INTERNATIONAL PLANT VIRUS DISEASE NETWORK (IPVDN):

toward the effective integrated pest management of plant diseases caused by viruses in developing countries

global program

PRINCIPAL INVESTIGATOR: Sue A. Tolin, Virginia Tech

CO-PRINCIPAL INVESTIGATORS: Judith Brown, University of Arizona | C. Michael Deom, University of Georgia | Robert Gilbertson, University of California-Davis | Naidu Rayapati, Washington State University

HOST COUNTRY COLLABORATORS: Central Asia — Nurali Saidov (Tajikistan) | South Asia — G. Karthikeyan, N. Balakrishnan, K. Manoranjitham (TNAU), Nutan Kaushik (TERI) (India); A. Muqit, Z. Karim, M. Muzahid-E-Rahman, M. Afroz, M. Mahfuzur Rahman, T.K. Dey (Bangladesh) | Southeast Asia — Sri Hendrastuti Hidayat, Tri Asmira Damayanti (Bogor Agricultural University) (Indonesia); C. Cheythyrith (Cambodia) | Latin America and the Caribbean — Margarita Palmieri, University del Valle de Guatemala (Guatemala); Mauricio Rivera, J. Melgar, H. Espinoza (FHIA), Alfredo Reuda (Zamorano) (Honduras); Jose Ochoa, R. Delgado, M. Insuasti, V. Barrera (INIAP) (Ecuador) | East Africa — J. Karungi, M. Ochwo-Ssemakula, S. Kyamanywa (Uganda); M. Otipa, Z. Kinyua (Kenya); P. Sseruwagi, D. Mamiro (Tanzania) | West Africa: Moussa Noussourou (IER Ghana), Michael Osei (CRI), Eric Cornelius (University of Ghana) (Ghana)

International Plant Virus Disease Network (IPVDN)

program summary

Plant virus diseases transmitted by insect vectors and through seed or germplasm are some of the major constraints to vegetable production in IPM CRSP countries. Approaches to management of viral diseases designed from information gained by the Plant Virus Disease (PVD) Global Theme project were conveyed through the IPM CRSP-organized symposium, "Research and Management of Insect-transmitted Virus Diseases in Vegetables in the Tropics and Subtropics." held in July 2012 in India (listed in training section).

Many of the reports of virus detection and identification were conducted in the host countries by using commercially available immunological test kits or sample submission for commercial testing. Several collaborators now also have the capacity to perform nucleic acid analysis by polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR), but standard operating procedures (SOPs) are needed. Collection of samples is also done for analysis by U.S. scientists' labs The crops in which viruses were detected and identified were tomato, pepper, eggplant, potato, sweet potato, okra, onion, pumpkin, and other cucurbits, five types of local gourd, country and yardlong bean, tree tomato, passionfruit, and others, including weeds. Viruses were identified belonging to eleven different genera, with whitefly-transmitted begomoviruses and aphid-transmitted potyviruses being the most prevalent. A new tomato disease in Ghana and Mali was found to be caused by a viroid. Weeds in several locations were found infected by the viruses of local crops.

Appraisals of virus ecology and temporal and spatial dynamics of aphid and whitefly vector species and populations were continued. The efficacy of host-free periods to reduce incidence of whitefly transmitted begomoviruses is presented from long-term monitoring in the Dominican Republic and Mali. Effects of IPM packages on the incidence of viruses are reported from experiments in India on tomato and in Guatemala on pepper, potato, and tomato. The importance of selecting or generating seed and other propagules free of virus is demonstrated in yardlong bean, tomato, passionfruit, potato, and sweetpotato. Progress is reported on establishing programs to: develop clean sweet potato planting material; reduce seed transmission of virus in yardlong bean; reduce virus incidence and impact by early roguing symptomatic tomato to reduce spread by thrips; and select for virus resistance in certain crops.

**Each program's focus, either of "important plant virus diseases and their ecology and transmission" (E+T) or "implementation of applied research on specific virus diseases in selected crops" (AR), is noted after each project title beneath the regions.*

SOUTH ASIA

Diagnostic evaluation of virus diseases in vegetable crops and weeds in India (E+T)

Rayapati, Karthikeyan, Manoranjitham

Samples from vegetable crops, namely tomato, eggplant, chili/pepper, snake gourd, ribbed gourd, bitter gourd and pumpkin showing virus-like symptoms were collected from farmers' fields in select regions of Tamil Nadu. These samples were pressed onto FTA cards and brought to the lab. They will be shipped to Rayapati (WSU) for further analysis by cloning and sequencing to identify virus(es) present.

