidence and Parasitization Studies of *Anthonomus grandis* Boh. in Paraguay

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(Presented by Iván Gallo)

Introduction

The boll weevil has been observed in Venezuela since November of 1949, and in 1950 it was detected in Colombia's Atlantic coast, where until 1952, it had spread through the areas of Cartagena, Barranquilla and Valledupar (Marengo et al, 1987). For more than 30 years the boll weevil was restricted to Northern Latin America.

In February of 1983, it was detected in Brazil, in Santa Barbara do Este, close to Campinas, in the State of São Paulo. From there it advanced to the north, towards Paraíba, and to the west, appearing in Maringá (State of Paraná) in April of 1986, at less than 200 km from the Paraguayan border, and moving in the direction of Saltos de Guaira (IASCAV 1993).

In April 1991, the boll weevil entered Paraguay in the District of Saltos de Guaira, State of Canindeyú (Gómez 1996 and IASCAV 1993). In 1992, it had colonized Canindeyú. Moving in the NE-SW direction, it arrived in Concepción, San Pedro, Caaguazú, and Alto Paraná in 1993. By 1994, the boll weevil had already attacked Coronel Oviedo, Guayra and Caazapa and expanded to Concepción, San Pedro and Coronel Oviedo. In 1995, it arrived in Itapua and in 1996 occupied all of Itapua and parts of Misiones (Gómez 1996). The boll weevils' dissemination occurred in Paraguay at an average speed of 66 km/year (Gómez 1996). Currently, as of 1997, only the Chaco and the State of Ñeembucú are free of the boll weevil.

In Paraguay, most of the cotton cultivation is performed by small farmers who cultivate <3 ha individually (Morel 1987). Cotton is their highest income crop. Social well-being and farmers' income depend on cotton. Before the arrival of the boll weevil they harvested 1,400 kg/ha of raw material and performed three applications of insecticide on average; protection of the crop represented 12-15% of production cost (Morel 1987). By 1997, they were applying insecticide 8-10 times per season and domestic output diminished to 1,200 kg/ha (Gómez 1996). During 1992/93 area infested with the boll weevil only obtained 1/3 of the liquid assets from cotton cultivation compared to areas without boll weevil infestation (Duarte, 1993).

Materials and Methods

Information about the boll weevils' biology and ecology is still scarce in Paraguay. The "Centro Experimental Agrícola" (Agriculture Experiment Center) of the MAG in Choré (CEA) has performed related studies since 1995. They describe parasite aspects in two field studies in 1995 and 1996.

Inspection of Fallen Buds, 1995/96

Between June 11, 1995 and February 20, 1996 we dissected fallen buds (blossoms) in fields in the area of Choré, classifying the boll weevils' stages as 1) alive, 2) infected with parasites, and 3) dead for unknown reasons.

Life Cycle, 1996

On 20 January 1996, we removed all the blossoms infected with the boll weevil and damaged by other causes, from central sections of 1/4 ha. of a cotton crop, thereby enabling us to date subsequent egg deposits. Beginning on the next day we inspected the blossoms daily and tagged a greater number of egg deposits while we eliminated those with other kinds of damage. The small cardboard tags, tied to the flowerbuds, showed the date of egg deposit. Every day another sampling of egg deposits of the same age was formed. The last day of tagging was February 22, 1996. For reasons beyond the scope of the study this had to be discontinued on 24th of February; thus, later samples appeared in increasingly shorter periods. The sampling of 22nd of February appeared in only two days.

Every day, except on Sundays, we took a sample of an average of 10 buds from the field specimen and dissected them under a stereoscope. Immature boll weevils were identified in their larva or pupa stage and declared healthy, infected with parasites, or dead for unknown reasons. The larva's cephalic coating was measured (and reported on another occasion). The petals that were damaged after being tagged (by lepidopterous larva, for example) were discarded.

Hypotheses

After analysis of the data we came to the following hypotheses:

The females deposit eggs only once on a petal; they avoid petals that already contain boll weevil traces (in the US < 5% of buds contain more than one stage [Sturm & Sterling 1986].)

Each place attacked receive only one egg per deposit.

Because of the aforementioned, each bud only shelters one stage of the boll weevil.

More than one parasite can be found on one boll weevil larva or pupa.

When analyzed in detail, immature parasites were found by themselves on the petal, apparently having consumed the host in its entirety. These cases were classified as "larva parasites."

Petals with places attacked by egg deposits but without the presence of any stage of the boll weevil or parasites indicate "egg mortality." The egg did not hatch for unknown reasons (sterility, non-biotic reasons, proliferation of the petal wall that squashes the egg).

An irregular incision in the bud indicates "depredation," the entrance of an organism that removed the boll weevil.

A larger round incision indicates "hatching = survival" of an adult boll weevil.

A smaller round incision indicates the development of a parasite = "existence of parasites."

Results

Parasite Identification

We tentatively determined the existence of two different parasites: 1) *Bracon* spp. and 2) a ptero-malignant parasite, possibly *Heterolaccus grandis*. The species were sent to the US to be identified and results are pending.

Parasites on Fallen Buds 1995-96

Very high levels of parasites were found in boll weevil larvae and very few in the pupa (Figure 1). The presence of parasites was higher in stubbles and diminished with the new cotton crop, probably because of 1) destruction of the habitat with land preparation, 2) scarcity or absence of flowering.

Presence of Parasites During the Immature Cycle

Of a total of 4,096 places affected with egg deposits, 91 boll

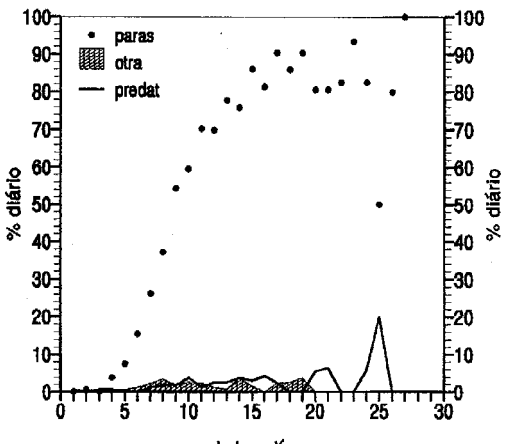

Figure 1. % of parasites present in boll weevil

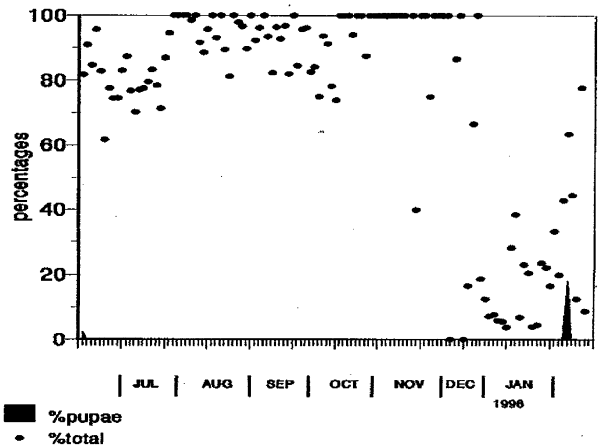

Figure 2. Boll weevil: Mortality in Flower Buds

weevils finally appeared, at survival rates of 2.22% or a mortality rate of 97.8%. With such a low survival rate there is very little or no population growth. Of the same 4,096 egg deposits, 1,116 did not produce larva indicating a mortality rate of 27.2% of the egg. The remaining 70.6% was caused mainly as a result of the presence of parasites. Depredation and unknown factors contributed very little to mortality (Figure 2).

Being that the amount of stages differed on each sampling date, the mortality analysis over time proceeded by percentages, as if 100 stages were present each day. Each day adds up to 100%. A large amount of parasites consisted of "parasites alone"; therefore we assembled all the parasites and did not separate them by immature stage of the boll weevil. The graph in Figure 2 shows these percentages after egg mortality.

The presence of parasites was the most important factor, beginning after four days of larva age and increasing exponentially until ten days, when it grew in asymptote form up to about 90%, tracing a sigmoid curve overall (Figure 2). The second most important factor was "egg mortality."

In conclusion, the presence of parasites and egg mortality were key factors of mortality in immature stages of the boll weevil in Choré. Depredation and other causes made little difference.

Discussion

The presence of parasites affecting the boll weevil in Paraguay seems to be much more prevalent than in other countries although Paraguay had not had boll weevils for five years. This is one of the rare cases where a pest introduced into a new region found already established parasites. In Nicaragua, the presence of parasites is generally low, although it can reach up to 60%, but only in the dry season when cotton is not cultivated. (G. León in Daxi,1996). In Paraguay, the presence of parasites seems to be strong throughout the year and the stumps seem to serve as a reservoir for parasites.

Cotton pest management should try to protect and develop boll weevil parasites by using insecticides very carefully. The "Tubo Mata Picudo" (grandlure) is even more significant in this situation. The combination of natural parasites, with proper use of the grandlure, can suppress the boll weevil to a point where it no longer affects cotton financially. Insecticide application against the boll weevil can become unnecessary.

A stump management program should be developed to maximize parasite survival without promoting cotton pests. If this impressive natural resource in Paraguay is damaged by inappropriate practices, the boll weevil will be transformed from a serious pest into a calamity.

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Cotton Leaf Curl Virus Epidemic in Pakistan: Virus Characterization, Diagnosis and Development of Virus Resistant Cotton through Genetic Engineering

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(Presented by Yusuf Zafar)

The cotton leaf curl virus (CLCuV), a whitefly-transmitted geminivirus (Mansoor et al, 1993) has caused heavy losses to the cotton crop and still remains the most important constraint for the development of the cotton sector in the country. The symptoms produced on the cotton plant are leaf curling, thickening of veins, enations and stunted plant growth. Cotton leaf curl disease was recorded as early as 1967 from Pakistan (Hussain et al, 1991). Since 1991/92, cotton leaf curl disease

that was only curiosity previously is now a major threat to the sustainability of this crop. The epidemic of CLCuV in Pakistan is one of the best examples of the dramatic shift in importance of an unimportant endemic disease in the past. It is estimated that the disease has resulted in a loss of 4.98 million bales of cotton with an estimated value of US$7.4 billion.

Recent advances in molecular biology and genetic engineering have opened new avenues in understanding and controlling the disease epidemics. Genetic engineering of crop species such as cotton allows introduction of a specific character such as disease resistance to be incorporated in existing varieties without compromising other agronomic characters. This technology is superior to conventional plant breeding as breeding for disease resistance using resistant germplasm may result in some undesirable characters contributed by resistant germplasm.

The National Institute for Biotechnology and Genetic Engineering (NIBGE) is a federal research institute with a mandate to apply modern and innovative techniques in agriculture, health, environment and energy. Realizing the potential of molecular biology and genetic engineering in solving the CLCuV problem in the country, NIBGE initiated the research program with the following objectives.

