

IPM CRSP Trip Report

Country Visited: Dominican Republic

Dates of Travel: 16-19 May 2011

Travelers Names and Affiliations: Dr. Sue A. Tolin, Virginia Tech
Dr. C. Michael Deom, University of Georgia

Purpose of Trip: To meet with Teresa Martinez and others at IDIAF to review virus work.

The purpose of the trip was two-fold: (1) To observe virus-like symptoms in greenhouse grown pepper and tomato crops and identify causal viruses. (2) To make a follow-up visit to the virology laboratory at CENTA that Tolin and Deom had previously visited and advised on construction, equipping, and operation, and assess capabilities for virus diagnosis.

Sites Visited:

16 May - IDIAF headquarters in Santo Domingo (Deom only – Tolin was delayed in flight)

17 May - IDIAF station in Constanza, including greenhouses producing tomato and pepper.

18 May - IDIAF CENTA research station in Santo Domingo – virology laboratory.

19 May – Attempted to visit IDIAF Director and USAID Mission, but were unable to schedule.
Worked on reports until noon departure.

Description of Activities/Observations:

The Constanza area is a high-elevation valley with a highly developed, diversified agriculture for production of high-value horticultural crops including onion, garlic, as well as potato and tomato. Fields along the roadside were in various stages of establishment (tomato) and harvest (potato, onion). This valley appeared to be much more prosperous than the Ocoa region. Because of the elevation and cooler temperatures, this area has minimal pressure from whiteflies and the viruses they transmit, in contrast to the Ocoa Valley area that we had visited on previous trips and is the main focus of the IPM CRSP work.

Recently, large greenhouses have been constructed in Constanza. The apparent advantage of this system is a longer harvest time and greater profitability for a single establishment cost. Although the greenhouses have restricted entry and utilize foot baths to rinse shoes before entering, they are not meant to provide high confinement. Martinez arranged to meet the IDIAF extension agent whom she has worked with addressing virus diseases in the greenhouses. We were taken to a commercial greenhouse producing **tomato** and an IDIAF-owned greenhouse growing **pepper** for a commercial producer, likely through rental of space. A field visit had been scheduled to a grower's greenhouse in Jarabocoa north of Constanza, but heavy rains began while we were in the tomato (first) greenhouse that closed the Jarabocoa road and precluded further travel.

Tomato plants were over 2 m in height and producing large round tomato for slicing. Both red and green tomato fruits were being harvested. There were a few plants of a Roma-type with smaller fruits. Several plants showing unusual symptom types had been noticed by the grower and the IDIAF personnel and were brought to our attention.

Type 1 – extreme growth abnormalities including thickened, curled primary stems, curled and distorted leaves, swollen nodes, and small non-ripening fruit or aborted fruit. New shoots were chlorotic. This was not similar to any virus disease described, and may be a genetic abnormality. However, symptoms were definitely virus-like. Plants with these symptoms were isolated with no nearby plants having the syndrome, making it unlikely that it was chemically induced. (Fig. 1)



Type 2 – Necrotic flecking on upper leaves, suggested by Martinez to be caused by TSWV that she had seen in other plants testing positive by immunostrips to this virus. Fruit symptoms also occurred, mainly on Roma-type, but were unlike chlorotic circles usually caused by this virus. A few thrips were observed on plants in this area of the greenhouse. (Fig. 2)



Pepper plants in the second greenhouse were producing harvestable bell peppers. Some foliar fungal lesions were observed on upper leaves of plants in rows growing along the outer greenhouse plastic wall, but did not appear to be damaging production. Plants with symptoms of *Pepper mild mottle virus*, a seed and mechanically-transmitted Tobamovirus, were observed at a low incidence (Fig. 3). This seed-borne virus had caused major disease losses in other areas of Dominican Republic in previous years, until resistant cultivars were introduced.