Similarly, different weed samples showing virus-like symptoms in the vegetable ecosystem of Tamil Nadu were also collected and analyzed by PCR / RT PCR and by cloning and sequencing for the documentation of plant viruses. The results of the testing are given in table 1. Begomoviruses (whitefly vector) were detected in the three weed species, and a tospovirus (thrips vector) in eggplant.

Table 1. Documentation of viruses from eggplant and weeds in a vegetable ecosystem.

Sample	Type of symptom(s)	High similarity with
Brinjal (Eggplant)-*Solanum melongena*	Necrotic spots on leaves	*Peanut bud necrosis virus*
Malvastrum coromandelianum (False Mallow)	Yellow mosaic on leaves and vein clearing	*Malvastrum yellow vein virus*
Passiflora foetida (Stinking Passionflower)	Yellow mosaic on leaves	*Cotton leaf curl Bangalore virus*
Clitoria ternatia (Butterfly pea)	Yellow mosaic on leaves	*Rhynchosia yellow mosaic India virus*

Impact of *Peanut bud necrosis virus* (PBNV) infection on the nutritional quality of tomato fruits in India (E+T)

The PBNV infection of tomato produces various types of symptoms including yellow/chlorotic rings or patches on the fruits. Experiments were conducted to determine the effect of virus infection on nutritional quality of tomato fruits. Tomato fruits were harvested from healthy and PBNV infected tomato of four different cultivars (Laxmi, US 618, 3041, and Vaishnavi), and their chemical composition was compared to determine the influence of virus infection on fruit quality.

As shown in table 2, the mean values of key nutritional quality components in fruit from PBNV infected plants were determined relative to healthy fruit. Lycopene, ß- carotene, vitamins A and C, calcium, zinc, total sugars, and caloric value were significantly reduced, and only slight reductions were measured in sodium, potassium, and protein. In contrast, percentage total iron, fat, antioxidants, phenol, and fiber were slightly increased in virus-infected tomatoes.

Table 2: Effect of PBNV infection on the nutritional quality of tomato fruits

Component	% reduction in infected fruit
Lycopene (mg/100g)	41.5
Vitamin A (IU)	30.4
Vitamin C (IU)	54.5
ß- Carotene (mg/100g)	30.3
Total Sugars (%)	19.8
Calcium (mg/100g)	14.8
Zinc (mg/100g)	38.5
Calorific Value (kCal)	8.4

IPM package development: Implementation of IPM components for the management of insect-transmitted virus diseases in tomato in India(AR)

Karthikeyan, Rayapati

Thrips-transmitted *Peanut bud necrosis virus* (PBNV) and whitefly-transmitted *Tomato leaf curl virus* (TLCV) are the two major virus diseases affecting tomato production in Tamil Nadu and neighboring states of South India. In order to develop environmentally benign management approaches, we initiated farmer-participatory IPM trials in farmers' fields for the management of these two virus diseases in tomato. Trials were conducted at four different locations, and the following components were included in evaluating IPM tactics. Each plot (approximately one acre) was divided into two equal parts, with one plot designated "IPM plot" and another, "farmer practice." In the IPM plot, the following components were implemented:

- Application of Neem cake @ 250kg/ha
- Seed treatment with *Pseudomonas fluorescens* @ 10g/kg and *Trichoderma viride* 4g/kg of seeds
- Soil application of *Pseudomonas fluorescens* @ 2.5kg/ha
- Selection of healthy and virus disease free seedlings for planting
- Roguing out of virus infected plants up to 45 days of transplanting
- Installation of yellow sticky traps
- Spraying Neem formulations / Neem seed kernel extract (Need based)

The farmer practice plot was managed with no IPM components. In each trial, the incidence of PBNV and TLCV was monitored bi-weekly, based on visual symptoms. The results for the four trials are shown in table 3. Trials 1 and 2 were begun in Year 2 (2010-2011) and completed in the current reporting year. Trials 3 and 4 were conducted in January 2012 and at different locations. Trials 2 and 3 used the same cultivar, Ruchi, and had a similar virus incidence in both trials. Trials 1 and 4 were at the same location, but at different times and with different cultivars.