- Biological and molecular characterization of CLCuV which includes virus purification, cloning and sequencing of the genomic components and generation of infectious clones.
- Development of PCR/DNA probe-based diagnostic test for the detection of virus in insects and plants and use of this diagnostic test for the identification of alternate hosts of CLCuV.
- Development of virus-resistant cotton through genetic engineering.
- Molecular diversity and distribution of virus in cotton growing areas of Pakistan.

The Cotton Group of NIBGE extensively studied leaf curl disease and made vital contributions to solve this problem.

Biological and Molecular Properties

A basic understanding of the causative agent is essential for devising control strategies. Some of the biological properties such as insect transmission, transmission of virus to indicator host plants, detection of natural alternate hosts, virus particle morphology and detection of virus coat protein by Western and ELISA was carried out. The molecular characterization of virus is essential for genetically engineered resistance as well as development of molecular diagnostic methods. The work on molecular characterization of CLCuV includes cloning of full length genomic components, determination of complete nucleotide sequence, generation of infectious clones and evaluation of molecular diversity of CLCuV.

Biological Properties

Insect Transmission Studies

Our research has shown that CLCuV can be transmitted from cotton to cotton and tobacco by whiteflies. Factors affecting insect transmission efficiency have been studied. The efficiency of virus transmission was greatly affected by temperature. At higher temperatures (40-45C^{o}) the virus efficiency was increased ten fold and CLCuV could be transmitted by a single whitefly. Aphids and jassids were also tried but were unable to transmit the virus, thus whitefly is the only insect vector of CLCuV.

Experimental Host Range

Traditionally plant viruses are experimentally transmitted to some indicator host plants for the observation of symptoms produced. Tobacco plants which were infected by whitefly transmission were used in graft transmission. Infected tobacco leaves were grafted onto *Nicotiana benthamiana*, *N. tabacum* var. Samsun, datura and tomato var. moneymaker. Grafting from cotton to cotton was done to maintain virus infected plants. For sap inoculation a young infected leaf was ground in 0.06 M phosphate buffer pH 6.5, centrifuged in an Eppendrof tube for 3 minutes and supernatant was collected. Sap mixed with carborundum was rubbed on young leaves of tobacco, *N. benthamiana* and tomato plants. CLCuV was successfully transmitted by grafting from tobacco to *N. benthamiana*, tomato and datura and all these plants showed identical symptoms. Mechanical inoculation did not show symptoms on indicator plants suggesting that CLCuV could not be transmitted by sap.

Preparative Scale Purification of Geminivirus Particles from Infected Plants

Cotton is a woody plant and upon homogenization of tissues produces a lot of phenolic compounds which interfere with virus purification. Conditions were optimized for the purification of intact geminivirus particles from infected cotton plants. One to three months old healthy and infected cotton plants were used in this study. The geminivirus particles isolation was performed according to Czosnek et al. (1988) with some modifications. The samples were treated with 2% aqueous uranyl acetate (negative staining) and observed on JEOL transmission electron microscope at a power of 40 Kv. Intact geminivirus particles were observed in one of the fractions. The optimization of isolation procedures of CLCuV from the cotton plant itself is the first report of its kind in the world. The purification of intact virus particles has allowed development of antisera which can be used for the detection of virus in infected plants by ELISA.

Molecular Properties of CLCuV

DNA Isolation, PCR Amplification and Cloning of PCR Amplified DNA

Total DNA was isolated from tobacco leaves by the Kirby

method. For the isolation of total DNA from cotton a method modified from Doyle (J.K.Brown, personnel communication) was used. Universal primers for dicot-infecting geminiviruses were used in PCR for the amplification of CLCuV DNA. African Cassava Mosaic Virus (ACMV) and Indian Cassava Mosaic Virus (ICMV) were used as a positive control where as DNA isolated from healthy *N. benthamiana* was used as a negative control. The PCR product of the expected size was obtained both from infected cotton and tobacco plants. The amplification of viral DNA was confirmed by using ACMV DNA A as a probe. PCR amplified DNA was digested with SaII and EcoRI and a fragment of about 1.2kb was cloned in SaII-EcoRI site in Bluescript vector. For the amplification of DNA B degenerate primers reported by (Rojas et al, 1993) were used in PCR. ACMV which is known to be amplified by these primers was used as a positive control. However, no amplification was obtained with primers for DNA B.

Cloning of Components DNA-1 and DNA-2

Double stranded replicative form of CLCuV was purified from infected cotton plants. Clones of DNA 1 of CLCuV were selected by southern hybridization. Full length clones of genome A were obtained either by cloning at unique restriction site or by combining two cloned fragments in such a way that desired open reading frames (ORF) remained intact. Sequencing of several clones was carried out and two variable clones of DNA 1 were identified named as CLCuV Pak-1 and CLCuV Pak-2. Analysis of clones identified another clone which did not hybridize to DNA-1clone but hybridized to viral DNA. The sequence analysis of this clone did not show homology to know geminiviruses. The clone did not hybridize to intergenic probe. This clone has been named as CLCuV DNA-2. PCR primer designed on the basis of this clone suggest that this genomic component is associated with both whitefly-transmitted geminiviruses associated with the disease.

Complete Nucleotide Sequence of CLCuV Pak-1

CLCuV Pak-1 was completely sequenced by dideoxy chain termination method using radioactive 35S or 33P dATP. The sequence data were assembled and open reading frames were identified. The two variable clones have genome organization typical of old world geminiviruses. There were two open reading frames AV1 and AV2 in the virus sense where as five open reading frames in complementary sense namely AC1, AC2, AC3, AC4 and AC5 were identified. The two strains (Pak-1 and Pak-2) differed in their genome size and predicted amino acid sequence of complementary sense genes. However, the most striking differences were in the intergenic or common region. The sequence data has been submitted to EMBL data bank and this is the first sequence of CLCuV in databank.

Total No. of nucleotides = 2749
Length of common region = 278

Table 1. The Genome Organization of CLCuV Pak-1. EMBL Accession No. X98995 (Mansoor et al, 1996)

ORF	Reading frame	Start	Stop	No. of a.a
AV1	+1	124	432	103
AV2	+2	284	1051	256
AC1	-3	2594	1506	363
AC2	-1	1606	1155	150
AC3	-3	1461	1060	135
AC4	-1	2437	2137	100
AC5	-1	592	70	174

Sequence of several clones identified two geminivirus species. The comparison of these viruses is given in Table 2. The two viruses are named CLCuV PK-1 and CLCuV-PK2.

Table 2. Putative Open Reading Frames (ORFs) of Cotton Leaf Curl Virus (CLCuV) species

ORFs	Start	Stop	No. of Amino Acids	Protein Mr (kD)	Similarity Index (%)
A	1	2750			71
AV2	139 (124)	495 (435)	118 (103)	13.7 (12.1)	90
AV1	299 (284)	1069 (1054)	256 (256)	29.7 (29.7)	74
AC1	2600 (2594)	1518 (1503)	360 (363)	40.3 (40.8)	87
AC2	1615 (1606)	1211 (1154)	134 (150)	15.3 (17.4)	66
AC3	1470 (1461)	1066 (1058)	134 (134)	15.7 (15.6)	66
AC4	2445 (2680)	2144 (2135)	100 (181)	11.1 (20.6)	72
AC5	814 (592)	290 (68)	174 (174)	19.9 (19.3)	68

Values outside parenthesis represent CLCuV-Pk2/Fsd/1.
Values inside parenthesis represent CLCuV-Pk1/Fsd/3.
V in an ORF represents virion sense strand and C denotes the complementary strand.

Biological Variability in Symptom Expression

Biological and molecular variability of CLCuV was also studied. The important variation observed in the field is the upward or downward curling of the leaves. It is not known whether the curling is determined by different viruses or is a plant response to virus infection. To study this phenomenon, cotton plants showing upward or downward curling were passaged by grafting to healthy cotton plants and the symptoms on the plants were recorded. It was found that plants showing downward curling during passage produced plants with upward curling. Similarly, one of the plants where graft was showing upward curling produced downward curling symptoms. The results show that symptom phenotype was not maintained and that curling is either a plant response or may be due to the dominance of one strain.

Molecular Diversity: Evidence of Presence of Two New Geminivirus Species in CLCuV Pak-1

For the evaluation of molecular diversity several clones were obtained either by ccc DNA cloning or PCR. As discussed earlier, the common region is the most diverse region among CLCuV isolates. A common region of six clones was completely sequenced. Four of the isolates have a common region sequence of PK-1 strain while 2 of the clones had a sequence of Pak-2. The data suggested that considerable variability exists in CLCuV. Based on the sequence data, Pak-1 and Pak-2 specific primers were designed and the desired product has been confirmed by cloning and sequencing. These primers are being used for the assessment of distribution of two strains of CLCuV.

Detection and Differentiation of Geminiviruses

Previously we have used the polymerase chain reaction (PCR) for the identification of alternate hosts of cotton leaf curl virus. Surveys were conducted for the collection of additional plant species which were not reported previously to be infected with geminivirus. Samples from infected cotton plants were also collected from different cotton growing areas of Pakistan. DNA A of ACMV or CLCuV was used as a probe for the detection of whitefly transmitted geminiviruses. A number of well characterized geminiviruses both from the old and new world were used as positive control while DNA extracted from healthy plants of various species was used as negative control. It was found that use of ACMV or CLCuV as probe was able to detect geminiviruses by dot blot hybridization both from the old and new world viruses. Similarly the use of ACMV or CLCuV as probe detected geminivirus in 30 plants species out of 40 plant species suspected of whitefly-transmitted geminiviruses.

For the differentiation of an alternate rate of CLCuV from other geminiviruses, a probe of CLCuV common region was prepared. The common region of whitefly-transmitted geminiviruses is the most diverse region and may serve as a virus specific probe. It was found that under high stringency level the use of a common region as probe specifically detected CLCuV both in cotton and alternate hosts and further confirms that members of the Malvaceae family are the alternate hosts of CLCuV.

Development of a Diagnostic Test

Development of PCR Based Diagnostic Test

PCR/DNA probe-based test has been developed for CLCuV by designing two sets of primers. One set of primers is capable of amplifying a portion of viral genome from common region to N-terminal sequence of coat protein. The other set amplifies part of viral genome from replication associated protein (AC1) to common region. Newly developed sets of primers were used to detect presence of CLCuV in cotton plant leaves collected from different cotton growing areas. The amplification of viral genome was confirmed by cloning and sequencing of amplified product. This is a highly sensitive assay for the detection of virus in the plant. Recently, we have developed a multiplex PCR for the detection of two geminiviruses species associated with cotton leaf curl disease in Pakistan. A simplified method is used for the isolation of template suitable for PCR. A rapid profile used PCR primers designed to specifically detect these two virus species. The two species could be found independently or co-infecting the same plants. This is a unique example where two geminivirus species could cause the same disease in a geographical area.

