USDA APHIS INTERNATIONAL SERVICES AND USDA AGRICULTURAL RESEARCH SERVICE
QUARTERLY MEETING ON THE SCREWWORM ERADICATION PROGRAM
Knipling-Bushland US Livestock Insect Research Laboratory
Kerrville, Texas
9-10 June 2009

Personnel attending:

USDA APHIS IS

Lou Vanechanos, Associate Deputy Administrator, Riverdale, MD
Shannon Hamm (absent due to injury that occurred during TDY), Associate Deputy Administrator, Riverdale, MD
Dale Maki, Agricultural Science Officer/Screwworm Program Director, Pacora, Panama
Cheryle Cannady, Associate Deputy Administrator, Washington, DC
Frank Vollmerhausen, Acting Screwworm Program Director (Chiapa de Corzo)

USDA ARS

Daniel Strickman, National Program Leader Veterinary, Medical, and Urban Entomology
Adalberto Peréz de León, Director, KBUSLIRL
Steve Skoda, Research Leader, Screwworm Research Unit, Pacora, Panama
John Welch, Field Entomologist, KBUSLIRL
Felix Guerrero, Research Physiologist, KBUSLIRL
Roger Leopold, Research Entomologist, Insect Genetics and Biochemistry Research Unit, Fargo, ND
Muhammad Chaudhury, Research Entomologist, Screwworm Research Unit, Pacora, Panama
Pamela Phillips, Remote Sensing Specialist, Screwworm Research Unit, Kerrville, TX
Ju Wang, Post-Doc to Felix Guerrero, KBUSLIRL
Anna Rachinsky, Post-Doc to Felix Guerrero, KBUSLIRL
Mario Vasquez Mendiota, Technician, Screwworm Research Unit, Pacora, Panama
Kathy Li, Summer Intern, KBUSLIRL

Purpose: To review ARS research that has been funded by APHIS for development of a male-only strain of screwworm fly and for adaptation of cryopreservation for preservation of large numbers of screwworm embryos. The meeting also provided an opportunity to discuss progress in the Screwworm Eradication Program as a whole and to explore the possibility of other collaborations.

Action Items:

1. Biological Control Center of Excellence: Produce concise description of Center of Excellence concept (Dan Strickman and Shannon Hamm).
    a. Develop list of potential participating groups.
    b. Start off with focus on

screwworm
fruit fly
ticks
mosquitoes

c. Visit Navy Center of Excellence in Jacksonville and ARS Mosquito and Fly Research Unit in Gainesville.

d. Set up meeting with COPEG management to discuss concept.

e. Set up meeting with MIDA to discuss concept.

2. Risk assessment (Shannon Hamm, Lou Vanechanos, Steve skoda)

a. Develop group email address.

b. Invite ARS into teleconferences with CEAH/invite CEAH to participate in quarterly meetings.

c. Inventory list of historical data available

3. Blood as component of diet (Frank Vollmerhausen, Muhammad Chaudhury)

a. Distribute findings from lab tests of varying percentages of fractionated blood/plasma.

b. Oversee and monitor floor testing in Methods.

c. Verify results.

d. Communicate results energetically to ARS/APHIS.

e. Coordination of microarray analysis of nutritionally stressed larvae.

4. Male only strain (Frank Vollmerhausen, Felix Guerrero)

a. Request extension of Anna Rachinsky's appointment.

b. Review resource requirements.

c. Report on progress and barriers to progress.

5. Regulatory considerations for release of transgenics (Shannon Hamm, Ed Gersabeck)

a. Set date for premeeting with Dave Bergsten.

b. Report out to ARS and APHIS.

c. Set up meeting with MIDA (in conjunction with next quarterly meeting in Panama).

6. Eradication Program in Cuba (Dan Strickman, Lou Vanechanos)

a. Evaluate the need for funding for samples from Guantanamo Bay (APHIS funds)

b. Schedule collection and transport of samples to Panama or Tuxtla.

c. Develop strain development plan.

d. Consider addressing this situation (i.e., cooperation with Cuba) at future Commissioners' Meetings.

