

PN. ACA-270
93378

PROEXAG II



COMPONENTE AGRICOLA DEL PROYECTO DE APOYO TECNOLOGICO PARA LAS
INDUSTRIAS DE EXPORTACION DE CENTROAMERICA Y PANAMA

WHITEFLY / GEMINI VIRUS Mini-course in the Detection of Whitefly Biotypes by Gel Electrophoresis

A

Assignment Number: ST-166B

PREPARED BY:

Donald R. Frohlich, Ph.D.

THROUGH

Chemonics International Consulting Division
2000 M Street, Northwest
Suite 200
Washington, D.C. 20036

UNDER THE AUSPICES OF

The Non-Traditional Agricultural Exportation Support Project
(Project No. 596-0108-3-60011)
United States Agency for International Development (USAID)
Guatemala City, Guatemala

June 12 - 23, 1993

WORKSHOP FINAL REPORT AND SUMMARY:

Donald R. Frohlich, Ph.D.

12 June 1993 - 23 June 1993

Detection of Whitefly Biotypes by Polyacrylamide Gel Electrophoresis
(PAGE)

The first workshop, covering detection of whitefly biotypes by PAGE, was conducted at Escuela Agricola PanAmericana (EAP), Zamorano, Honduras, from 14-16 June. Prior to the workshop, electrophoresis equipment and enough reagents and supplies to stock an electrophoresis center for approximately one year were purchased by Proexag II, in the United States, and transported to the course site by myself (see list enclosed).

The afternoon previous to the first day (13 June) was spent setting up the laboratory and unpacking supplies. On opening the electrophoresis unit I noticed that the manufacturer (Hoeffer Corp., San Francisco) had not included part of the electrophoresis chamber. Mr. Rafael Caballero, however, had made contingency plans in the case that the transported equipment was not allowed to clear customs, so a spare electrophoresis unit was obtained that was used throughout the course. The Hoeffer Corp. was contacted the next day by FAX and responded immediately by phone (Ms. Corona Rivera) and the missing part was shipped out that day by DHL. Because I left before the scheduled delivery date, I do not know if the part was delivered. I have not been contacted by either R. Caballero or C. Rivera if there have been further problems.

Day 1 of the workshop involved a morning of lectures and discussion (0800 - 1130 hrs), and an afternoon and evening of laboratory work (1300 - 2200 hrs). In all, 7 students representing 4 Central American countries participated (see participant list). Topics covered the first day included 1.) general whitefly biology and systematics, 2.) background and current status of biotype problem, 3.) history of geminivirus transmission and global perspectives, 4.) principles of

allozyme analysis, 5.) classification of esterases and usefulness in distinguishing biotypes, and 6.) principles of gel electrophoresis. Participation in lecture/discussions was excellent and R. Caballero and K. Ufer translated. All students were given a packet that included recipes and protocols for all laboratory procedures, a list of some 70 references about whitefly/geminivirus biology and pertinent to lectures, a recent paper on whitefly-transmitted geminiviruses and global perspectives by Dr. J.K. Brown, and photocopies of gels showing the A and B biotype esterase banding patterns. Copies of figures and tables used during the lectures were made available to students on request.

The laboratory on Day 1 involved all procedures from preparation of buffers and stock solutions, and preparation of whitefly homogenates to running the first sets of gels and visualizing esterase activity. Again, participation by all students was excellent and the first day, though long, was successful.

Day 2 began with preparation of gels and fresh whitefly homogenates (0800 hrs - 1730 hrs). By the time the laboratory portion of the workshop was completed in late afternoon, three separate sets of gels had been run and all students had participated in all aspects of gel pouring and polymerization, sample preparation and application, gel staining and esterase detection.

The evening portion of Day 2 involved a 2 hour discussion regarding actual implementation of the electrophoretic techniques demonstrated to monitor whitefly populations in Central America that I would like to recap in some detail. Because it had been agreed that EAP would be the central clearing house for monitoring Central American whitefly biotypes by PAGE, I felt that there were issues in 3 key areas that had to be settled in order to make the investment in time and materials productive: 1.) sample type and preservation, 2.) gel standardization, and 3.) sampling strategy.