In the opposite end of the house, several plants exhibited tip die-back and necrosis. Light green and dark green mottling was observed on green fruit. Fruit that was beginning to turn red showed dramatic circular red and orange color breaking. (Fig. 4).



Tomato and pepper samples were collected from the Constanza greenhouses and tested the next day using immunostrips from Agdia. Both tomato and pepper leaf and fruit tissue were positive for *Tomato spotted wilt virus* (TSWV). The method of testing fruit by inserting the immunostrip directly into a tomato was demonstrated by Tolin, with results (Fig.5A) comparable to grinding necrotic leaves (Fig. 5B). Use of this method, first suggested by R. Naidu, will be more cost effective for their testing program. Pepper had a higher titer of virus but had to be ground (Fig. 5C).



A tomato fruit collected by Martinez in an experimental plot in the Ocoa Valley showing a gray-wall type of symptom tested positive for *Tobacco mosaic virus* (TMV) (Fig. 9). Previous immunostrip testing of tomato plants from this location were TMV positive, and also detected mixed infections with *Cucumber mosaic virus* (CMV).



The virology laboratory at CENTA was found to be fully functional and capable of performing several diagnostic tests for viruses and bacteria. They have funding for a project to monitor for the presence of the citrus greening liberobacter pathogen by PCR, which they are doing successfully. Results are entered by hand in a logbook. Tolin suggested that data would be better searchable for summarizing their work if a database program was used. She set up spreadsheets for the virus and bacteria diagnosis and instructed them in data entry.

The laboratory has some limitations on space, observable in Fig. 8 where bench space to perform immunoassays has to be shared with balances. The window in the figure has a southwest exposure so afternoon sun is quite bright. Some changes in configuration were suggested.



The facility lacks space to grow plants for biological culture of viruses, which is important for planned research to select pepper resistance to virus. Martinez showed us a location – a concrete slab extension at the building – on which an enclosure is planned to grown plants in a protected environment. An adjacent building that has been refurbished to contain plant tissue culture and elemental analysis is well equipped, as is the primary plant protection laboratory.

The information presented by the CENTA group at the recent LAC planning meeting was reviewed with Tolin and Deom. One of the main activities was monitoring pepper plots in Ocoa for aphid-transmitted viruses, aphid species present, and weed species as possible reservoir hosts for virus. Such information is needed to design IPM packages for virus disease management.

Training Activities Conducted

Program type	Date	Audience	Number of Participants		Training Provider	Training Objective
			Men	Women		
Tips for testing for virus by immunostrips	5/18/2011	CENTA lab personnel	0	3	Virginia Tech, Univ of Georgia	Diagnostic methods enhancement
Using Excel for digital records of diagnostic tests	5/18/2011	CENTA lab personnel	0	3	Virginia Tech	Conversion of hand-written log books to digital data

Suggestions, Recommendations, and/or Follow-up Items:

The capacity for virus detection and diagnosis using immunological methods has increased remarkably since our first visit to CENTA. Some additional chemicals are needed before they can utilize the more economical tissue blot immunoassay. Ms Martinez is well-trained and enthusiastic, has capable assistants, and is recognized by growers and IDIAF agents as an authority on viral diseases. We met the new director of CENTA, Genaro A. Reynosa, who also has an active biotechnology project to engineer banana resistant to the Black Sigatosa disease. He seemed to be quite supportive of the program of Ms Martinez and of the association with the IPM CRSP, both the IPVDN global theme and the LAC regional project. Linkage between the activities appears to be good. Plans were discussed to send Mrs. Xiomara Cayetano from this laboratory to train with Margarita Palmeiri in Guatemala. During the LAC planning meeting, J. Brown arranged to assist the CENTA lab in diagnosis of geminiviruses.

List of Contacts Made:

Name	Title/Organization	Contact Info (address, phone, email)
Genaro A. Reynosa	Director of CENTA, IDIAF	greynosa@idiaf.org.do
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