7. Communication (Cheryle Cannady, Ed Gersabeck, Dan Strickman)

a. Set up web site.

b. Post quarterly presentations to web site.

c. Set up group emails (one with CEAH, one without CEAH)

d. Set up cross departmental and cross agency meetings in Pacora.

e. Publish and follow up on test findings.

f. Enact more formal and frequent communication between ARS and COPEG in Pacora.

g. Explore possibility of using ARS labs as rotational posts for APHIS.

8. Enhanced use of GIS/remote sensing (Pamela Phillips, Frank Vollmerhausen)
   a. Develop model for most efficient aerial releases based on risk.
   b. Develop most economical placement of field inspectors based on risk.

Next meeting: Proposed for October, 2009 in Panama; to include major purpose of discussing environmental analyses with the Panamanian government and to have discussions with the new staff of the screwworm plant

Summary of the meeting's content:

Development of Male-Only Strain: Following a summary of the activities and history of the KBUSLIRL by Dr. Peréz de León, Drs. Guerrero, Rachinsky, and Wang described progress on the development of a male-only strain of the screwworm fly. The program completed its second funded year on 20 March 2009. The major change in the project has been a switch from the horn fly (*Haematobia irritans*) as the model to the secondary screwworm fly (*Cochliomyia macellaria*). The problem with the horn fly had been that it is very difficult to inject its embryo with the DNA plasmids used to transform the fly (95% of the injected embryos die). The work accomplished to date on the horn fly is valuable in itself, particularly the successful indexing of an extensive Expressed Sequence Tag (EST) library. The secondary screwworm fly is much easier to inject (50% of the injected embryos survive), and some individuals (2 of 604 injected) have already (permission from the Institutional Biosafety Committee was received 13 Feb 09) been transformed successfully to express green fluorescent protein. Because survival of the secondary screwworm following injection of the embryo is good, it has been necessary to rear the individuals in groups. Dr. Wang has been working on the DNA cassette that will transform screwworm flies to male-only strains. All parts of the cassette (transposons, promoter, and binary parts of lethal protein gene) have been established in plasmids except the transformer. The transformer is only activated by females and allows the binary lethal protein gene to assemble. The next steps are to confirm transformation of the secondary screwworm flies, develop transgenic lines of the secondary screwworm flies, train the Panamanian ARS staff to inject embryos, inject the secondary screwworm fly with parts of the DNA cassette that will result in a male-only strain, and assemble the complete DNA cassette using the transformer gene based on the Mediterranean and olive fruit flies.

Year 2 (ended 20 Mar 09) Objectives and Completion:

Demonstrate horn fly transformation: Completed (in secondary screwworm fly).
Synthesis of horn fly genomic libraries: Completed.
Assemble components of the conditional lethal gene system: Slowed by delay in hiring Ju Wang (August 2008). Only one step left to accomplish, which should be completed in the next 3-9 months.

Year 3 (ends 20 Mar 2010) Objectives and Completion:

Transform model fly (secondary screwworm fly) with conditional lethal system
Evaluate fitness of transformed model fly: Currently in process

Train Panamanian to perform transformations: Mario Vasquez currently being trained in Kerrville for two weeks; a second trip has been budgeted for later in the year; a four-week trip for Anna Rachinsky to train personnel in Panama is budgeted

Initiate cloning New World screwworm fly DNA system components: In progress

Questions and issues:

Strickman: Have we under resourced the transformation process? We heard that this is a matter of transforming many isofemale lines in order to find those strains that combine the necessary desirable qualities, not just the conditional lethal condition. The original green fluorescent protein transformed flies required 12,000 embryo injections to get three successful strains.

Welch: I believe that it became obvious for the need of additional personnel to help with the rearing of both the model secondary screwworm and the primary screwworm in Kerrville and Pacora, respectively. From the discussions, I do not believe this was budgeted for. This is an issue. I think we need to figure this out directly as it will take time to hire and train the technicians in both places.

Gersabeck: 1) Warns that successful use of the male-only strain will not cut production costs in half and we should be careful not to raise expectations too high on the efficiencies this development will bring. 2) It may not be too early to explore the agreements that will be necessary to use Trinidad and Tobago as the trial area for the transgenic screwworm fly. 3) Is tetracycline the antibiotic of choice to deactivate the lethal protein?

Skoda: When working with the New World screwworm fly, it will be necessary to use isofemale lines resulting from embryo transformation.