Table 3: Effect of IPM tactics on the incidence of PBNV and TLCV in tomato (four trials)

Trial 1: Tomato Hybrid: Lakshmi Date of planting: 13-06-2011. Location: Veerakeralam, Coimbatore.				
Days after planting	**% PBNV incidence**		**% TLCV incidence**	
	IPM Plot	**FP**	**IPM Plot**	**FP**
15	0.5	0.9	0.2	1.0
30	1.9	3.4	1.0	3.8
45	5.6	11.6	2.3	4.6
60	9.8	16.9	3.7	6.2
75	13.1	22.7	4.3	8.9
90	16.9	29.4	6.9	9.3

Trial 2: Tomato Hybrid: Ruchi. Date of Planting: 16-06-2011. Location : Marappanaickenpatti, Dharmapuri				
Days after planting	% PBNV incidence		% TLCV incidence	
	IPM Plot	FP	IPM Plot	FP
15	0.2	1.1	0	0
30	1.7	3.9	2.2	5.3
45	3.2	7.4	9.1	21.7
60	5.6	12.3	16.5	49.6
75	8.7	16.1	19.8	63.2
90	12.4	21.7	22.6	69.5

Trial 3: Tomato Hybrid: Vijaya Date of planting : 04-01-2012. Location: Veerakeralam, Coimbatore				
Days after planting	% PBNV incidence		% TLCV incidence	
	IPM Plot	FP	IPM Plot	FP
15	0.3	0.8	0	0
30	1.6	3.1	0.2	0.9
45	2.9	6.7	0.8	3.1
60	5.1	11.5	2.5	4.2
75	8.2	15.3	3.7	6.9
90	12.7	23.4	4.3	8.2

Trial 4. Tomato Hybrid: Ruchi Date of Planting: 20-01-2012 Location: Polayampatti Post- 635 305 Dharmapuri Dt. Tamil Nadu				
Days after planting	% PBNV incidence		% TLCV incidence	
	IPM Plot	FP	IPM Plot	FP
15	0.5	1.1	0.3	0.8
30	1.9	3.3	1.7	3.3
45	4.2	9.8	6.5	14.4
60	9.6	15.4	13.8	32.2
75	13.7	21.9	18.6	46.5
90	17.5	30.8	23.2	58.7

In four independent trials, the progress of disease, as measured by percentage of plants showing PBNV or TLCV symptoms at 15-day intervals, was delayed in plots receiving IPM tactics, in comparison with untreated farmer practice. The final disease incidence was consistently lower in the IPM plots. The incidence level attained by 90 days in IPM plots was 30 days or so earlier than in many of the farmer practice plots. The incidence of virus was lower in Trials 1 and 3 at the Coimbatore location, and it was about the same in both varieties and at the two planting times, with more PBNV than TLCV. The IPM plots yielded 28.6 and 27.7 t/ha of fruit, respectively, and the farmer practice plots' yields decreased to 24.7 and 24.1 t/ha of fruit, respectively.

At the lower incidence sites (or with a cultivar less susceptible to TLCV) in trials 2 and 4, IPM tactics improved yields by 2.5%-3%. In trials 2 and 4 and using the susceptible Rushi cultivar at two sites, the IPM plots yielded 18.7 t/ha and 17.5 t/ha, respectively, and farmers practice plot yield was decreased to 13.1 t/ha and 13.3 t/ha. Comparison of these two plots indicates a yield benefit of IPM tactics of 3.2%-4.3%.

Diagnosis of viruses in vegetable crops in Bangladesh (E+T)

Rayapati, Muqit

A small-scale survey of vegetable fields in Comilla area of Bangladesh was conducted in April 2012. Symptomatic samples from yardlong bean, country bean, okra, and gourds were collected and processed for testing by serological and molecular assays. Leaf samples showing mosaic symptoms from country bean, ash gourd, and bottle gourd tested positive in ELISA for universal antibodies to potyviruses, indicating that these samples were infected with a potyvirus. Symptomatic leaves of yardlong beans and okra were pressed on FTA® cards in the field and brought to Rayapati's lab for further processing and testing for different viruses. Total nucleic acids eluted from FTA cards were subjected to reverse transcription (RT)-polymerase chain reaction (PCR) with universal primers specific to the cytoplasmic inclusion body protein of potyviruses. Total nucleic acid eluted from FTA cards pressed with okra samples were tested by PCR using universal primers for begomoviruses. In both cases, the amplified DNA fragments were cloned separately and nucleotide sequence determined. A comparison of sequences from yardlong bean with corresponding sequences in GenBank showed close affinity with *Bean common mosaic virus* (BCMV; *Potyvirus*) obtained previously from yardlong beans in Indonesia. The nucleotide sequences derived from okra samples showed high affinity to *Bhendi yellow vein mosaic virus* (*Begomovirus*) infecting okra in India.