Screening by Southern Hybridization

CLCuV samples were collected from different cotton growing districts of the Punjab and full length cloned viral DNA was used as probe in southern hybridization. The cloned DNA probe hybridized with the samples and thus can be used for screening purposes.

Development of Polyclonal Antisera

Purification of intact geminate particles paved the way for the production of polyclonal antisera against CLCuV. For this purpose rabbits were immunized with viral particles mixed with Freund's complete adjuvant (first dose). Subsequent subcutaneous booster doses were given with Freund's incomplete adjuvant at an interval of 15-20 days. Polyclonal antisera has been distributed to Ayub Agriculture Research Institute — AARI and National Agriculture Research Center — NARC for evaluation by ELISA. We received feed back from both research institutes and they recommended that this material needs further purification for reliable testing for the amplification such that the process is completed in two hours. This method is being used to assess the distribution of two geminivirus species in cotton growing areas of the Punjab, Pakistan.

Development of Monoclonal Antibodies and ELISA Test

As a first step for the development of rapid immunological test for screening, sets of primers based on the sequence to genome A were synthesized for PCR amplification and cloning in expression vectors. These primers amplified 1.1kb fragment from cloned as well as from total DNA isolated from infected cotton leaves. This PCR product has been cloned in bacterial expression vector for large scale protein production and purification. The expressed protein has been used for the generation of monoclonal antibodies. Two clones of hybridoma cell lines producing antibodies against CLCuV have been identified and are being tested for the specificity of monoclonal antibody by ELISA. These studies are being done in collaboration with Department of Pharmacology, John Hopkins University, Baltimore, USA.

Development of Virus-resistant Cotton Through Genetic Engineering

Several approaches have been reported for the development of transgenic resistance against whitefly transmitted geminiviruses.

The approaches that are being used for CLCuV at NIBGE are the following

- Expression of antisense RNA against complete or fragments of AC1 gene
- Over-expression of AC1 in transgenic plants
- Expression of a virus-induced cytotoxin gene in transgenic plants

Tissue Culture of Cotton

The genetic engineering of a plant is heavily dependent on transformation technology whereby a functional foreign gene could be inserted into the genome of the cotton plant. Currently, the two most widely used methods for plant transformation involve agrobacterium-mediated transformation and bombardment of cells with DNA coated particles. These two methods appear to be most important for genetic engineering of cotton both in terms of success and current efforts. Unfortunately, transformation with Agrobacterium requires that the cotton genotype, be regenerable from callus tissues, a feature which so far appears to be limited to some Coker lines and an Australian cultivar Siokra 1-3 among the commercial cultivars. Because of this limitation, researchers have strong interest in alternative transformation processes such as direct transformation of meristems with Agrobacterium.

Tissue Culture of Local/Exotic Varieties of Cotton

Nineteen local/exotic cultivars of cotton were evaluated for in-vitro callus induction and plant regeneration. Varieties S-12, NIAB-78, AEM-1-85, FH-682 and BH-36 produced significantly better calli than other varieties. However, induction was observed to be highly variable not only among different genotypes but also among various explants of the same genotype. Successful attempts were made to control contamination, auto-inhibitory response and decay of calli. Embryoids were observed in some varieties but regeneration was obtained only in Coker-312 and Sikora 1-3 varieties. Further work is in progress.

Meristem Tip Culture of Local Varieties of Cotton

Meristem shoot tips of ten cultivars of cotton *Gossypium hirstum* were cultured on several media formulated for shoot and root development. The best shoot development was observed on media containing 0.1 mg/liter Kinetin, while rooting was observed on media containing 0.5 mg/l NAA and 0.1 mg/l Kinetin. No inter-varietal variability was observed. A complete protocol was developed from meristem tip culture to field transfer for biolistic gun transformation of cotton.

Development of a Recombinant DNA Construct Based on Sense or Antisense Expression of AC1 Gene

Several technologies have been reported for the development of genetically engineered resistance against cotton leaf curl virus. One of them is the production of antisense RNA against replication associated protein or over-expression of ACI gene in a transgenic plant driven by CaMV S35 promoter.

Open reading frame coding for ACI was identified on clone PS1. Primers (V2417, V2418) were designed such that an NCoI site was incorporated at 5' end of both primers. A product of expected size (1.2 k) was obtained. The PCR product was digested with NCoI enzyme and was cloned in PJIT 166 (a plant expression vector) both in the sense and antisense orientation. The orientation of clones was confirmed by restriction analysis. The clone was also confirmed by sequence analysis. GUS gene from the vector was removed by digestion with SmaI and relegated to give pSJITAC1. The construct was lifted from pSITAC1 by digestion with Sph1 and Sst I and ligated in pBin plus vector digested with the same enzyme and vector pSBinJIT AC1 was obtained. Electro-competent cells of agrobacterium strain C58 (disarmed strain) were used for electro transformation and transformed cells were selected by kanamycin resistance.

Development of Recombinant DNA Construct for Resistance Against CLCuV Based on Virus-induced Expression of a Cytotoxic Gene

During recent years, several techniques have been reported for the development of genetically engineered resistance against geminiviruses. Pioneering work on the use of ribosome-inactivating proteins (RIPs) has been initiated at John Innes Centre. RIPs are naturally occurring plant toxins that are presumed to provide a defense mechanism against pathogens or predator by disrupting protein synthesis in damaged eukaryotic cells. One of these RIP, dianthin has been exploited in these studies. The expression of dianthin is driven by viral coat protein promoter and is activated in-trans by one of the viral gene product (AC2). The activation of transgene expression during virus infection avoids the constitutive expression of the transgene.

A set of primer was designed to amplify the coat protein promoter of CLCuV. Necessary restriction sites were introduced to ensure that the promoter is in frame with the dianthin gene. The primers were successfully used and the desired product was cloned in pGEMT vector and checked by restriction analysis. The cloned fragment was further checked by sequence analysis. The clone was sequenced by use of a T7 sequencing kit using 33P labeled nucleotide. The sequence analysis confirmed that the desired product has been cloned. A plant expression vector pJIT163 was used for the expression of transgenic dianthin. Double 35S promoter in the vector was replaced by coat protein promoter and dianthin was cloned in the correct reading frame. The construct was lifted by digestion with Sst I and ECoRV and cloned in plant transformation vector pBin Plus. The clone was transferred to agrobacterium strain C58 by electroporation.

Development of Constructs Based on Antisense RNA Expression of Parts of Complementary Sense Genes

To express the viral antisense RNA in transgenic plants, an expression cassette was constructed. A ~1.5 kb fragment containing the double CaMV 35S promotor and poly A terminal sequence was isolated from the plasmid pJit 60 (gift from Dr. P. Mullineaux, J11, Norwich, UK) with Xho1 and Sst1 and subcloned into plasmid pBluescript 11 KS to get pSQMW1. The fragments of ~ 460 bp (Start of AC1-end of AC4 gene), ~520 bp (end of AC4- start of AC2 gene) and ~ 540 bp (Start of AC2-end of AC3 gene) were isolated from the pYASF clone using specific primers (SH1 and SH2, and SH3, SH4, SH5 and SH6 respectively) by PCR. The amplified DNA fragments were end filled with T4 DNA polymerase. The individual fragments were then cloned in PSQW1. More than 60 recombinants were screened for sense and antisense insertions of the above mentioned gene sequences. Confirmation was obtained by cutting the plasmid insert with restriction enzymes. PstI (AC1-AC4 and AC1/AC2/AC3 gene) and Sa11 (AC1 middle region)

The fragments of 2.0 kb harboring the above mentioned genes in both sense and antisense orientation were isolated by cutting the pSQW1 with Sst1 and EcoRV. Finally these fragments were cloned into the plant transformation vector PGA 482 at Sst1/ Hpal site to yield PGS clones. Later these plasmid carrying the respective genes were transformed into agrobacterium tumefacient strain LBA4404 by electroporation.

Transformation of Constructs in an Elite Pakistani Cotton Variety S-12

A protocol was developed for Agrobacterium mediated transformation of an elite cotton variety S-12. The method that uses 3-day-old mature embryos for transformation and selection was made on kanamycin. Analysis of the first batch of plants suggests that the gene has been integrated in these putative transgenic plants. The plants are being tested for the presence of genes and the ability of plants to resist geminiviruses.

Cotton Genome Project

A majority of the present day commercial cotton varieties grown in Pakistan belong to *Gossypium hirsutum* L. (upland cotton) a very few to diploid species *G. arboreum* L. Breeders have evolved these varieties through selection based on morphological and physiological features (yield, fiber quality, resistance against certain pests and diseases, etc.).

Most of the varieties grown in Pakistan originated from intraspecific crosses of *G. hirsutum* L. at various research centers around the country. These hybridization practices resulted in narrow genetic base of the new varieties. Any crop with a narrow genetic base is more prone to natural disasters such as outbreak of a disease.

Morphological features are indicative of the genotype but are represented by only a few loci because there are not enough number of characters available. Moreover, they can also be affected by environmental factors and growth practices. To have an accurate and reliable estimate of genetic relationships and genetic diversity, a large number of polymorphic markers are essentially required.

Random amplified polymorphic DNA (RAPD) technique of Williams et al (1990) provides an unlimited number of markers which can be used for various purposes. In addition to technical simplicity and speed of RAPD methodology, its level of genetic resolution is equivalent to restriction fragment length polymorphism (RFLP) for determining genetic relationships.

RAPD analysis was used to evaluate the genetic diversity of elite commercial cotton varieties in addition to the intravarietal studies. Twenty individual plants of cotton variety S-12 were analyzed with 10 primers for any polymorphisms. No polymorphisms were observed with any of the ten primers indicating that the technique can be used for the analysis of purity of seeds in cotton.

Twenty-two varieties belonging to *Gossypium hirsutum* L. and one to *G. arboreum* L. were analyzed with 50 random decamer primers using polymerase chain reaction (PCR). Forty-nine primers detected polymorphism in all 23 cotton varieties, while one produced monomorphic amplification profiles. A total of 349 bands were amplified and 89.1% of which were polymorphic. Cluster analysis by unweighted pair group method of arithmetic means (UPGMA) showed that 17 varieties can be placed in two groups with a similarity ranging from 81.51% to 93.41%. *G. hirsutum* L. varieties S-12, V3 and MNH-93 showed a similarity of 78.12, 74.46 and 69.56% respectively with rest of the varieties. One variety CIM-1100 showed 57.02% similarity and was quite distinct. The diploid cotton *G. arboreum* L. var. Ravi was also very distinct from rest of its tetraploid counterparts and showed only 55.7% similarity. The analysis revealed that the intervarietal genetic relationships of several varieties is related to their center of origin. The results also showed the genetic relationship of elite commercial cotton varieties with some standard "Coker" and diploid *G. arboreum* L. var. Ravi (old world cotton). As expected, most of the varieties have a narrow genetic base. The genetic similarities obtained can be used for the selection of possible parents to generate a mapping population. The polymorphic profiles can be used for the identification of different varieties and the protection of breeders propriety rights.