Regarding samples, some discussion arose over exactly what is to be sent to Zamorano. In the end it was agreed that if at all possible, leaves with live nymphs should be sent. Only in the case where it is really impossible to find nymphs should adults be sent. This is to ensure that the host plant associations that are being determined are accurate. That is, presence of adults on specific plants does not

necessarily mean that insects are utilizing them as hosts or feeding on them. A number of methods are available for preserving the enzyme activity in samples but it was felt that, based on prior experiences, leaves containing live nymphs should be wrapped in a moist paper towel and closed in a plastic bag. Nymphs so handled are able to live for 2-5 days. In the case where adults are to be sent the method of preservation is less clear. R. Caballero feels that based on experiences in Colombia adults can be ground in the actual homogenization buffer and shipped cold. We (Arizona) have also been able to recover enzyme activity from adults that have been dried over a desiccant such as calcium chloride. R. Caballero has agreed to conduct an experiment to resolve which method is better. Workers will also have to be very careful that only samples that are Bemisia tabaci are sent. Shipment to Zamorano could potentially be on DHL or TACA but probably the best way will be to send them with a colleague or other person going to visit Zamorano.

With respect to sample and PAGE standardization, it has been left up to the people in Zamorano to decide how many insects will be run per sample. However, gel conditions should be as follows: 7.5% separating gels (pH 8.8) with gels run at a constant 30 amperes until the tracking dye reaches the bottom of the gel. Although the gels run by the class produced clear and readable results I suggested that Rafael try some separating gels topped with a 4% stacking gel (pH 6.8). This will likely improve resolution if he finds that he is uncovering a myriad of complicated biotypes. .

I am also concerned that gels are interpreted in a standardized fashion relative to each other. To that end, we have agreed that all gels shall be run with at least 2 standards against which field populations can be compared. This would ideally include a standard made from the Zamorano population in culture and one made from the Arizona 'A' biotype. I have given Rafael several tubes of Arizona 'A' and have also agreed to run samples that he sends us in order to determine if we are all making the same diagnosis at the outset.

Much of the discussion also involved sampling strategy. It seems that a project of this sort could become very diffuse very quickly if some direction is not agreed upon. To that end, I asked the students to

identify the most important plants and areas to sampled. It was agreed that at the outset agronomic plants should be concentrated on with weed hosts being secondary. Specifically, workers in each country will particularly concentrate on tomato, beans, and melon. Sampling will take place in each country over as wide a geographic area as possible but with major consideration toward the most economically important areas with respect to the above 3 crops.

At the end of the evening discussion a class picture was taken and I will provide copies when they are developed.

Day 3 consisted of only Rafael and myself setting up the new electrophoresis apparatus in so far as possible without the missing part, going over the ins and out of programming the power unit, looking at the Zamorano whitefly colonies and, touring the biocontrol facility.

In summary, I was quite impressed with the facility at Zamorano and the dedication and independence of the course participants. One of my bigger concerns had been the actual implementation and smooth operation of sample processing at Zamorano. Rafael Caballero has a position at EAP that makes very heavy demands on his time. Additionally he is planning to pursue a Ph.D. in the United States which will take him away from the facility for large blocks of time. Apparently, however, the person actually doing the sample processing will be Ms. Elena Maria Perdomo. Elena was probably the best student in the class and demonstrated a significant amount of skill and familiarity with the lab. Everything is in place for the crew at EAP to operate and conduct an efficient and effective regional whitefly biotype diagnosis program for the next year. After that time some mechanism for getting additional disposable supplies to Zamorano will have to be ironed out.