Screwworm Risk Assessment Project

APHIS IS (Shannon Hamm is POC) has partnered with APHIS CEAH to perform a risk assessment of screwworm for the United States, especially in light of the probable opening of trade with Cuba. CEAH is performing pathway analysis and formulated a data request. They hope to complete the assessment by December 2009.

Questions and issues:

This project will be added to the agenda for quarterly meetings between APHIS and ARS, with participation by CEAH personnel.

Cryopreservation

Roger Leopold reported on the progress of adapting cryopreservation of screwworm flies to mass preservation in order to preserve particular strains and to serve as a backup colony at production plants. The work has included development of new substrates for the embryos and a cryoprotectant that causes less cracking of the embryos. They are also looking at methods for handling greater numbers of eggs at one time, as well as automation. Once the liquid nitrogen machines are in place in Chiapa de Corzo and Pacora, then it will be possible to quit rearing Panama 95 and maintain that strain under cryopreservation. Muhammad Chaudhury reported that

he was able to rear just three cryopreserved individuals to adults, an accomplishment that was thought to be impossible because of the need for screwworm larvae to develop in masses of many individuals. Roger is going to be working with the Mediterranean fruit fly group in Guatemala in August and with the Malaria Branch, CDC Atlanta.

Questions and issues:

Cochliomyia macellaria as a model for the male only strain will require development of cryopreservation techniques for this species.

Biological Control Center of Excellence

Shannon Hamm, Frank Vollmerhausen, and Dan Strickman had discussed the concept of a new initiative to make the Pacora, Panama, plant and laboratory a center for biological control efforts. The center would pull in resources from other areas, keep research grounded in operations, keep operations grounded in science, and potentially attract other funding. John Welch suggested that rather than biological control, the theme of such an effort might better be expressed as area-wide pest management. The initial effort would be budget neutral in an attempt to use current resources in a more integrated and less stove-piped manner.

Questions and issues:

Welch: Rather than limiting it to only SIT, perhaps it should be broadened to IPM and area-wide pest management. It may have more interested parties that way. Insecticides and cultural controls are also used in both the Screwworm and Medfly programs.

Following the discussion on this idea, Dan Strickman, John Welch, Shannon Hamm, and Frank Vollmerhausen will write a short, cohesive description of the concept for further consideration.

Regulatory Treatment of Transgenic Screwworm Flies

The APHIS document entitled "Use of Genetically Engineered Fruit Fly and Pink Bollworm in APHIS Plant Pest Control Programs – Final Environmental Impact Statement—October 2008" (Agency Contact: David A. Bergsten) was rated "Lack of Objections" by the US EPA. This document covers many of the same techniques proposed for the male-only strain of the New World screwworm. David Bergsten is drafting a similar document for the screwworm, with input from APHIS IS and ARS.

Shannon Hamm will be holding a meeting or two on the environmental documentation for the GE screwworm prior to the October meeting to be held in Panama. The goal is to go to MIDA at that time to go over the timeline and proposed plan for documentation.

Screwworm Eradication Program in Cuba

Steve Skoda reviewed the distribution of the screwworm fly in Cuba and preliminary efforts to plan an eradication program. The problem is significant on the island with 17,000 cases recorded per year as of 2006. It is logical to assume that when trade restrictions with Cuba are lifted, there will be a considerable threat of importation of screwworm flies into the U.S. unless the species is eradicated from the island. Discussions within APHIS and following production of a thematic plan in 1998 and a regional meeting in 2000 have produced a detailed plan for an eradication program. This plan would start with the Isla de Juventud and then proceed west to east in Cuba in four zones. APHIS and ARS have already made an analysis of the spatial distribution of risk throughout the island based on distribution of hosts and vegetation (data from remote sensing and Cuban records of animal distribution). It has been estimated that eradication would cost $85-90 million and require no more than four years. ARS research could contribute in the following areas: 1) Strain development; 2) Sterile male release strategy based on habitat and host distribution; 3) Determination of initial populations of flies; 4) Placement of monitoring traps; and 5) Other research as required by the operation. ARS might require two addition scientists to accomplish this work.