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Global Diversity and Distribution of Cotton-Infecting Geminiviruses: An Essential Requisite to Developing Sustainable Disease Resistance

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Introduction

Until quite recently, whitefly-transmitted (WFT) geminiviruses were restricted primarily to weed hosts in the subtropics and tropics, with rare exceptions. For example, in Africa, cassava mosaic virus (ACMV) has been problematic in cassava since the turn of the century, primarily because the virus has been

maintained in cropping systems by the practice of vegetatively propagating infected cassava from slips. In Sudan, since the 1950s, leaf curl of cotton was reported as an annual disease, though the magnitude of yield loss varied from year to year. Tomato-infecting geminiviruses were documented in India and the Middle East at about the same time and were periodically yield limiting. In the Western Hemisphere, geminivirus incited diseases were of little importance until the 1960s when disease outbreaks occurred in bean and tomato crops in Brazil, the Caribbean Basin, Mexico, and Venezuela. However, by the 1980s, geminiviruses became recognized as emergent virus pathogens on a global scale, and are of widespread importance in cotton-vegetable agroecoystems.

Bemisia tabaci (Genn.), known as the cotton or sweet potato whitefly, is the sole whitefly vector of geminiviruses. Until the 1960s, *B. tabaci* was recognized as an infrequent pest and virus vector, and hence, was considered of little consequence to crop production. The increased importance of geminivirus diseases and their whitefly vector have been exacerbated by two recent events: the nearly global introduction and establishment of an exotic whitefly vector type of *B. tabaci*, the B biotype and the widespread and simultaneous development of insecticide resistance in local whitefly vector populations throughout the subtropics/tropics. High levels of vector populations in these agroecosystems, many only recently expanded with the aid of irrigation projects, has resulted in the mobilization of many new geminiviruses from weed to crop species, and subsequently, routine and damaging diseases caused by geminviruses in cropping systems for the first time. Among the crops most damaged by geminivirus diseases are beans, cotton (*G. arboreum, G. barbadense, G. hirsutum*), all cucurbits, pepper, tomato, and watermelon.

This global upsurge in *B. tabaci* in agroecosystems has greatly facilitated higher rates of virus transmission, subsequently leading to increased disease incidence and a wider biogeographic distribution of geminiviruses. Consequently, the growing trend is toward increased baseline levels of virus inoculum in agroecosystems leading to higher disease incidences and unprecedented yield losses due to infection by essentially unknown or poorly studied plant viruses. Therefore, most problematic to controlling these new virus pathogens is a general lack of knowledge about their identity, distribution, and host range, and about the biogeographic diversity and distribution of whitefly vector populations that can vary considerably with respect to host range, vectoring capacity, reproductive abilities, and frequency of resistance to particular insecticides. Finally, the unavailability of disease resistant crop cultivars does not promise feasible solutions to combat economic losses in the near future.

Background

History of Geminviruses in Cotton

During the 1950-1980s, decreased cotton production in Central America and the Caribbean Basin was attributed in part to whitefly-transmitted geminviruses (J. Bird, per comm.). In addition, following the establishment of the B biotype whitefly, enormous losses were felt due to feeding damage and honeydew contamination of lint, and subsequently to appearance of many new geminivirus diseases. Indeed, cotton production in Puerto Rico, the South Coast of Guatemala, and Dominican Republic is now either non-existent or greatly reduced, and in Nicaragua, although production continues, geminiviruses are a growing constraint (Brown et al., 1991; Kraemer, 1966; P. K. Anderson, pers. comm.). Several geminivirus diseases of cotton have been described in Brazil and possibly, in Paraguay (Costa, 1976), but these viruses are as yet uncharacterized. In the US (Arizona and California) and northern Mexico (Sonora and Sinaloa), cotton leaf crumple disease occurred sporadically since the 1950s when it was first described, however, typically late season infections have precluded substantial crop losses in most years. Cotton-infecting viruses associated with whitefly infestations have also been reported in Texas since the 1950s, and although preliminary data suggest they are distinct from leaf crumple, these viruses are also poorly studied.

The most worrisome virus diseases of cotton occur primarily in the Eastern Hemisphere. Particularly serious losses have been documented in India, Pakistan, and in Sudan, Egypt, and several other African countries. In this part of the world, geminivirus diseases in cotton began to escalate in the 1980s, increasing steadily in distribution since then. Whitefly-transmitted geminivirus diseases of cotton now occur annually in these areas and widespread epidemics have become a common occurrence (Brown, 1992; Idris, 1990; Mansoor et al., 1993; Varma, pers. comm.). For example, in Pakistan in 1993/94, about two million hectares of cotton were infected by cotton leaf curl virus and production was reduced to 1.4 million tons from an all time high of 2.2 million tons in 1991/92 (ICAC estimates). In 1994, leaf curl, or a related geminivirus, first threatened cotton production in the Indian Punjab, while at the same time, there were increased reports of cotton leaf crumple virus in Mexico and the SW US. Although geminiviruses were likely present or are 'indigenous' to these areas, the contemporary diseases in cotton appear to be more widely distributed and to cause substantially more damage than their previous counterparts, perhaps because varieties grown for contemporary markets lack disease tolerance or resistance that may have been a characteristic of varieties grown in the past. Nonetheless, the effects of these diseases are compounded by rising vector populations and the likely possibility that new geminiviruses may yet emergence. Increased disease pressures and whitefly populations have made necessary heavy applications of insecticides to reduce whitefly populations, pointing to the urgent need for virus disease resistant cotton varieties.

The rising costs of managing the whitefly vector, coupled to losses due to geminivirus diseases now hinder cotton produc-

tion by the demand for inputs beyond economic feasibility. The clear need for virus-resistance in high yielding, high quality cotton varieties that are tailored to disease prone areas presents a new challenge. If this challenge is not met, there is no certainty that cotton production at present day levels will be possible, nor is there a guarantee that market demands will be achieved in the absence of crop sustainability. To meet the growing demand for geminivirus resistant cotton varieties and to achieve their development, baseline information is required concerning the identity, the distribution, and the relevant characteristics of the most threatening geminivirus pathogens of cotton. Only then, can germplasm be developed with sustainable disease resistance against the specific geminivirus pathogens in a region. Also essential is sound knowledge about the range of virus strains against which cultivars are effectively protected, and about the distribution of commonly occurring geminviruses, given that tolerant or resistant cultivars will be highly desirable in other cotton growing regions where similar and possibly distinct strains of geminivirus pathogens and whitefly vector complexes occur.

The Impact of *B. tabaci* Vector - Geminivirus Complexes

Indigenous viruses such as CLCV in cotton have caused sporadic disease in the southwestern US for over thirty years. Epidemics have typically been associated with mild winters that yield early season whitefly populations and the cultivation of ratooned cotton infected with CLCV during the previous season (Dickson, 1954; Allen et al., 1960; Brown et al., 1983; Erwin, 1959; Russell, 1981). In epidemic years, cotton yields are reduced by 50-80%, particularly in those fields near ratooned cotton which serve as virus and whitefly reservoirs. Although the precise reasons for periodic epidemics of CLCV have not been ascertained, years of high disease incidence are associated with mild winters that promote early season increases in vector populations. Until recently, disease resistant varieties have not been considered worth the economic investment and programs to eradicate ratooning practices have been implemented.

During the late 1970s early 1980s, sporadic geminivirus epidemics in cotton and vegetables were generally regarded as anomalies that would not establish as persistent diseases of annual importance. However, the growing inability to reduce whitefly vector populations in cotton-vegetables agroecosystems, partially due to insecticide resistance, rapidly resulted in increased baseline virus inoculum levels in the US and Mexico. The establishment of the exotic Old World B biotype whitefly in SW cotton in 1988-90 resulted in such dramatic direct feeding damage and honeydew-contaminated lint that losses due to CLCV went largely unnoticed. Today, there is growing awareness of the persistence of this disease and incidence has risen over time. At about the same time, similar upsurges in local (or endemic) whitefly vector populations led to serious disease situations in many world locations, and cotton leaf curl virus emerged as an economic threat to production in Pakistan. In addition to the rising impact of locally occurring virus diseases, it is also feared that new geminiviruses will either emerge or be introduced from neighboring locales due to the widespread cultivation of high-yielding, disease susceptible varieties. These predictions are driven by observed patterns of increasing virus disease incidences in the US and the Caribbean Basin following the establishment of the highly prolific B biotype whitefly vector. Indeed, such trends have been born out by recent reports of geminiviruses in cotton in Guatemala (Brown et al., 1993), India (Varma, pers. comm.), Nicaragua (R. Caballero, pers. comm.), Pakistan, Paraguay, The Dominican Republic (Brown et al., 1991), and the US-Texas, (Brown, unpublished).

The global increase in whitefly pressures and local, or 'indigenous' WFT geminiviral pathogens (Brown, 1990; Brown and Bird, 1992), suggests that epidemics caused by WFT geminiviruses will continue to rise. Clearly, WFT geminivirus epidemics have already become routine in certain vegetable-cotton agroecosystems, and although information is incomplete, these viruses are likely harbored in a variety of plant genera or species that may be related or unrelated to cotton. As a result, substantial efforts have been underway to achieve stringent control of the whitefly vector, combining new insecticide chemistries with biological control agents. As yet, there has been little effort toward reducing the impact of geminivirus diseases through the development of host plant resistance. Indeed, the documentation of many new and emerging WFT geminiviruses in cotton has launched an effort to critically examine the identity, distribution, and biogeographic and genetic variation among cotton-infecting geminiviruses, rapidly becoming of importance on a worldwide basis.

Geminiviruses of Cotton

In general, WFT geminiviruses have been poorly studied, despite their recent emergence as important pathogens. Factors that have hindered their characterization include the requirement to rear the whitefly vector for experimental virus transmission in the laboratory, their limitation to phloem tissues which makes virus particle purification difficult, an abundance of secondary products and polysaccharides in cotton leaves that interfere with virus isolation, and their characteristically narrow host ranges that limit the potential to discover alternative, less recalcitrant host species for experimental studies. The overall knowledge about virus diseases of cotton has been reviewed recently (Brown, 1992; 1997); and it is quite clear that whitefly-transmitted geminiviruses are among the most limiting and poorly studied pathogens of cotton on a global basis.

Biological characteristics such as virus host range, disease symptomatology, and virus-vector relationships remain ill-defined

for most geminiviruses of cotton. Among the best studied is the CLCV from Arizona, which has a narrow host range within the Malvaceae and Leguminosae (Brown and Nelson, 1984). The discovery that common bean was an alternate host of CLCV facilitated visualization of geminivirus particles by transmission electron microscopy, resulting in the first confirmation that the CLCV was morphologically like other geminiviruses. This unique virion morphology is now accepted as a sole trait of the Geminiviridae, together with that of a single-stranded, circular DNA genome.