SOUTHEAST ASIA

Diagnosis of viruses in vegetable crops in Indonesia (E+T)

Rayapati, Hidayat, Damayanti

Surveys were conducted in November 2011 to document viruses in vegetables in West Java and few areas in Central Java. Serological and PCR techniques were used. Four tomato samples and one leek sample from Lembang, and three tomato samples from Pengalengan tested negative by PCR for *Crinivirus* and *Tospovirus*, and were also negative to *Cucumber mosaic virus* (CMV) by ELISA. Three potato plant samples from Lembang were positive by immunostrips for *Potato virus Y* (PVY) but negative for *Potato leafroll virus* (PKRV). Three potato samples from Pengalengan were negative for both viruses in immunostrip tests.

In some cases, DNA amplified by PCR was sequenced to identify virus(es) present. The PCR results from yardlong bean (tab. 4) showed samples were positive for *Bean common mosaic virus* (BCMV) and Geminiviruses in most samples and were also positive for CMV in four samples that were also positive for BCMV. Samples 1-4 from Bogor were also negative in tests for the following five genera of viruses known to infect legumes: *Carlavirus*, *Comovirus*, *Crinivirus*, *Luteovirus*, and *Sobemovirus*. Further studies are in progress to identify viruses in other samples.

Coat protein sequence of *Bean common mosaic virus* (BCMV) from yardlong bean used to identify strain in Indonesia (E+T)

Previous studies have shown that BCMV is widely prevalent in yardlong beans and affects crop yield. Therefore symptomatic samples were collected from yardlong beans in farmers' fields from different areas, the coat protein gene was amplified, and the nucleotide sequences were obtained and compared with corresponding sequences from GenBank. The results shown in table 5 indicate that two distinct strains of BCMV are present, each with wide variability among the isolates from yardlong bean. The Blackeye cowpea mosaic

Table 4. Viruses detected from yard long bean samples in Indonesia

No.	Location	RT-PCR/PCR			
		BCMV	CMV	Geminivirus	Five virus genera
1	Bubulak-Bogor	+	+	-	-
2	Cikabayan-Bogor	-	-	+	-
3	Cibeureum-Bogor	+	-	+	-
4	Leuwikopo-Bogor	+	-	-	-
5	Darmaga- Bogor	+	+	+	Not tested
6	Jatisari -Karawang	+		+	Not tested
7	Subang	+	+	+	Not tested
8	Indramayu	+	+	+	Not tested
9	Cirebon	+	-	+	Not tested
10	Tegal	+	-	+	Not tested
11	Pekalongan	+	-	+	Not tested

Table 5. Identification of BCMV strain infecting yardlong bean based on sequence of the coat protein gene.

No.	Origin of sample	Identity	Homology (%)	Gen Bank Accession No.
1	Darmaga, Bogor	BCMV-BlC	97	AY575773.1-Taiwan AF395678.1- Taiwan FR775796.1-Thailand
2	Leuwikopo, Bogor	BCMV-BlC	90	DQ925423.1-Vietnam
3	Cirebon	BCMV-NL1	90	L15331.1 AF083559 (NY15)
4	Sidorejo, Pekalongan	BCMV-NL1	94	GQ850881 [C-6 India] FJ491262 [N-1 India]

strain (BCMV-BlC) was detected in two fields in Bogor and was related to other Asian BCMV sequences. Further studies are required to develop a comprehensive analysis of BCMV, including the possible origin of the virus from fields.