For several reasons I have also asked each participant to send me some samples dried over desiccant so that I can compare DNAs. During the afternoon of Day 2, Rafael had the opportunity to show slides of esterase gels obtained from several localities in Central and South America that he had not included in his Master's thesis. Although not all of the gels had been run under exactly standardized conditions they were still clear enough to indicate to me that the whitefly variability in Latin America is probably considerably more complex than is currently understood. My own research at the University of Arizona involves

developing and using specific DNA markers to genetically characterize whitefly populations. Esterase banding patterns are excellent 'markers' by which to monitor the spread of the B biotype. However, they really can tell us nothing about the variability of the genotypes within each biotype. This may on the surface seem a purely academic question. However, as the B biotype has spread throughout northern Mexico and the United States, populations in several areas have exhibited considerable insecticide resistance. One of the things that we would like to know is if those populations are genetically homogeneous or variable. We would also like to know if those insecticide resistant B populations are genetically the same as the B populations reported from Latin America. Approaches to chemical control will vary depending on the future outcome of such studies. Consequently each student was provided with several desiccant collecting tubes and an aspirator. I asked them to send me duplicates of samples that are sent to EAP so we can begin to assess genetic variability and K. Ufer stated that there were funds to send the samples to the U.S. Although I intend to keep Chemonics apprised of any data that result from said samples I think that a systematic survey of genotypes in the future might be the best way approach. Initially this might involve Guatemala and Nicaragua, as the first and last geographic regions to be invaded. I hope that samples sent in by the students will begin to shed some light on the diversity of the populations.

Detection of Whitefly-Transmitted Geminiviruses by DNA-DNA Hybridization Assays

The second workshop was conducted largely by Drs. Judy Brown and Ramon Lastra. My input was generally to take care of purchasing and supplies prior to the workshop and assist with sample and solution preparation during the course of the laboratory. Additionally, on the last day of the workshop I gave one lecture entitled "Bemisia tabaci: More than one species or more than one biotype?" Specifically, the lecture examined 4 areas of evidence that have led some workers to conclude that the A and B biotypes of B. tabaci are really two species and not biotypes. The areas examined included: 1.) behavioral studies,

2.) mating and crossing studies, 3.) isozyme and allozyme analyses and the measurement of biochemical polymorphisms, and 4.) evidence from randomly amplified polymorphic DNAs (RAPDs). We then had a discussion concerning interpretation (especially statistical) of molecular and biochemical data and a number of the students shared their observations of host plant and behavioral differences between the different biotypes encountered in the region.

I also took time during the second workshop to meet on two occasions with Dr. Luko Hilje and discuss implementation and coordination of biotype monitoring in the region. He is in general supportive of the effort to be made at Zamorano and will do what he can to facilitate matters in Costa Rica. We also discussed areas that may be developing insecticide resistance and his involvement in a joint CRSP to be proposed between CATIE, Arizona, and two other institutions. I also discussed obtaining samples from several areas in the region with a couple of participants and will consequently be sending collecting equipment and vials to Julio Bourbon (Dominican Republic) and Hilda Blanco (CATIE). Hilda Blanco also obtained some local whitefly samples for us and I curated samples from Ramon Lastra's colonies.

Post-Workshop Activities

After the workshop at CATIE was completed we met with the IPM group to discuss their participation in the afore-mentioned CRSP proposal. The response was favorable and they are supposed to be organizing their efforts in anticipation of a call for proposals. Some discussion (and warning) took place regarding the fact that the granting agency is more interested in development of a model with social and economic components than in the design of an empirical scientific study.

The same day we also took a local collecting trip to an area called Tucurrique where we found numerous whiteflies (including nymphs) and virus symptoms, on both tomato and squash and surrounding weeds despite the fact that it was rainy season and populations were supposed to be low. We spent some time talking with a local grower (for local consumption) about insect problems in general and I was surprised to see

that he, and supposedly his neighbors, are making up to 3 applications weekly of compounds such as lannate and thiodan. Apparently the applications have done little to control insects including whitefly, and as a former pesticide consultant I am surprised that growers of such meager means can afford such a steady supply of insecticides. We collected numerous whitefly samples for DNA and enzyme analysis, and I will keep both CATIE and Chemonics informed of results.

Our last day in Costa Rica involved a meeting at US/AID headquarters in San Jose concerning the CRSP proposal. Basically the message was: "That's nice ... let us know when you're here. Please don't ask us to do anything. Let's do lunch sometime."