Recent advances in molecular cloning have facilitated a preliminary study of viral gene sequences, required to achieve virus identification. In this study, the conserved viral coat protein gene of CLCV from Arizona was compared to that of an isolate causing leaf distortion of cotton in Texas, and two isolates from Guatemala that cause leaf curl or yellow mosaic symptoms. Results indicated that at least three distinct viruses were involved, and that there may be several strains or close relatives of CLCV in the Americas. However, much remains to be learned about the similarities and differences among these New World isolates. The only other cotton virus for which genetic level information is available is an isolate of CLCuV from Pakistan. A geminivirus has been cloned from infected cotton and the genome has been partially sequenced. But, in the absence of additional virus sequences needed for comparison, it was not yet possible to predict the relationship of the Pakistan leaf curl virus to others from other world areas.

Knowledge about the genetic variation and relevant biological characteristics of WFT geminiviruses are now imperative to permit identification and to discover the distribution and degree of importance that geminiviruses pose to cotton production. A panel of well-characterized viruses are needed as sources of virus diversity against which germplasm may be screened. These same viruses, once cloned, can be sequenced in their entirety to learn more about their relationships to one another (i.e. genotype variability), and can also serve as sources of virus genes that can be engineered to produce disease resistant transgenic cotton using virus-derived resistance. Detailed comparison of select geminiviruses at the level of individual genes or key sequences involved in regulating the virus disease cycle (capsid protein, replicase, regulatory regions, movement proteins) will lend insights toward virus diversity and the global distribution of viruses and related strains utilizing molecular based information that is now accessible for the first time. Knowing who and where the most serious viruses are is clearly the first step toward developing sustainable disease resistance in cotton, irrespective of traditional plant breeding or genetically engineered plant approaches.

The Whitefly Vector

Members of the whitefly *B. tabaci* species complex are the only known vectors of subgroup III (whitefly-transmitted subgroup) geminiviruses, worldwide (Bedford et al., 1994). This whitefly has a potentially broad host range among (Brown et al., 1995) with certain populations expressing preferences for cotton, vegetables, and ornamentals. Geminiviruses are transmitted in a persistent, circulative manner by their whitefly vector, meaning that once virus is taken up from a host plant, transmission can occur within several hours, and continuously for the life of the vector. This type of virus-vector relationship makes persistently transmitted viruses as the most difficult to study, as well as to manage in the field. Possible future strategies for disease management also involve interference with this highly specific virus- vector relationship, however, at present, little is understood about the mechanisms involved in this process. A greater understanding of the mechanisms governing whitefly-mediated geminivirus transmission will lead to possibilities for interfering with those processes, feasibly through expression of anti-transmission factors in transgenic plants. Such factors, when expressed as transgenes in transgenic plants, are envisioned to 'neutralize' transmission by mimicking the virus and thereby, binding to sites in the whitefly vector that are essential to whitefly-mediated transmission. Saturation of essential sites with 'modified virus' will preclude virus binding, and hence, interfere with transmission.

Studies devoted to investigating the importance of host races, strains, or biotypes within the *B. tabaci* species complex have provided important insights necessary for managing whitefly and disease problems. These efforts will continue to shed new light on our understanding of the morphologically identical members of the *B. tabaci* complex are surprisingly, 'cryptic' in that they may differ entirely in such biological characteristics as host range, vector capabilities, fecundity, and insecticide resistance. Certain widespread and troublesome populations of the *B. tabaci* complex, for example, the B biotype, can be identified and tracked for the first time, using molecular gene markers, or biotype-specific DNA sequences that serve as an important fingerprint (Brown et al, in preparation). Sound information is available about the biological characteristics of this population, and can be obtained for others. Indeed, had it been understood that although *B. tabaci* is morphologically the same regardless of its biogeographic niche, while at the same time genetically heterogeneous and having likewise distinctive biological traits, the introduction of the B biotype could have possibly been prevented or at least recognized much sooner. The concept of whitefly biotypes or strains along with similar studies of virus strains and quasi-species also requires rigorous investigation at the biological and molecular levels in order to explain presently puzzling variation observed in whitefly vectoring capacities with certain geminivirus-vector-host complexes. Presently, however, very little is know about biology and genetic diversity for most whitefly biotypes or geminivirus strains or about the nature of the specific interactions that lead to either high, moderate, or low frequency transmission events.

Whether there are vector biotypes with a capacity to more efficiently transmit certain geminiviruses to and from cotton is not known. Also relevant to vector characteristics that affect transmission of geminiviruses is knowledge of unique behavioral characteristics that may be peculiar to a particular whitefly race or biotype, for example, long distance dispersal behavior and extremely broad host range (exhibited by the B biotype, for example) coupled to vectoring capacity and resistance to insecticides, that must be taken into account in implementing successful biological control programs that rely on release and/or enhancement of whitefly parasitoids, fungal or viral pathogens or predators to reduce whitefly populations.

Geminivirus Detection and Identification toward Disease Control

Very little is known about the identity or the distribution of the most geminivirus pathogens of cotton. Toward this end, the Arizona (JKB) laboratory has developed a polymerase chain reaction (PCR)-based method that permits highly sensitive detection of a gene fragment present in all whitefly-transmitted geminiviruses. Amplification of this diagnostic fragment (550 bp) and its visualization by agarose gel electrophoresis is the first available assay that confirms the absence or presence of geminivirus infection in plants. Obtaining the specific nucleotide sequence of the virus gene fragment readily permits a comparison of this viral sequence with sequences from well-characterized geminiviruses, providing an invaluable identification tool. Using this approach, we are archiving coat protein gene sequences of geminiviruses by compiling a database that can be accessed on the World Wide Web. After matching an input sequence to the most closely related sequence in the data base, relevant information about that virus and other close or more distant relatives can be located in data base linkages. This interactive component will permit the first comparative virus identification of geminiviruses of cotton. Information about biological and genetic diversity amongst these viruses can also be applied to study or track viruses over broader geographic areas and crop species. This is necessary because there is scant information concerning crops and/or weeds that serve as virus reservoirs to bridge the disease cycle. Further, there is no capacity for testing germplasm in resistance trials in the field or greenhouse with well-defined virus genotypes selected from disease prone areas. Reliable laboratory-based detection and identification methods are the cornerstones of developing disease resistance.

The following is a summary of the research undertaken in the US laboratory during 1996-97 as supported by the Common Fund for Commodities in a collaborative effort between scientists at John Innes Centre and collaborators at NIBGE, Faisalabad, Pakistan under the direction of Dr. Kauser Malik, Director of NIBGE. Supplemental funding for portions of this work has been provided by Cotton Incorporated, Raleigh, NC, USA, and through a donation by Delta and Pine Land Company, Scott, MS, USA.

DNA Sequence Database and Phylogenetic Inferences for Whitefly-Transmitted Geminiviruses of Cotton: Towards Disease Resistance

Project Activities and Progress to August, 1997

Objective 1

Subgroup III Geminivirus Isolate Collection

Shown in Table 1 are the geminivirus isolates of cotton currently obtained for archiving and for molecular evaluation in the Arizona laboratory. We have thus far, obtained isolates from the Western Hemisphere: US (Arizona, California, Texas), Mexico (Sonora), Guatemala, Puerto Rico, and The Dominican Republic. In 1997 we expect to obtain isolates from Brazil, Nicaragua, Paraguay, and from other cotton growing regions where viruses diseases prevail. The Eastern Hemisphere isolates we have obtained include viruses from India, Pakistan, and Sudan. We continue efforts to obtain additional isolates through the assistance of colleagues, cotton breeders, commercial companies, and travel to specific locations when opportunities arise.

Rationale

The continual acquisition of geminivirus isolates from affected areas is absolutely essential to achieve representative Old and New World viruses for the geminivirus collection archive, working collection, and for the WWW:// cotton geminivirus database (GEMINI DETECTive). The virus collection and database will be used for virus identification by comparison of input virus sequences and to classify viruses based upon phylogenetic comparison to yield a differential panel of virus genotypes for disease resistance efforts. Defining comparative genetic variability and relationships of geminiviruses and implementing sequence data to track virus distribution will permit the first global map of geminivirus genotype distribution in cotton. This tool is a key component of an optimal strategy toward resistance that will ultimately permit varieties to be tailored to resist specific viruses that cause diseases in the particular locale. This approach relies upon a capacity to select specific geminivirus isolates representative of those particular geographic areas for challenging germplasm in traditional breeding programs and as sources of virus genes in transgenic approaches to disease resistance. This initial goal involves the development of effective methods to determine the range and nature of virus genotypes that infect cotton germplasm. This objective has direct application toward developing virus-specific and/or broad spectrum resistance in cotton intended for specific or multiple sites where particular geminiviruses are

known to be yield-limiting pathogens of cotton.

Objective 2

PCR of Geminivirus Coat Protein Fragment for Detection and DNA Sequences for Virus Identification

Development of PCR Primers and Universal Detection of Whitefly-Transmitted Geminiviruses in Cotton

We have developed the first method that permits universal amplification of a geminivirus coat protein gene fragment 550 bp in size from all geminivirus-infected plants (Wyatt and Brown, 1996). Diagnostic size viral gene fragments have been cloned and the DNA sequence has been determined using automated technology. Geminivirus sequences are compared with those of previously studied viruses previously obtained, and entered into the Arizona GEMINI-DETECTive data base on the world wide web. Comparison of input sequences with those of well-characterized viruses provides a rapid, accurate, user-friendly approach for identifying whitefly-transmitted geminiviruses, and for establishing the discovery of a previously undescribed virus. New sequences are added to the database and the virus is further studied in biological and molecular terms. Using this approach, we are presently tracking and mapping the distribution of cotton- infecting geminiviruses, and will ultimately be able to point to the most abundant and widely distributed viruses that can be selected for disease resistance efforts. Consistent sampling over time will allow for the study of multiple samples per each major production zone and hence, accurate information about predominant virus genotypes. This approach will also facilitate the rapid discovery of new or emerging viruses.

Laboratory Analysis of Virus Samples Analyzed to 1996 by Universal Polymerase Chain Reaction and Future Work

All samples listed in Table 1 have been confirmed to contain geminivirus DNA, based upon the diagnostic subgroup III-specific PCR assay described above. From studies thus far, we are discovering many newly emerging geminiviruses in cotton, worldwide, and that most have not been previously studied. Because this is the only global effort of its kind, samples from all cotton growing locations in which suspect geminiviruses are present are needed for analysis. To accomplish this effort, we have solicited the help of many colleagues and hope in the upcoming seasons, to receive additional material. The UA laboratory will also engage in local and regional collecting trips to accomplish this goal, and we will continue to rely on our collaborators in Pakistan, India, the United Kingdom (Rothamsted Experiment Station), and elsewhere throughout the world to assist in obtaining additional samples. Samples will be received in the Arizona laboratory from any colleague in any world location wishing to provide material for this study under our recently awared USDA PPQ permit to import plant leaf material for this effort. Sampling both cotton and nearby weed species are desirable. The UA laboratory has the responsibility to obtain and assemble coat protein gene and viral common region sequences for all cotton infecting geminiviruses into the data base.