Demonstration of seed transmission of BCMV in yardlong bean in Indonesia (E+T)

Rayapati, Hidayat, Damayanti

In experiments to confirm seed transmission of BCMV, 100 seeds of the following varieties — NJT-New Jaliteng, PLR-Pilar, PRD-Parade, LS-Long silk, MHR-Maharani, 777, LS-Lousiana — and one local variety were planted in small pots. Three weeks after germination, leaves from individual seedlings (100 per variety) were harvested and tested by ELISA and tissue blot immune assay (TBIA) using commercially available antibodies. Percent seed transmission was calculated. As shown below, BCMV can be readily detected by ELISA (figure 1) and TBIA methods. In TBIA, dilutions of antiserum (obtained from Agdia, Inc.) to BCMV up to 1:10,000 were effective in detecting positive samples. In general, BCMV was detected serologically from all commercially available seed and the percent positive samples varied with variety. In four varieties, less than 5% of plants were infected. In three varieties, 20-70% seed transmission was detected. These results provide definitive evidence that BCMV is disseminated via seed supplied from commercial sources, and suggest that a management strategy must begin with clean seed.

IPM package development: Evaluation of Chitosan application for managing BCMV in yardlong bean in Indonesia (AR)

Rayapati, Hidayat, Damayanti

This study was conducted in the greenhouse to evaluate the effect of chitosan application on plant growth and disease incidence caused by *Bean common mosaic virus* (BCMV). The study was conducted using two concentrations of chitosan (0.1 % and 1%) and three times of application (seed treatment, before virus inoculation, and after virus inoculation). Plant height was measured at 2-week intervals. Other data were collected 8 weeks after inoculation. The eight treatments were:

- PB0.1: seed treatment with 0.1% chitosan
- PB1: seed treatment with 1% chitosan
- SB0.1: application of 0.1% chitosan before virus inoculation
- SB1: application of 1% chitosan before virus inoculation
- ST0.1: application of 0.1% chitosan after virus inoculation

Figure 1.Seed transmission of BCMV from several yardlong bean varieties detected by ELISA and TBIA of individual plants from grow-out tests.

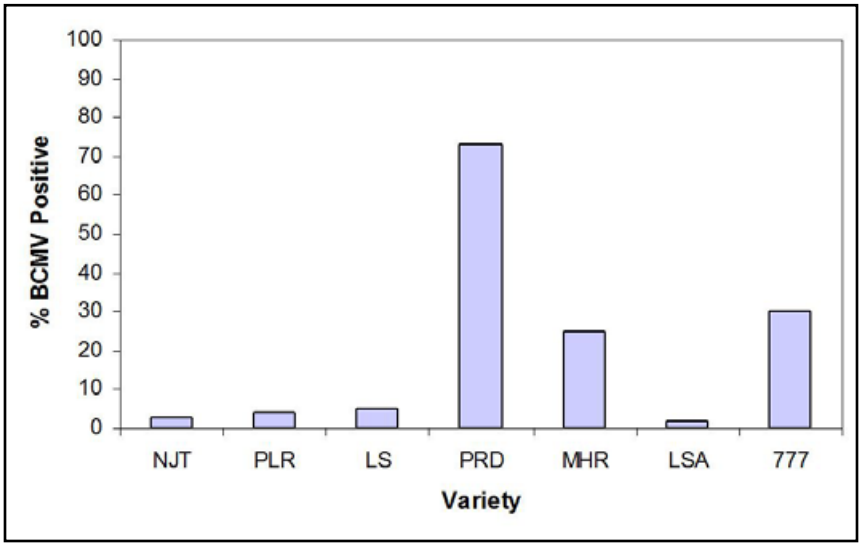

Table 6. Effect of chitosan on plant height

Treatment	Plant height (cm)			
	2 WAI*	4 WAI	6 WAI	8 WAI
PB0.1	64.9 ± 8.5 a	141.6 ± 24.8 ab	195.3 ± 22.0 a	215.4 ± 31.3 a
PB1	73.6 ± 17.2 a	171.1 ± 37.1 a	216.6 ± 38.8 a	233.6 ± 47.6 a
SB0.1	67.7 ± 20.5 a	154.0 ± 36.6 ab	188.5 ± 42.0 ab	207.6 ± 43.5 a
SB1	67.2 ± 18.2 a	146.1 ± 14.4 ab	190.5 ± 20.1 a	209.8 ± 35.2 a
ST0.1	72.4 ± 7.1 a	150.0 ± 21.2 ab	191.8 ± 22.6 a	213.1 ± 27.6 a
ST1	69.4 ± 7.8 a	154.5 ± 28.9 ab	200.2 ± 40.9 a	218.4 ± 30.4 a
K-	69.3 ± 14.8 a	167.2 ± 4.,1 a	211.1 ± 51.9 a	225.3 ± 53.3 a
K+	62.9 ± 12.0 a	119.9 ± 19.1 b	156.2 ± 21.4 b	164.5 ± 24.4 b

