DEFINICIÓN DE ESPECIES CRÍPTICAS EN *Anastrepha fraterculus* Y *A. obliqua* (Dip., Tephritidae),
EN COLOMBIA: ALTERNATIVAS DE MEJORAMIENTO DE LA TIE Y LOS SERVICIOS
CUARENTENARIOS

(Resolution of cryptic species of *Anastrepha fraterculus* and *A. obliqua* (Dip., Tephritidae) in
Colombia: Opportunities for improve the SIT and quarantine services.)

PROGRESS REPORT 1

Part of CRP: “Resolution of cryptic species complexes of Tephritid pests to overcome constraints
to SIT applications and international trade."

Research Contract No. 16069

Contracting Institute: UNIVERSIDAD DEL TOLIMA

Chief Scientific Investigator: NELSON A. CANAL

Species of the genus *Anastrepha* are the most important fruit flies in Latin America. Seven species
had been quarantined for international market, including *A. fraterculus* and *A. obliqua*. Sterile
Insect Technique had been improved for *A. ludens* and has been performed for species like *A.
fraterculus* and *A. obliqua*. The South American Fruit Fly (*A. fraterculus*) must be a complex of
cryptic species with so different economic and quarantine importance. In order to improve the SIT
for *Anastrepha* species and to remove some quarantine barriers, the taxonomic status of the
complex has to be defined.
Both *A. fraterculus* and *A. obliqua* are the most important Colombian fruit flies and there are not
studies carried out in order to define variations among Colombian population of these species
and/or with other Latin-American populations.

Research carried out
1. Ubiquity of Places: We were looking for the places with high populations of *A. fraterculus* and *A. obliqua*. Data from formal publications or informal papers were obtained, data from institutes like CORPOICA, ICA, CENICAFE, museums and technicians were obtained too. According to the obtained data, to date we have selected six populations of *A. fraterculus* and seven of *A. obliqua* (Table 1), however, other populations will be selected after.

Table 1. Collection data of selected Colombian populations of *A. fraterculus* and *A. obliqua*.

<table>
<thead>
<tr>
<th>SPECIES OF FRUIT FLY</th>
<th>HOST SPECIES</th>
<th>STATE</th>
<th>CITY</th>
<th>COUNTY</th>
<th>PROPERTY</th>
<th>OWNER</th>
<th>LOCATION N</th>
<th>W</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. obliqua</em></td>
<td>Mango</td>
<td>Cundinamarca</td>
<td>Anicaima</td>
<td>El Imbon</td>
<td>Arturo Oropeza</td>
<td>4º 43’ 23.6”</td>
<td>74º 25’ 4.3”</td>
<td>972</td>
<td></td>
</tr>
<tr>
<td><em>A. obliqua</em></td>
<td>Chincha amambro</td>
<td>Quindío</td>
<td>Calabaza</td>
<td>La Tebaida</td>
<td>Carretera</td>
<td>5º 54’ 22.8”</td>
<td>75º 41’ 35.8”</td>
<td>1409</td>
<td></td>
</tr>
<tr>
<td><em>A. obliqua</em></td>
<td>Chincha roja</td>
<td>Tolima</td>
<td>Guamo</td>
<td>Lombia Luisa</td>
<td></td>
<td>4º 18’ 55.0”</td>
<td>74º 54’ 16.6”</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td><em>A. obliqua</em></td>
<td>S. mombos</td>
<td>Tolima</td>
<td>Cuello</td>
<td></td>
<td></td>
<td>4º 11’ 33.695”</td>
<td>74º 57’ 42.200”</td>
<td>387</td>
<td></td>
</tr>
<tr>
<td><em>A. obliqua</em></td>
<td>Mango</td>
<td>Tolima</td>
<td>Espinal</td>
<td></td>
<td>Corpoica Natalia</td>
<td>5º 32’ 27.5”</td>
<td>75º 9’ 29.6”</td>
<td>2052</td>
<td></td>
</tr>
<tr>
<td><em>A. fraterculus</em></td>
<td>Finoas</td>
<td>Cundinamarca</td>
<td>Anicaima</td>
<td></td>
<td>Tocoroma</td>
<td>Olay</td>
<td>Rafael Romero</td>
<td>4º 45’ 1.8”</td>
<td>74º 22’ 1.3”</td>
</tr>
<tr>
<td><em>A. fraterculus</em></td>
<td>Cafe</td>
<td>Tolima</td>
<td>Ibagué</td>
<td></td>
<td></td>
<td>El placer</td>
<td>Jesus Antonio</td>
<td>4º 24’ 53.5”</td>
<td>75º 18’ 50.6”</td>
</tr>
<tr>
<td><em>A. fraterculus</em></td>
<td>Cafe</td>
<td>Valle del Cauca</td>
<td>Aridenillo</td>
<td>La Agudo</td>
<td></td>
<td></td>
<td>Norbert Añez Leiva</td>
<td>4º 23’ 05.8”</td>
<td>75º 13’ 20.30”</td>
</tr>
</tbody>
</table>

2. Sampling: Fruits had been collected according to the season; however samplings of populations referred in the table had been obtained. Fruit were collected in the field, put in vermiculite and taken to the laboratory of entomology in the University of Tolima (Ibagué) and processed following standard protocols for obtaining the adults.

3. Rearing: Artificial rearing processes are necessary in order to obtain adequately immature stages or for sexual compatibility testes. Emerged adults are confined in cages (30x30x30) and reared following standard protocols from our laboratory. Our protocols include artificial food, oviposition place and larval diet, however, fresh fruit could be offered when artificial subtract is not accepted.

4. Karyotypes: Some larvae obtained from laboratory rearing have been collected in order to study karyotypes. Karyotypes will be studied in ganglia and imaginal discs prepared according to standard protocols. 20 larvae will be studied for each population.

5. Adult morphometric studies: Adult female structures have been mounted in order to perform morphometric studies. 20 female are obtained after be fed five days. Ovipositor,
oviscape (rasper included) and right wing are mounted in permanent slides. Structures will be photographed and measured according Hernández-Ortiz et al. (2004).

6. Molecular studies: Five males and five females were obtained from the colonies culture and fixed in 95% methanol. The protocol standardization was our first objective. Primers were designed according to published data and they were tested. Degenerated oligonucleotids for amplify mitochondrial gen COII or ITS1 were designed; DNA was obtained according to salting-out methodology from legs and thorax and the DNA concentration obtained by Dot-Spot.

Results

1. Four populations of A. fraterculus and five of A. obliqua collected.
2. Protocols for obtain karyotypes were performed in our laboratory. Ganglia and imaginal discs from three populations of each species were prepared and stored for future studies.
3. Adults females of three populations of each species were mounted and stored for future morphometric studies. Females from other population and males from all populations were obtained and will be mounted.
4. Larvae from two populations of A. fraterculus and three of A. obliqua were obtained and stored for future studies.
5. Testes about artificial rearing had been done for all populations, however just one population of South American Fruit Fly could be rearing on our artificial methodology and other one could be rearing on fresh fruits. Two populations of A. obliqua could be rearing in artificial diet and one in fresh fruit. Rearing methodology will be performed on future time for other populations.
6. Testes performed for obtain DNA show amplified products of ~650pb for COII and of ~600pb for ITS1 in all samples. DNA sequencing of these products will be performed.