Objective 3

Biolistic Inoculation of Cotton with Geminivirus DNA Extracts and Full Length Infectious Clones of Cotton-Infecting Geminiviruses: A Working Collection

We previously developed a simple biolistic method for inoculating seedling cotton with DNA extracts from virus-infected material, and have shown this possible for the virus isolates from Arizona, California, Texas, and Guatemala (see Table 1). This method will be used to inoculate cotton seedlings with infectious clones when available from our laboratory and other laboratories working on this project. While perfecting this method to permit inoculation of cotton seedlings with full length infectious virus clones, we have found greater success when viral genomic clone inserts are excised from the cloning vector prior to inoculation. We have been successful with this method in demonstrating infectivity of infectious clones or DNA extracts containing viral DNA. This will be the preferred method for preliminary evaluation of infectivity of cloned virus genomes, and can also be applied to screening of elite germplasm in late stages of resistance efforts (it is too expensive and time-consuming to use in breeding programs where hundreds to thousands of plants must be screened). Select virus clones could feasibly be engineered into an *Agrobacterium* binary vector to facilitate inoculation of large numbers of plants.

Objective 4

PCR of Target Regions for Phylogenetic Comparisons PCR Amplification, Cloning, and Sequencing of the Core Region of the Coat Protein Gene of Cotton-Infecting Geminiviruses

All samples except those collected in 1996 have been cloned and the DNA sequences obtained for the core coat protein gene. Phylogenetic trees reconstructed from these sequence data indicate that viruses are likely endemic in the locations where they have been collected, and no evidence of introductions of exotic strains or viruses have been detected. Also, it is clear that cotton leaf crumple virus from Arizona is of New World origin (recent), whereas, the Pakistan cotton leaf curl virus is of Eastern Hemisphere origin. All DNA core coat protein sequences obtained thus far, have or will soon be placed into the data base. Ultimately, a file will be compiled for each well characterized isolate that will include the core coat protein gene sequence, geographic information, host range data, photos of symptoms in cotton and relevant hosts, and any other useful information that will facilitate virus identification and disease management in the short term. By November 1997, the GEMINI-DETECTive data base will be accessible on the World Wide Web via the IPM Network, hosted by the North Carolina State University National Science Foundation Center for Integrated Pest Management, under the direction of Dr. Ron Stinner,

Table 1. PCR Positive Whitefly-transmitted Geminivirus Isolates Under Further Study in the Arizona Laboratory

Isolate Designation	Symptom in Cotton	Geographic Source	Source Plant	Year
Sudan okra (cot leaf curl)	minor vein thickening	Sudan	okra	1995
txcot94*	mosaic, stunting	TX, USA	cotton	1994
clcvaz1	leaf crumple	AZ, USA	cotton	1982
cottex92*	foliar stunting	TX, USA	cotton	1992
hibis93*	N/A	AZ, USA	hibiscus	1993
txsida93	N/A	TX, USA	Malvastrum	1993
cot1guat*94	leaf crumple	Guatemala	cotton	1994
cotguat94ver*	mosaic	Guatemala	cotton	1994
cot2guat94*	leaf crumple	Guatemala	cotton	1994
clcvaz2	leaf crumple	AZ, USA	cotton	1993
cotguat92ym*	yellow mosaic	Guatemala	cotton	1992
cotdr*	mosaic	Dom Rep	cotton	1992
mx94okra*	yellow mosaic	Mexico	okra	1995
guatabut94	N/A	Guatemala	Abutilon (weed)	1994
abmvroth	N/A	West Indies	Abutilon (ornamental)	1800s
hibis94*	N/A	AZ, USA	hibiscus	1994
cotguatlc94*	leaf curl	Guatemala	cotton	1994
cotegypt95	leaf curl	Egypt	cotton	1995
cottex96	mosaic	TX, USA	cotton	1996
kenaftex96	leaf curl	TX, USA	kenaf	1996
cotmex96	leaf crumple	Sonora, Mexico	cotton	1996
cotmex96	yellow mottle	Sonora, Mexico	cotton	1996
cotmex96	leaf crumple	Sonora, Mexico	cotton	1996
cotindia96	leaf curl	Punjab, India	cotton	1996
cotpak1	leaf curl	Faisalabad, Pakistan	cotton	1996
cotpak2	leaf curl/mild	Faisalabad, Pakistan	cotton	1996
cotaz	leaf crumple	AZ, USA	cotton	1996
sidatex	mosaic	TX, USA	*Sida glabra*	1996
cotsud1	small vein	Sudan	cotton	1996
cotsud2	big vein	Sudan	cotton	1996
cotcabMx	CLCV	Mexico	cotton	1997
cotRMVPR	Cotton veinal yellows	Puerto Rico	*R. minima*	1997

* Denotes new disease report.

Center Director. As of August, 1997, the first phase of the data base has been accomplished, and the site contains relevant disease information and viral DNA sequences for ten geminivirus isolates that infect cotton and other Malvaceous plants.

Efforts are also underway to clone and sequence PCR products of the large viral intergenic region (LIR), viral regulatory sequences that are nearly identical on interacting A and B viral chromosomes, or are found in a characteristic location on the chromosome of viruses with a single chromosome. PCR can be used to amplify from single or multiple chromosomes by taking advantage of certain sequences flanking the LIR and are conserved or nearly identical in all subgroup III viruses. Obtaining two LIR sequences that are nearly identical that are associated with A and B viral chromosomes from the same plant indicates the virus has two genomic components (bipartite), as do viruses in the Eastern and Western Hemisphere, such as cotton leaf crumple (Brown et al., unpublished). Thus far, several cotton infecting viruses having a single chromosome (monopartite) have been found in the Eastern Hemisphere, and cotton leaf curl appears to be a likely candidate for a single chromosome virus. Inoculating plants with clones that have the same or compatible LIR sequence results in development of disease symptoms, permitting virologists to demonstrate that the virus clones are not only infectious when present at the same time (both are needed to achieve infection of bipartite viruses), but also establishes that the pathogen has been isolated in entirety, and can now be accurately named and subsequently characterized. These compatible clones can then be reliably used to artificially inoculate plants without the whitefly to mediate transmission. Clearly, this approach permits studies of virus host range, symptom phenotype, and the capacity to experimentally inoculate germplasm for resistance screening, or challenge inoculation of transgenic plants engineered for resistance.

This sequence is also an important indication of the genetic nature of a geminivirus to be targeted for transgenic plant mediated virus-derived resistance. Virus-derived resistance approaches to disease control rely upon the expression of an inactive or mutated, cloned viral gene in a transgenic plant, the gene having been obtained from the target virus, and hence

conferring protection of the territory by being present first. This approach can be thought of as a type of immunization of plants whereby, a mutated form of the protein is engineered and used to 'transform' the plants genome to include the virus gene. When a transgenic plant makes the mutant viral 'gene product' or protein, the presence of the protein infers with the function of that particular virus gene when the virus in inoculated to the plant by the vector whitefly. The result is a plant protected from virus infection. Because single and double chromosome-containing geminiviruses have distinct sets of genes with different functions, it is essential to know what type of chromosome organization applies. Hence, it is possible to target a viral gene shared in common between single and double chromosome viruses, or one that is found only in one type of virus organization. The approach described here to examine the coat gene and LIR sequences of geminiviruses, without having to clone and obtain the entire genome first, will permit us to rapidly determine if the pathogen in question has a single or double chromosome, further permitting diagnostic molecular differentiation among uncharacterized geminiviruses.

Marker Sequence-based Predictions of Geminivirus Relationships: An Essential Tool for Resistance Objectives

The DNA sequences of the geminivirus coat protein gene and LIR are useful to establish geminivirus identification. These sequences when compared among multiple viruses can provide important clues about the evolutionary relationships of whitefly-transmitted geminiviruses. Evolutionary histories of viruses can be viewed as 'trees' using aligned virus DNA sequences. Such trees can be used to predict virus relationships based upon sequence similarities and differences, and provide information about the geographic origin of the virus and about its biology. Trees generated with geminivirus coat protein or LIR sequences place most closely related viruses in the same cluster on a branch, while those that are not closely related are placed on a different branch with their closest sequence relative. These two regions of the virus gnome have been shown useful in making predictions about virus identification without cloning and sequencing the entire virus chromosome. Either of these two sequences yield trees that show relationships of viruses by separating viruses (sequences) into clusters or most closely related groupings. The large cluster of whitefly transmitted viruses are separated from two large clusters of related viruses transmitted by leafhopper vectors. Once placed in the whitefly subgroup, viruses are clearly separated by geography of origin (Eastern or Western Hemisphere), and at times by a further sub-geographic separation (Brown, 1996 (abstr); Brown and Wyatt, 1995 (abstr); 1996 (abstr); Padidam et al., 1995). Thus, the introduction of a geminivirus from one geographic world region can be readily detected using this approach. In addition, germplasm that is protected against an Eastern Hemisphere virus could be tested for its ability to protect against infection by other closely geographically related viruses, as well as the more distantly related relatives from another region. Clearly, the broad theoretical and practical utilities of this predictive tool should not be underestimated.

In the large intergenic region are found regulatory sequences that are postulated as important in predicting the likelihood of cross-replication or pseudo-recombination between compatible, and putatively, genetically similar or, the most closely related viruses. Proposed for use with these predictions are families of iterated sequences found in the intergenic region (sequences, directionality, and the specific number of repeated iterons) (Arguello-Astorga et al., 1994), and these viral sequences thought to be involved in binding of rep protein/host factor complexes during viral replication. We are currently obtaining these marker sequences to facilitate predictive inferences of virus genomic subclusters, i.e. clusters containing evolutionarily related viruses of cotton. These data will assist in determining if viral genomic groupings inferred by DNA sequences of viral marker gnome regions can be corroborated with responses of genetically diverse cotton germplasm when infected with our library of prototype isolates. Representative viruses will be selected from those listed in Table 1. We continue to obtain key viral DNA sequences for all isolates with which to infer relationships and further catalog identity and distribution of the viruses.

Objective 5

Cloning and Sequencing of Cotton Leaf Crumple Geminivirus and other New World Geminiviruses

The most prevalent virus in the US is cotton leaf crumple, but several new viruses including several from Texas and Sonora, Mexico appear to be of new importance. These viruses will be cloned and sequences of the entire genomes obtained beginning with the highest priority virus, CLCV. Likewise, the Pakistan laboratory has done extensive work toward cloning cotton geminivirus isolates from their locale. Molecular clones of the most prevalent cotton-infecting geminiviruses will be obtained as components of the working virus collection. As stated, infectious virus clones are required for disease resistance efforts, and to carry out additional investigations of virus-vector biology and the molecular epidemiology of priority viruses.