* WAI, weeks after inoculation of virus

Table 7. The effect of chitosan on flowering time, numbers of leaf, and dry mass of plant

Treatment	Days to flowering	Numbers of leaf	Plant dry mass
PB0.1	33.0 ± 0.69a	9.11 ± 0.50 bc	1.92 ± 0.34 ab
PB1	32.4 ± 3.38a	11.33 ± 0.33 a	1.98 ± 0.22 a
SB0.1	34.2 ± 1.34a	9.66 ± 0.66 abc	1.94 ± 0.32 ab
SB1	32.9 ± 1.26a	10.33 ± 0.33 ab	1.98 ± 0.55 a
ST0.1	34.0 ± 0.33a	10.66 ± 0.66 ab	1.90 ± 0.27 ab
ST1	32.0 ± 2.96a	11.33 ± 1.00 a	1.97 ± 0.44 a
K-	33.0 ± 1.92a	9.22 ± 1.64 bc	1.92 ± 0.25 ab
K+	34.4 ± 3.32a	8.11 ± 1.25 c	1.82 ± 0.18 b

Table 8. Effect of chitosan on virus accumulation, virus and disease inhibition

Treatment	ELISA value	Virus titer reduction (%)	Disease incidence reduction (%)
PB0.1	0.42 ± 0.12 b	82.3	55.3
PB1	0.42 ± 0.20 c	82.3	65.5
SB0.1	0.43 ± 0.16 cd	81.9	44.7
SB1	0.36 ± 0.21 b	84.8	62.1
ST0.1	0.57 ± 0.19 d	75.9	41.3
ST1	0.57 ± 0.19 d	75.9	41.3
K-	0.13 ± 0.01 a	100	100
K+	2.37 ± 0.56 e	0	0

- ST1: application of 1% chitosan after virus inoculation
- K-: no virus inoculation without chitosan application
- K+: virus inoculation without chitosan application

The results show that chitosan application either as seed treatment or leaf spray before or after inoculation with the virus showed significant positive effects on plant height at 8 weeks (tab. 6) and on days to flowering (tab. 7). The numbers of leaves per plant was significantly greater in plants following treatment with the higher concentration on seed or on leaves. Plant dry mass was greater with the higher chitosan concentration by any treatment (tab. 7). All chitosan treatments affected virus titer, reducing ELISA values to one-fourth or less that of the infected control, and reducing disease incidence by half or more (tab. 8). These results provide a rationale for using chitosan as a component of IPM packages for the management of BCMV, enhancing plant growth, and reducing disease incidence especially when the risk of virus infection is high.

CENTRAL ASIA

Detection and identification of viruses in potato and onion in Tajikistan (E+T)

Rayapati

***Potato virus Y* in potato:** In continuation of FY 2011 research, we conducted molecular studies to precisely identify *Potato virus Y* (PVY) strains from symptomatic potatoes collected in farmers' fields from the Buston and Dushanbe regions of Tajikistan. Total nucleic acids eluted from FTA® cards pressed with tissue samples from symptomatic plants were subjected to reverse transcription (RT)-PCR with primers specific to the coat protein of PVY. Samples infected with PVY ordinary strain (PVY^{O}), tuber necrosis strain (PVY^{NTN}), tobacco veinal necrosis strains ($PVY^{EU\text{-}N}$ and $PVY^{NA\text{-}N}$), and a recombinant strain ($PVY^{N:O}$) were included as references to validate RT-PCR results. The amplified fragments from two samples from Dushanbe and six from Buston areas were cloned separately and sequences were compared with corresponding sequences of reference strains available in GenBank. The results showed the presence of PVY^{O} in samples from Dushanbe and Buston regions and presence of PVY^{NTN} in samples from the Buston region. This study represents the first confirmed report of two distinct strains of PVY in potato in Tajikistan, of which PVY^{NTN} is a quarantine pathogen in many countries. These findings warrant further efforts to improve phytosanitary status of potato fields and to facilitate the availability of virus-free seed in clean plant programs for significant yield increases in Tajikistan.

