

## IPM Innovation Lab Trip Report

**Country Visited:** Vietnam

**Dates of Travel:** February 28 – March 11, 2017.

**Travelers Names and Affiliations:** Naidu Rayapati, Washington State University.

**Purpose of Trip:** This trip was undertaken to review current status of the Witches' broom syndrome in longan (*Dimocarpus longan*) and organize plans to resolve outstanding issues about this problem and implement strategies for management of the Witches' broom syndrome. This collaborative activity is aligned with the "Component 3: Research and development of new, bio-rational IPM technologies" in the project "Strengthening production and export of Vietnamese fruit crops through innovative and market-oriented IPM."

**Sites Visited:** Southern Horticultural Research Institute (SOFRI), Tien Giang.

### **Description of Activities/Observations:**

February 28-March 2: Naidu Rayapati traveled from Prosser (USA) to Ho Chi Minh City (HCMC) and checked into the hotel on early hrs of March 2<sup>nd</sup>.

March 2: In the morning, Naidu Rayapati met with Dr. R. Muni Muniappan (IPM-IL Director) and traveled from HCMC to My Tho City. After checking into the hotel, they traveled to Tien Giang and held a group meeting in the afternoon with SOFRI scientists (Dr. Nguyen Van Hoa, Mr. Mai Van Tri, Dr. Tran Thi My Hanh, and colleagues) to review different aspects of the Witches' broom syndrome in longan. Dr. Hanh, entomologist, presented research activities conducted by SOFRI team on Witches' broom syndrome. The results from grafting of healthy bud wood to infected rootstock indicated no Witches' broom symptoms on newly developing young shoots of the bud wood. This result suggested that no graft-transmissible agent (phytoplasma and virus) is associated with Witches' broom symptoms. Experiments were conducted to examine the role of mites (*Eriophyes dimocarpi*) in the induction of Witches' broom symptoms. When mites collected from shoots showing Witches' broom symptoms were released onto healthy longan seedlings, newly developing shoots showed typical symptoms of Witches' broom. In contrast, mites collected from longan trees with no apparent Witches' broom symptoms did not induce such symptoms on longan seedlings. These results suggested that (i) mites feeding could subsequently induce the expression of Witches' broom symptoms and (ii) there could be two species of mites, one capable of inducing Witches' broom symptoms and the other unable to induce such symptoms. Naidu has provided a brief account of his work at WSU in identifying causal agent(s) associated with Witches' broom symptoms. Samples from longan plants with Witches' broom were processed for total RNA extraction and the RNA subjected to high-throughput sequencing (RNA-Seq). Mining of the sequencing data using bioinformatics software and comparing with known phytoplasma and viral sequences in public database (GenBank) did not provide any indication for the presence of sequences related to either phytoplasma or virus in longan samples.

After extensive discussions, the group concluded that Witches' broom symptoms could be a consequence of mites feeding. Additional research was proposed to analyze more samples from longan trees collected from different locations to rule out the involvement of phytoplasma and/or virus. In addition, the group felt that mites on longan could represent distinct species or a species complex and suggested using DNA barcoding markers to resolve this issue towards a better understanding of Witches' broom syndrome.

March 3: Muni, Naidu, and SOFRI team (Dr. Hanh, Dr. Lan and Mr. Loc) visited longan farms at Cai Be-Tien Giang province and Long Ho-Vinh Long province to examine Witches' broom symptoms and mites associated with these symptoms, and observe farmer participatory IPM practices implemented to mitigate the syndrome. In the morning, they traveled to Long Ho-Vinh Long province (about 75 km from SOFRI) and met with the farmer (Mr. Duong Khac Hoang) and a group of government officials (Ms. Nguyen Huy Thao, Deputy Head and Mr. Le Tien Dat, Technical Officer of sub-plant protection department of Vinh Long province and Ms. Phan Bui Thi Huu Thuy, Technical Officer of plant protection station of Long Ho district-Vinh Long). In this orchard of 1 hectare, the farmer planted the longan variety Edor introduced from Thailand. This variety is gaining popularity for domestic consumption and export, compared to local varieties (viz. Tieu Da Bo), due to good tasting quality and high pulp and small seed. The trees were of different ages and at various stages of fruit development, ranging from flowering through mature fruit ready for harvest. A visit of the orchard showed very low incidence of Witches' broom symptoms. An observation of growing buds from symptomatic branches under microscope revealed the presence of mites.