Questions and issues:

An immediate possibility would be collection of screwworm fly samples from the U.S. Navy base in Guantanamo. These samples could be used for genetic analysis to help determine whether current strains would be useful in Cuba. John Welch has been to Guantanamo to advise the Navy on protection of horses there. Following the meeting in Kerrville, Dan Strickman coordinated with the U.S. Navy and Marine Corps Public Health Center to get the help of the Navy Entomology Center of Excellence (NECE, Jacksonville NAS, FL) for collection of the specimens. CDR George Schoeler, Officer in Charge of NECE, is working out details of the collections with Steve Skoda. The travel costs of Navy personnel from NECE will be paid by APHIS, as currently arranged.

Dale Maki suggested that the first operational goal should be eradication from the Isla de Juventud, a process that might cost about $500,000. He also asked that ARS put something formal about the goal of examining Cuban screwworm eradication into the ARS NP104 plan.

Ed Gersabeck pointed out that only a GS-14 or lower rank can go to Cuba for discussion at this time.

Dan Strickman and Lou Vanechanos agreed to work up a package to present to Department of State representatives as a way of introducing the idea that screwworm eradication in Cuba would be feasible and desirable. The first step is coordinating with both APHIS and ARS components, including on the ARS side the Deputy Administrator for Animal Production and Protection and the Office of International Research Programs.

USDA ARS Remote Sensing Program

Pamela Phillips described the effort to develop and apply geospatial analysis and remote sensing to the screwworm eradication program. She has been able to assign priorities of risk throughout the current release zone in eastern Panama, based on host distribution and vegetation. She will do

ground-truthing work in Panama in July, 2009. This work could be used to economize on the number of flies released by releasing more flies where there is greater risk and fewer flies where there is less. It might also influence the placement of field inspection personnel. Eventually, integration of the modeling effort with actual releases (recorded with AgNav software) could form an important archival record for the program.

Questions and issues:

Pamela Phillips should contact the USDA ARS Area-Wide Pest Management Research Unit, College Station, TX, that specializes in the engineering aspects of aerial applications.

Blood Supply for Screwworm Larval Medium

Muhammad Chaudhury gave a presentation on the sources and nutritional contributions of blood in the larval diet. Currently, spray dried blood is 6% of the larval diet, but the sole source of this material, the California Spray-Dry Company, has quit producing the product in favor of making a greater profit by marketing dry plasma and cell components separately. Frank Vollmerhausen reported that APHIS has made a contract with the APC Company that ties the price of the spray-dried whole blood to the price of dried plasma, protecting the company from an unexpected decrease in profits. Muhammad Chaudhury discussed the advantages and disadvantages of eggs, milk, and soy protein, concluding that blood remains an essential component for unknown reasons. He is currently doing tests with dried plasma and cells separately, including products from both California Spray Dry Company and APC Company. He reported that in the laboratory, blood cells alone are sufficient and that whole blood products are not necessarily needed. Longer term, he is working on identifying larval phagostimulants, which may help improve the flexibility of diets.

Questions and issues:

APHIS had been under the impression that only whole blood would be sufficient. The possibility that blood cells alone would be adequate changes their plan on future diets. Reducing the costs of diet components is a priority.

The ARS lab results must be completed and then tested on a larger scale with the help of the Methods Development Section at the plant. This might be accomplished in Chiapa de Corzo, taking advantage of unused capacity there.

Welch: It probably should be tested in both Mexico and Panama as the cellulose and gel based diets have enough differences that results may show that it could possibly be useful with one and not the other diet.

Roger Leopold suggested simultaneous testing of flies using Felix Guerrero's microarrays. In this way, the specific genes (or marker sequences) associated with survival and growth could be studied.

Other Topics

Frank Vollmerhausen:

Research on optimal placement of field inspectors would be useful.
Suggests more formal means of communication within the Pacora facility, perhaps regularly scheduled meetings that include cross section of ARS and APHIS departments.
The Screwworm Task Force needs have been met, except for an evaluation of the benefits of the Whorley towers for harvesting flies. The question is whether the Whorley towers increase longevity and therefore reduce the number of flies required. Steve Skoda stated that studies to date have not shown that the Whorley towers increase longevity significantly.

Ed Gersabeck:

Offers QuickPlace software as a convenient medium for sharing information within and outside government.
Suggested ARS labs as hosts for APHIS personnel who rotate from overseas posts and who would benefit from exposure to the research environment. Felix Guerrero's laboratory is open to such collaboration.