***Iris yellow spot virus* in onion:** The bulb onion is an important crop for farmers' income and overall economy

and food security of Tajikistan. During a limited survey of onion fields near Dushanbe in June 2011, we observed onion plants showing characteristic diamond-shaped lesions (with or without green islands) on leaves and scapes. Total nucleic acids eluted from FTA® cards pressed with tissue samples from symptomatic plants were subjected to RT-PCR with primers specific to the nucleocapsid protein (NP) gene of the Tospovirus *Iris yellow spot virus* (IYSV). A single DNA product of approximately 896 base pairs was amplified from all samples, cloned and sequenced. A comparison of these sequences with corresponding NP gene sequences of global IYSV isolates revealed that IYSV isolates from Tajikistan are more closely related to isolates from Serbia and Italy. *Thrips tabaci*, a known vector of IYSV, was observed in onion flowers. To our knowledge, this is the first confirmed report of IYSV in onions in Tajikistan. In recent years, IYSV has been reported in many onion-growing regions. Our finding of IYSV occurrence in Central Asia, considered as the center of origin for onion, expands our knowledge of its global distribution.

WEST AFRICA

Identification of a viroid as the cause of an unusual disease phenotype in tomato in Mali, Ghana, and Senegal (E+T)

Gilbertson, Keo, Melgarejo

Since 2006, surveys of tomato fields conducted as part of the IPM CRSP project revealed an unusual disease symptom phenotype in which plants were stunted and leaves were curled and yellow and showing necrotic veins. During surveys of tomato fields in Ghana and Mali in March 2011, these unusual disease symptoms, including stunted growth, epinasty and chlorosis of leaves, and necrosis of leaf veins and stems, were observed in multiple fields. The symptom incidence was sporadic (~1%–5%), but distinct from those associated with known viral diseases in the region. Representative leaf samples were applied to FTA® cards, and DNA and RNA extracts were prepared. RT-PCR tests for tospoviruses, bromoviridae, tobamoviruses, torradoviruses, and potyviruses as well as PCR tests for begomoviruses were all negative. Similarly, PCR-based tests for phytoplasma and *Candidatus* Liberibacter infection were also negative.

However, a putative virus-like agent was transmitted to tomato seedlings by rub-inoculation with sap from a sample from Niono, Mali. This agent induced stunted growth and severe epinasty of leaves, followed by necrosis of leaf veins, petioles and stems, similar to those observed in field-collected plants. When RNA extracts from leaves of these infected tomato plants were rub-inoculated onto healthy tomato seedlings, similar symptoms developed, suggesting the causal agent might be a viroid. RT-PCR tests of RNA from symptomatic tomato leaves with universal and various specific pospiviroid primer sets were negative, but with the pCLV4/pCLVR4 primer pair, specific for *Columnea latent viroid* (CLVd), a DNA fragment of the expected size (~370 bp) was amplified. The sequence of this DNA fragment (GenBank accession no. JQ362419) was 99% identical with those of CLVd isolates from the Netherlands (AY373446 and AY372396). This is the first report of CLVd in tomato in West Africa, which was perhaps introduced in association with tomato seed.

In host range studies, the African CLVd isolate induced symptoms in sap-inoculated tomato plants, but no symptoms developed in inoculated *Chenopodium quinoa* and *C. amaranticolor*, *Nicotiana benthamiana*, *N. tabacum* (cvs. Havana and Turkish), *N. glutinosa*, *N. glurk, Datura stramonium*, common bean (cvs. Topcrop and Pinto bean), pumpkin (cv. Small Sugar), pepper (cv. Yolo Wonder), or cucumber (cvs. Emparator and Poinsett 76) plants. However, symptomless infections were detected in pepper (*Capsicum annuum*), *Nicotiana benthamiana,* and *N. tabacum* cv. Turkish plants.

Characterization of a weed-infecting begomovirus in Mali, Ghana, and Senegal (E+T)

Gilbertson, Keo, Melgarejo

Previously, our work in Mali established that symptoms of leaf curling, vein swelling, and yellowing in the common weed *Sida* spp. were associated with infection with the begomovirus, *Cotton leaf curl Gezira virus* (CLCuGV), and the betasatellite, *Cotton leaf curl Gezira betasatellite* (CLCuGB). Sequence analysis of the PCR-amplified fragments revealed infection with CLCuGV-Sida strain (94-95% identity). These results extend the concern that this very common perennial weed could serve as a reservoir for begomoviruses with the potential to infect okra or even cotton.