The farmer is implementing a combination of IPM tactics recommended by SOFRI through the IPM-IL project, consisting of pruning and incinerating infected shoots, spraying with Sulfur and Pyriproxyfen before fruit set, orchard hygiene and soil enrichment with organic fertilizer for good plant growth. The farmer expressed satisfaction with recommended IPM tactics and found beneficial in reducing Witches' broom symptoms and getting a good crop (about 20 tons per hectare) with high quality fruit. The farmer hosted an excellent lunch for the entire group.

In the afternoon, Muni, Naidu and SOFRI team met with a second farmer (Mr. Nguyen Thanh Hoa) and Mr. Nguyen Van Hai-Head of sub-plant protection department of Cai Be district, and visited the longan farm (0.7 hectare) planted with the local variety Tieu Da Bo. We observed several trees showing severe symptoms of Witches' broom in this local variety. Many trees were totally affected with the Witches' broom and these trees produced no fruit at all. Apparently, the farmer did not invest time and resources to control mites and consequently incurred severe crop loss. In contrast, less symptoms were observed in Edor variety planted in the same farm. In a neighboring farm planted with Tieu Da Bo variety, less infection was observed due to the control of mites. These observations indicated that planting Edor variety and controlling mites can effectively reduce the incidence of Witches' broom in longan.

March 4: The entire day was devoted to review IPM-IL project activities in Vietnam. Scientists and project staff from SOFRI, Plant Protection Department of the Ministry of Agriculture and Rural Development (Mr. Nguyen Hong Chau), Plant Protection Research Institute (Dr. Bui Xuan Vi) and Fruit and Vegetable Research Institute (Mr. Nguyen Duy Hung), Hanoi, participated in this meeting. Vietnamese scientists presented project reports related to longan, mango, dragon

fruit and lychee crops. After the presentations, discussions were held about the work plan and other logistics of the project. Muni collected presentations made by Vietnamese collaborators and will be providing more details about this meeting in his trip report.

March 5: In the morning, Muni and Naidu visited tourist spots in nearby islands (Thai Son island and Vinh Trang pagoda) and spent rest of the day in reviewing project activities and preparing a draft on Witches' broom news article.

March 6: Muni and Naidu visited entomology lab to review research on Witches' broom. Dr. Hanh showed experiments on Witches' broom in the lab and in screenhouses. Plants grafted with bud wood from symptomatic trees did not show any symptoms of Witches' broom. Conversely, plants infested with mites showed Witches' broom symptoms. She also showed two morphological forms of mites – short and long forms. Further research is needed to determine if they are two distinct species of *Eriophyes* or represent a species complex. We also found heavy infestation of scale insects in papaya fruits and Muni collected samples for ID of these scale insects. Afterwards, Muni and Naidu met with SOFRI team to review invoicing, budgeting and other operational aspects of the project. The entire team also discussed about strategies for increasing awareness of Witches' broom, including writing popular articles on Witches' broom for publication in trade magazines, local Ag newspapers, a Fact Sheet and a peer-reviewed article in a scientific journal. After lunch, Muni and Dr. Hoa left to HCMC to meet with USAID staff. Naidu spent rest of the afternoon with Dr. Hanh and Dr. Bui Thi Ngoc Lan (Deputy Head of Biotechnology Department, SOFRI) and discussed strategies and protocols for DNA barcoding markers to resolve whether there are two distinct species of *Eriophyes* or represent a species complex. Afterwards, Dr. Lan showed her lab facilities to conduct proposed activities. Naidu shared with Dr. Lan cost-effective virus diagnostic protocols that are currently used in his lab for sample extraction and PCR assays. Dr. Hanh, Dr. Lan and Naidu are planning to develop PCR assays for DNA barcoding of mites collected from longan trees.

March 7: In the morning, Naidu, accompanied by SOFRI entomologist Mr. Huynh Thanh Loc, visited SOFRI farm (36 hectares) to observe canker disease in dragon fruit (5 hectares) and anthracnose in mango (2 hectares). Symptoms due to canker were observed sporadically in dragon fruit. However, heavy damage due to sunburn was observed in dragon fruit. Not much of anthracnose was observed in mango. In the afternoon, Naidu and Loc visited longan farm in SOFRI campus and collected samples with and without Witches' broom symptoms. These samples were brought to SOFRI and packed for shipment to WSU for RNA-Seq analysis. Afterwards, Naidu visited the tissue culture lab Floriculture and Ornamental Division, where the staff members are propagating a variety of ornamentals for commercial purposes. Ms. Nguyen Van Anh showed the facilities and explained about tissue culture activities. Later, Naidu, Dr. Hanh and Dr. Lan discussed about DNA barcoding strategies for mites and made plans for setting up PCR assays to amplify COI sequences from mites.