To this end, we have cloned and nearly completely sequenced the cotton leaf crumple virus from Arizona. In 1997, we will rectify the several areas of sequence discrepancy in the cloned virus. Because symptoms incited by the cloned CLCV are milder than expected, we will re-clone the virus to obtain cloned A and B components of a more typically virulent virus. We have also obtained partial clones of two geminivirus isolates (Brown, 1992; 1994) from the south coast of Guatemala, and will continue to investigate the molecular characteristics of these two isolates. Presently, one isolate appears to be closely related to cotton leaf crumple, while the other appears to be a distinct virus. A Texas-USA isolate from cotton will be the third priority isolate for the New World viruses, while isolates from India and Africa are Eastern Hemisphere priorities. To date, it has

been impossible to obtain quality extracts for such work with Indian or African (Sudan, Mali, South Africa) isolates, however, efforts are underway to circumvent this problem.

Related Scientific Cooperation and Travel for the Project

Pakistan

To obtain important collections virus isolates associated with the recently reported leaf curl outbreak in the Indian and Pakistan Punjab regions, the US team traveled to Pakistan and India in November 1996. In Pakistan, we visited with the National Institute of Biotechnology and Genetic Engineering (NIBGE), met with research scientists and the director of the institute, Dr. Kauser Malik. During our visit, we also obtained virus samples, reviewed research progress in the US and Pakistan laboratories, and exchanged information on the status of cotton leaf curl in Southern Asia. This travel was supported primarily by the Common Fund for Commodities-Cotton and in part by funds from USDA/OICD/FAS for scientific exchange.

India

Important contacts were made at Punjab Agricultural University, Ludhiana, Punjab, India with Dr. A.S Khehra, Vice Chancellor, and Dr. K.S. Aulakh, Director of Research, and with Dr. L.S. Randhawa, Principal Cotton Breeder. Dr. Randhawa is interested in collaboration on cotton virus/student exchange/supply of DNA extracts/possible joint publications. Dr. Gupta, Dept. of Biotechnology is interested in identification of geminiviruses of vegetable crops and possible alternate weed hosts. He is interested in detection techniques and co-operative efforts on virus characterisation. Dr. Dhanju, Vegetable Virologist studying under Dr. Gupta is excellent at field identification of disease symptoms, and he is undertaking variety trials assaying for virus resistance, based upon symptom development, only. Our hosts generously provided transportation, housing, and essential assistance in making collections of research materials (cotton, okra, and malvaceous weeds near cotton fields though to harbor geminiviruses).

CLCuV-infected cotton was first noticed in 1994 close to the Indo-Pakistan border. In 1995, 1000 ha were infected in the Punjab, which increased to 5000 ha in 1996 with a concomitant infection of 10,000 ha in Rajasthan (state SW of Punjab). Symptoms include upward and downward leaf curling, vein thickening and enations. Although the farmers are not yet worried, the cotton breeders are becoming concerned. PAU cotton breeder, Dr. Randhawa, has identified two dominant CLCuV resistance genes in *G. hirsutum* and will release cotton variety LHH144 (reputed to be resistant), containing at least one resistance gene, next year. In the future, PAU, as the main provider of commercial seed (free of charge) to the Punjabi farmers, will only release resistant varieties. As an interim measure, PAU have recommended the growing of *G. arboreum* (known to be resistant to CLCuV) instead of *G. hirsutum* in a 5 km wide area along the Indo-Pak border in order to establish a CLCuV-free buffer zone. If, in the future, CLCuV develops into a major threat to Indian cotton production, farmers have plans to change over to sugar cane, or rice in areas where the water table is rising. These farmers are therefore less reliant upon cotton for economic viability than their Punjabi counterparts in Pakistan.

Workers in PAU's cotton breeding department have identified approx. 10-20 weed species as CLCuV virus and/or whitefly hosts (including *Sida* spp. and *Abutilon* spp.) following vector transmissions from weeds to cotton and back to the weeds. Details are not clear, however, with no confirmation of whitefly biotype nor accurate virus identification. Local citrus orchards provide favourable for establishing whitefly populations, therefore PAU tests all cotton breeding lines in these conditions. Also, transmission tests are undertaken in the greenhouse with the use of clip cages on young cotton leaves.

The main breeding sites are at Ludhiana (main campus) and Faridkot (sub-station), where the initial crosses are made, along with varietal and hybrid trials (F_1 and F_2 generations). Progeny is then sent out to testing centres at Bathinda (not visited), Muktsar and Abohar. At Muktsar, segregating populations are grown, and hybrid seed production undertaken. Alternate blocks of *G. hirsutum* and *G. arboreum* are planted in order to reduce the potential for cross-pollination between hybrids of the same species (NB. *G. hirsutum* and *G. arboreum* are sexually-incompatible). Pollination is carried out by honey bees. At Abohar, testing of intra-*hirsutum* hybrids and production of varietal seed is done, along with agronomic trials. All promising varieties/hybrids are tested at each of these sites prior to release in an assessment of their characteristics under different climatic conditions. Once a variety/hybrid is deemed suitable for release, the seed is sent to selected larger farmers for use in large-scale demonstration plots. Smaller farmers are invited to see the improved material, and hence the seed is distributed from PAU throughout the state.

The summer monsoon (kharif) season is also a time during which farmers grow other vegetable crops, including okra, tomato, pepper (*Capsicum* spp.) and eggplant (brinjal). Severe geminivirus infections are reported to have occurred in okra, tomato and pepper in the preceding years. By the time of our visit, most tomato and okra had been harvested but plots of pepper breeding lines were severely affected by a geminivirus-like disease. The symptoms included leaf curl, yellowing and shortening of internodes. We also observed geminivirus-like symptoms in eggplant which had not been previously noted. Whiteflies were also evident in eggplant plots. Other crop species with suspected geminivirus infections were also sampled. Weed hosts were also plentiful, and several were collected from Ludhiana and Abohar. The most commonly infected weed was *Ageratum* with a yellow vein symptom. Whiteflies were abundant on stands of this plant. At other times, congress grass (a

dicot) is reputed to be frequently infected with a leaf- and stem-distorting symptom. There is a report that the B biotype is present in Pakistan and possibly across the border into the Indian Punjab as well.

Brazil

J. K. Brown traveled to Brazil in late November and visited a cotton growing region near Campinas to establish contacts through which to acquire virus samples from infected cotton in the spring when the diseases are anticipated. During my (JKB) visit to Brazil in November 1996, I traveled to the cotton growing area to see newly planted cotton, visit with the retired virologist, Dr. A. S. Costa to learn more about the whitefly-transmitted geminiviruses in cotton in Brazil and surrounding locales, and made arrangements to receive relevant materials when available from assistants in the research station in Campinas. According to Dr. A.S. Costa (Emeritus) in the Virology Department, at least three distinct geminiviruses infect cotton in the region, annually. One is found toward the end of the season in conjunction with the foliar reddening disease, currently of unknown etiology, but postulated to be a luteovirus. This trip was funded in part by local concerns, and partially by the USDA OICD/FAS in support of international cooperative exchange of scientists and information, and toward the geminivirus database project.

Nicaragua

A Ph.D. graduate student from the laboratory, Rafael Caballero, visited his homeland in 1997 and traveled to the cotton growing area to collect geminiviruses samples for the project. The UA laboratory has a USDA PPQ Federal Quarantine permit to transport plant material into the laboratory from Nicaragua for extraction of viral DNA.

Sudan

A Ph.D. student in the laboratory, A. M. Idris, spent six months in Sudan during 1996 on a Rockefeller Foundation Fellowship. During this time, he obtained DNA extracts of several cotton infecting geminivirus isolates, and these isolates are currently under study in the AZ laboratory.

Benefits of Travel to The Project

Many rewards were gained from personal visits by project scientists to discuss and gain new insights into geminivirus disease problems around the world, and these will clearly benefit the Common Fund project. During past and most recent field visits to Pakistan, India, Brazil, Mexico, Puerto Rico, The Dominican Republic (1990-92), and within the US (Arizona, California, Texas), our laboratory made contact with several key colleagues that aided us in obtaining virus infected materials toward the stated goals. For example, several faculty members at the University of Sao Paulo, at NIBGE in Faisalabad, and in the Punjab Agricultural University indicated they would assist us in future collections, and that we could depend upon them for laboratory facilities, and for extraction of cotton and virus-infected weed samples. In turn, we have provided these laboratories with protocols for DNA isolation from cotton and weed samples and will maintain contact during the next several years to stay abreast of current and new problems and assist them in their work, as we are able. These efforts will help all involved to remain in productive and cooperative contact, thereby facilitating early communications of timely disease phenomena in the field.

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Improvement of the Marketability of Cotton Produced in Zones Affected by Stickiness

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Introduction

The factors contributing to the stickiness of cotton are many but the most important one in the Sudan is honeydew. Honeydew consists of sugary deposits secreted by two insect pests: whitefly [(*Bemisia tabaci*, (Genn)] and aphids [*Aphis gossypii,* (Glover)]. In the case of both insects, the honeydew consists of two isomers of fructose, α and β glucose and α -mannose. In addition to these monosacchrides, it also contains sucrose and an oligosacchride with mobility on TLC similar to that of melizitose (Ali and Khalifa, 1980). Earlier, Gameel (1969) reported the presence of two other unidentified components to which he designated the symbols X and Y. These last two unidentified components were absent from the aphid honeydew. Analyses have shown that the major part of these honeydew secretions was identical with that of the whitefly. This would suggest that whitefly is more important than aphid in causing stickiness in the Sudan (Ali and Khalifa, 1980).

Both insects feed on the underside of the leaf. They have sucking mouthparts by which they draw the plant cell sap and do much harm to the plant. Honeydew is their excreta which drops on the leaves and open bolls thus contaminating the lint. The sugary deposits are later dispersed by the ginning process, specially saw ginning.

Mealy bugs *Ferrisia virgata*, *Nipaecoccus vastator* and *Phaenacoccus* which are quoted by some authors to be involved in stickiness are not known in the Sudan to do the same.

Other factors of minor contribution are physiological sugars (originating in the plant), immature fibers, broken seeds, trash and other foreign matter.

During the last two decades stickiness has become an important problem facing all processes of the cotton fabric assembly line from ginning to weaving. All who are involved in the various processes of the cotton industry are, in one way or another, concerned with this problem to some degree.

Like elsewhere, rather intensive researchhas been conducted in the Sudan with regard to the stickiness problem and the causal insects (Gammel, 1964, 1968; Idris, Gameel and Mudathir, 1968 and Khalifa, 1980). Many recommendations were issued addressing control of the pests and resistant varieties were developed against them.