In summary, my overall impression of both institutions was good. Physically all of the tools and skills necessary for a good scientific evaluation of the whitefly/geminivirus problem are in place. At this point it will simply take the willpower and dedication of local participants to make them work on a regional basis.



Donald R. Frohlich, Ph.D.
28 June 1993

- Encl: List of Participants - Whitefly Biotypes Work Seminar
- Supply List - EAP Whitefly Esterase/Biotype Detection Facility
- Lecturers, Overheads - Course given in the Zamorano, EAP
- Student Packet - Prepared by D. Frohlich for the "Whitefly Esterase Mini-Course Perspectives - Detection of Whitefly Biotypes by Gel Electrophoresis"

LISTA DE PARTICIPANTES
TALLER SOBRE BIOTIPOS DE MOSCA BLANCA

NOMBRE	INSTITUCION	DIRECCION
Karl Ufer	PROEXAG II	5a. Ave. 15-45 Edificio Centro Empresarial, Torre I Nivel 9, Guatemala Guatemala, C. A. Tel: 33-7082-4 Fax: 33-7081
Daniel Coto	CATIE, Costa Rica	Apartado 7170 Turrialba, Costa Rica Tel: 56-1632 Fax: 56-1522
Ana Leticia Ochoa Gale	Recursos Naturales Sanidad Vegetal	Lab. Fitopatología 1. Ave. 17-20 calle S.E. Las Palmas, San Pedro Sula, Honduras, C. A. Fax: 52-1797
Elena María Perdomo	Escuela Agrícola Panamericana	D.F.V. Apartado Postal 93 Tegucigalpa, D. C. Honduras, C. A. Tel: 76-6140 ó 50 Fax: 76-6240
Dennis Alpizar Monge	Ministerio de Agricultura y Ganadería Departamento de Entomología	Apartado 869 Tibás, Costa Rica Tels: 35-0105 24-2005 24-2113 Fax: 24-2113
Nicolás Valle	Escuela de Sanidad Vegetal Universidad Nacional Agraria	Km. 12, Carret. Norte P.O. Box 453 Managua, Nicaragua Tel: 505-2-31501 Ext. 104 Fax: 505-2-31950
Don Frohlich	Universidad de Arizona, U.S.A.	Plant Sciences Dept./Forbes Bldg. University of Arizona Tucson, AZ 85721 U.S.A. Tel: (602) 621-1230 Fax: (602) 621-8839
Rafael Caballero	Escuela Agrícola Panamericana Entomología DPV	Apartado Postal 93 Tegucigalpa, D.C. Honduras, C. A. Tel: 766140, Fax: 766240

SUPPLY LIST - EAP WHITEFLY
ESTERASE/BIOTYPE DETECTION
FACILITY

EQUIPMENT

Hoefer 600E Dual Electrophoresis
Unit

combs, spacers, glass plates
BioRad Power Unit 165-4710

CHEMICALS AND SUPPLIES*

lauryl sulfate sodium (SDS)
sodium hydroxide pellets
EDTA (disodium dihydrate)
Triton-X-100
Trizma base
electrophoresis grade acrylamide
N,N methylene-bis-acrylamide
sodium phosphate monobasic
sodium phosphate dibasic
N,N,N,N-
tetramethylethylenediamine
ammonium persulfate
alpha naphthyl acetate
beta naphthyl acetate
fast blue RR salt
calcium chloride desiccant
glycine
microfuge tubes
pipette tips

sample buffer components**

boric acid
sucrose
beta mercaptoethanol
bromophenol blue

*all chemicals are available from:
Sigma Chemical Co., PO Box 14508, St.
Louis MO, USA Tel 314-771-5750, FAX
314-771-5757

**enough sample buffer was
prepared for approximately 2000
samples and delivered to EAP. This
was because of the difficulty of
transporting pure reagent grade
mercaptoethanol by passenger jet.
When those supplies are exhausted
raw materials will have to be
ordered.

9