March 8: Naidu, along with Dr. Hanh and Mr. Loc visited longan farms in Phu Qui commune-Nhi Qui Town-Tien Giang province (about 20 km from SOFRI) to observe Witches' broom symptoms and collect representative samples for RNA-Seq analysis. Many trees in a longan farm planted with a local variety (Tieu Da Bo) were showing Witches' broom symptoms. Apparently, the farmer is not paying due attention to manage the problem! In the adjacent farm, longan trees

were pruned and nearly 99% of new shoots were free from Witches' broom symptoms. Only a few new shoots were showing Witches' broom symptoms. This sporadic distribution of young shoots with Witches' broom symptoms would suggest localized/restricted infection, unlike systemic infection of all new shoots due to infection with either phytoplasma or viral infections. Naidu collected samples from symptomatic and non-symptomatic shoots, brought to SOFRI and prepared packing for shipping to WSU for RNA-Seq analysis.

March 9: In the morning, Naidu held extensive discussions with Mr. Tri, Dr. Hanh and Dr. Lan about writing a review article on the current status of Witches' broom in Vietnam. Based on the previous work done in different countries and the data available at SOFRI, they outlined contents of the article and shared with SOFRI team. It is anticipated that this review article will be completed and submitted to a peer-reviewed journal by end of 2017. In the afternoon, Naidu presented a seminar "An Overview of Virus Diseases" to SOFRI scientists and technical staff (total attendance = 22), showcasing the significance of virus diseases in vegetables and the work done through IPM IL to manage viral diseases in vegetable crops in subsistence agriculture.

March 10: Naidu held summary discussions about longan Witches' broom with Dr. Hoa and other SOFRI scientists. He then visited with Dr. Tran Thi Oanh Yen, Head of Division of Fruit Breeding and Selection. Her current research includes breeding for canker resistance in dragon fruit using marker-assisted breeding. She is currently making crosses and developing segregating populations, and anticipates field trials and selection of canker-resistant varieties in a few years. Naidu has spent rest of the day in writing the trip report and left SOFRI around 3:00PM to HCMC, and checked-in to a hotel at about 6:00PM.

March 11: Naidu traveled from HCMC to USA.

**Training Activities Conducted:** None

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

Based on our field visits and discussions with SOFRI scientists, it is apparent that Witches' broom is indeed a major constraint to longan in Vietnam. In addition, Witches' broom is emerging as a serious problem in rambutan. During the past few years, SOFRI scientists have generated valuable data on Witches' broom in longan. Based on field visits and reviewing on-going research at SOFRI, it is highly likely that Witches' broom is a consequence of mites feeding damage to growing buds of young shoots. It can be hypothesized that specific 'compound(s) in mite's saliva released at the feeding sites triggers metabolic changes in longan leading to phenotypic expression of Witches' broom symptoms. This is further supported by the absence of phytoplasma and viral sequences from high-throughput sequence analysis of longan samples showing Witches' broom symptoms. Concerted team efforts are, therefore, required to generate research-based knowledge for resolving the enigma of Witches' broom syndrome in longan and implement sustainable management practices.

Summary Recommendations:

- This is my first visit to Vietnam and I'm impressed with SOFRI. It has an excellent team of scientists and facilities to address the intrigues of Witches' broom syndrome. SOFRI team should continue collaborative research to generate science-based knowledge for practical applications to mitigate crop losses in longan due to Witches' broom.
- Research should be continued on RNA-Seq analysis of longan samples collected from different locations and across seasons to rule out any lingering questions about phytoplasmas and viruses as causal agents of Witches' broom in longan.
- The likelihood of more than one species of mites of the genus *Eriophyes* associated with Witches' broom needs further investigation. A combination of studies involving morphological characteristics of mites and DNA Barcoding of mite's genome should be pursued to resolve whether there are two distinct species of *Eriophyes* mites or mites causing Witches' broom represent a species complex.
- SOFRI scientists have generated valuable data on Witches' broom and are encouraged to publish this research in peer-reviewed scientific journals for increased visibility of their excellent productivity. They are encouraged to seek guidance from IPM IL team with regard to English language while preparing manuscripts for publications.
- Field studies should be continued to validate IPM tactics (cultural methods, mites control by chemical and biological means, use of tolerant/resistant varieties, etc.) for mitigating crop losses in farmers' fields due to Witches' broom.
- Demonstrating benefits of IPM tactics at target sites and outreach activities showcasing benefits of IPM strategies will enhance raid uptake of technologies.

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