Also, producers were advised to stratify their picking and keep different picks separate. Stickiness was measured for each lot (300 bales) using physical (minicard) and chemical standard methods. The sample size for the lot (No. of bales sampled) was increased, and the lot size (No. of bales per lot) was decreased from 300 to 100 to add credibility to the measurement taken and to the certificate issued for each lot (fiber measurements and stickiness degree).

The table below shows very clearly the trend of improvement in the level of stickiness, especially for the badly reputed Sudan Acala resulting from concern and efforts in control. However, even for this category and during the last five years the percentage of the total crop having stickiness degree between free and light (0 - 1) was never less than 42% and went in some years up to 91%. For Barakat, on the other hand, the range for the five-year period was 84-99%.

All the measures taken and facts published did not succeed in putting sales and prices of Sudan cotton back to normal. The country's product continued for years to be stamped with stickiness whether it was really contaminated or not, and priced less than its equivalents produced elsewhere.

The same kind of difficulties, we believe, has been experienced by other countries for similar periods. This happens in spite of the fact that the identification of producer countries with such a stigma is very difficult because the nature and intensity of contamination changes from place to place and from one season to another.

In situations like this one, cotton farmers in developing countries face the double difficulty of general reduction in income from cotton, because of its eroding share in world fiber market and further reductions in price for suspected contamination. In this case, cotton farmers in these countries will have to make a

choice among three alternatives:

- Increase lint yield per acre and/or
- Solve the contamination problem
- Give up cotton growing altogether

As far as the first alternative is concerned, agricultural research and other national efforts succeeded in formulating research technical packages securing substantial increases in lint yield per unit area if the farmer would be able to abide by them. But the difficulty in the Sudan and possibly other developing countries is that the farmer is financially unable to pay for the whole package which could have compensated for part of the decline in his real income from cotton. Also, increases in yield through research, or otherwise, cannot go indefinitely.

Level of Stickiness in Sudanese Cotton

Season	Variety and Region	Stickiness Level Tested with Minicard (% per category)			
		Free	Light	Medium	Heavy
1992/93	Barakat roller ginned	51	40	9	0
1993/94	Gezira Region	71	25	4	0
1994/95		66	28	16	0
1995/96		55	44	16	0
1996/97		73	26	1	0
1992/93	Acala roller ginned	26	66	8	0
1995/96	Gezira Region	29	55	16	0
1996/97		56	41	2	1
1992/93	Acala saw ginned from	23	19	28	19
1993/94	Rahad and Halfa	32	23	30	15
1994/95	Regions	36	49	12	2
1995/96		14	52	34	0
1996/97		52	37	9	2

Concerning alternative number 2, it is clear that a farmer by himself or a developing country alone can not provide the necessary inputs or infrastructure to deal with cotton contamination. As such, it becomes an international cotton community problem.

This was, fortunately, recognized in due time by the ICAC. It recognized the fact that greater cooperation is needed among cotton producing countries for exchange of technical information in the solution of mutual problems. Within the context of its "wider policy objective of improving the quality of cotton" it found it sound and necessary to address the current issue of stickiness and have an immediate objective of 'Improving the Marketability of Cotton affected by Stickiness' and decided to initiate this project to work towards that end.

Project Objectives

The project is anticipated to serve the purpose of improving the marketability of cotton produced in zones affected by stickiness through the development of methods that will help in

- Separating sticky cotton from non-sticky cotton
  Initiation and monitoring of an evaluation method for stickiness aimed at separating the sticky part from the nonsticky part of the production.
- Dealing with sticky cotton
  This will be concerned with finding ways and means to utilize sticky cotton in spinning.

These are the two main objectives of the project which when the study is completed are expected to lead to very positive results for the producer and the spinner alike. It will save the producer a reduction in returns resulting from unjustified price discounts. On the other hand it will provide information for the spinner useful to make prior machinery and factory adjustments and avoid surprises from sticky cotton.

First Year Workplan

SCC and ARC - Sudan

In Sudan, the work to be done jointly by Sudan Cotton Company (SCC) and Agriculture Research Corporation (ARC) is as follows:

1. Investigate stickiness variability within a bale (roller ginned)

2. Investigate stickiness variability within a bale (saw ginned)

3. Select 60 cotton bales in national production for the industrial spinning tests

4. Investigate the relationship between stickiness within a bale and the pest infestation

5. Determination of number of stickiness measurements per bale (roller ginned)

6. Determination of number of stickiness measurements per bale (saw ginned)

7. Economic evaluation of stickiness thermodetector usage

ARC - Sudan

Dissemination of information through the African cotton network

CIRAD - France

1. Standardize the methodology to use the stickiness detector

2. Determination of the methods and analysis of data with SCC and ARC to assess the variability within a bale of cotton (roller ginned)

3. Determination of the methods and analysis of data with SCC and ARC to assess the variability within a bale of cotton (saw ginned)

4. Data analysis with SCC and ARC to determine the number of stickiness measurements per bale (roller ginned)

5. Data analysis with SCC and ARC to determine the number of stickiness measurements per bale (saw ginned)

6. Studies to assess the extent of variability from one bale to the other

7. Determination of the methods to take samples and measurements for the output 1-5 (technology package)

SCC, ARC and CIRAD

Provide ICAC with all the technical papers for annual workshop and Mediterranean and Middle East networks

Second Year Workplan (in brief)

SCC and ARC - Sudan

1. Strategy to monitor and evaluate the stickiness for the whole production

2. Use of stickiness detector on a representative part of the production

3. Investigate the relationship between stickiness within a bale and the pest infestation

4. Data analysis with CIRAD

ARC- Sudan

Dissemination of information through the African network

CIRAD - France

1. Identification and enumeration of yarn neps from yarn spun by Textile Institute of France (ITF)

2. Effect of mixing sticky and non-sticky cottons from different regions on stickiness potential as measured on the stickiness detector

3. Studies to assess the extent of variability from one bale to the other

ITF - France

1. Effect of stickiness on conventional spinning process

2. Effect of stickiness on rotor spinning process

3. Data analysis with CIRAD

SCC, ARC, ITF and CIRAD

Provide ICAC with all the technical papers for annual workshop and Mediterranean and Middle East networks

Third Year Workplan (in brief)

SCC and ARC - Sudan

1. Use of stickiness detector on a representative part of total production

2. Investigate the relation between stickiness within a bale and the pest infestation

3. Data analysis with CIRAD

SCC - Sudan

Organization of the marketing system in relation to stickiness potential of the cotton bales

ARC - Sudan

1. Organize a regional workshop in Wad Medani or Khartoum with the objective of demonstrating the usefulness of the project findings and find ways and means to apply results for large scale utilization

2. Dissemination of information through the African cotton network

3. Writing of the handbook for general use in various countries

CIRAD - France

1.Effect of humidity on cotton mini spinning

2. Studies to assess the extent of variability from one bale to the other

3. Utilization of findings

SCC, ARC, ITF and CIRAD

1. Provide ICAC with all the technical papers for annual workshop and Mediterranean and Middle East networks

Methodology and Equipment

The “SCT Thermodetector” of CIRAD will be used for measuring stickiness levels. This piece of equipment which was developed by the CIRAD technology laboratory provides a thermomechanical method by which stickiness is detected and measured. It applies heat and pressure to the cotton sample contained between two plates of aluminum foil. Heat will release water out of the cotton which will be absorbed by the dry droplets of the honeydew. The honeydew becomes sticky. Applying pressure will fix the honeydew to the plates. The sticky spots can then be counted.

Preparing the Cotton Sample

The cotton sample weighing 2.5 grams is processed at standard conditions of 65 % RH and 21°C. The 2.5 g cotton sample is opened on a manual opener and spread to form a web with a density of 3 g/m. The web is contained between the two aluminum plates which are then placed on the lower plate of the thermodetector. Heat with pressure is then applied for 12 seconds followed by pressure alone for 2 minutes at ambient temperature. The whole setup of aluminum with contents is left for one hour before the sticky points on the lower and upper sheets are counted.

Counting the Sticky Points

The cotton web is removed from the lower plate. The plate is then cleaned using a special non-woven pad impregnated with mineral oil and the sticky points on both sheets are then counted. The test is repeated three times for every sample.

Work in 1996/97

Field Work

Sampling the Test Material

The test material was collected and prepared in the following manner:

Sixteen (80 to 100 gram) samples of cotton were randomly drawn from each of ten randomly selected bales out of cotton coming from each of fifty blocks from the 118 blocks of the Gezira whose cotton is roller ginned. The same was repeated for saw ginned cotton from another 50 blocks. (In Sudan's agricultural production corporations, the smallest administrative unit is called a "block". Each block has an average area of 4,000 ha divided into "numbers," each with an area of about 36 ha. Numbers are divided into tenancies and each farmer grows about 2 ha of cotton).

The sixteen samples resemble the sixteen layers of the bale and are drawn at the lint slide, between condenser and water sprayers. The total number of samples is 16,000 (8,000 roller ginned and 8,000 saw ginned). Each sample was packed separately, wrapped in paper and labeled with sample number and bale number. According to the Gezira and other cotton producing schemes numbering systems, in the Sudan, the bale number codes all the information regarding origin, farmer, season and cultivar. Every 160 samples belonging to one block were put together in one big sack. All the material was transported to the ARC fiber and stickiness testing laboratory in Wad Medani.

Identification of 60 Bales for Spinning

Based on the stickiness measurements on the SCT, we should identify 60 bales of varying grades of stickiness for two main growths (Extrafine count Barakat and Medium count Acala) and two ginning methods for Acala (roller and saw) taking 20 bales for each category as follows:

Level of Stickiness	Number of Bales
free	2
light	8
medium	8
heavy	2

For the first year, the differentiation of these bales was based on whitefly and aphid infestation levels of the respective blocks and minicard assessment of stickiness degree. This was dictated by uncontrollable factors; the six thermodetectors required for the job did not arrive in June 1997 as was scheduled in the original plan and it was not possible to keep the 1,000 bales indefinitely.

A sample was drawn, however, from each of these sixty bales and sent to CIRAD, France, for similar measurements. The bales are kept separate and will be shipped to France for spinning tests by ITF (Textile Institut of France) in order to study the effect of stickiness on both, conventional and rotor spinning processes. This is part of the second main objective of the project which concerns defining ways and means of dealing with sticky cotton in spinning. Cottons with varying levels of stickiness will be mixed with varying amounts of nonsticky cotton, under different machine and factory condition adjustments to establish thresholds for spinning sticky cotton.

Laboratory Work

Measurement of the stickiness degree of the samples collected waited for the arrival of the thermodetectors which were delayed for some necessary financial rearrangements but have been received now.

Pest Survey and Agronomic Data

Whitefly and aphid counts (together with other pests) are taken in periodical surveys in all agricultural production corporations at short and regular intervals along the season. This will provide insect population data which will be used in correlation studies with stickiness level.

Agronomic data is also available in the field books of every block, a detail information on all aspects of the variety, sowing date, irrigation, fertilization, and chemical pest control.

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