We have now cloned the complete genomes of the begomovirus DNA component and the betasatellite associated with the leaf curling and yellowing symptoms in *Sida*. The complete sequence of the begomovirus DNA is 88.6% identical to the closest known begomovirus, CLCuGV from Mali that infects okra. However, inspection of the genome revealed highly conserved regions of the genome and more divergent regions, suggestive of recombination. The betasatellite is more divergent and is only 63% identical to the most closely related betasatellites, raising the interesting possibility that the betasatellite may confer host specificity to CLCuGV complexes. We are now seeking to test this hypothesis as well as determine the host range of the CLCuGV from Sida by obtaining infectious clones and conducting agro-inoculation and particle bombardment experiments with cotton, okra, and *Sida* spp. plants.

IPM package development: Host-free periods for management of whitefly-transmitted viruses in tomato in West Africa (AR)

Gilbertson

The implementation of the host-free period in two locations in Mali, Baguineda and Kati, has resulted in a substantial reduction in the incidence of *Tomato yellow leaf curl virus* (TYLCV) and populations of whiteflies. Combined with the introduction of early maturing and high-yielding hybrid and open-pollinated varieties, the tomato yields for growers in these areas have increased dramatically. Thus, at the January 2012 West Africa Regional Planning meeting, plans were made to extend the host-free period to additional regions in Mali, including Segou and Konanbougou (a rainy season production area). Unfortunately, with the suspension of the activities in Mali, this part of the project was suspended.

In Ghana, plans have been made to establish a host-free period in the northern production region, where there is irrigated tomato production (Tono irrigation project). This is an ideal location, because it is an irrigated vegetable-rice production system, with tomatoes being grown in the dry season (October-April). Thus, a 2-3 month tomato/pepper host-free period could be established in June-August. Similarly, the possibility of establishing host-free periods in

Senegal is now being assessed, and the location(s) to be targeted will depend on the results of the tomato IPM plots as part of the West African Regional Project.

EAST AFRICA

Virus identification and workshops in Kenya, Uganda, and Tanzania (E+T)

Sseruwugi, Tolin, Otipa, Mamiro

Work was initiated in the East Africa region by Tolin attending a meeting in early March 2012 of representatives of the Regional Project and the IPVDN in Morogoro, Tanzania. She reviewed the work funded by Africa Food Security Initiative (AFSI) and the International Plant Diagnostic Network (IPDN) for a student from Makerere University to survey for viruses in tomato using available ELISA kits. Severe symptoms were observed in over 60% of the fields examined. In 71 samples, ELISA-positives included *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus*, *Cucumber mosaic virus*, Potyvirus, and *Tomato spotted wilt virus*, with mixed infections in 85% of the samples. The full-length sequence of ToMV-Uganda was determined, confirming that the virus is ToMV and not TMV, and suggesting that it is a distinct strain of this virus. This work was done at OSU under the supervision of F. Qu at Wooster (Ohio State) and the IPDN. Research done in Kenya on passion fruit viruses was also reviewed, including the thesis work done by M. Otipa with F. Qu. Suggestions made for developing membrane-based ELISA assays that could be used as an alternative to ELISA to expedite virus screening by nursery operators and seedling stock providers to provide management through clean seedlings.

Additionally, we collaborated with the IPDN in planning an April 2012 Diagnostics Workshop in Morogoro. Viruses included in the workshop were *Tomato yellow leaf curl virus* and *Passion fruit woodiness virus.* Draft SOPs were reviewed and suggestions made for revisions. The IPVDN also contributed expendable laboratory supplies to the IPDN for use in the workshop and the hands-on exercises at Sokoine University of Agriculture, Tanzania.

LATIN AMERICA AND THE CARIBBEAN

Prevalence of Viruses in Solanaceous Crops in Three Regions of Guatemala (E+T)

Palmieri

Samples of tomato, pepper, potato, and surrounding weeds were collected in three areas of Guatemala: Occidental (western highlands), Oriental (eastern), and north central. Potyviruses were detected in tomato in all regions but only detected in pepper in north central. Begomoviruses were detected in pepper only in eastern and north central regions. TMV/ToMV was the predominant virus complex in tomato and weeds in all three areas. *Tomato spotted wilt virus* (TSWV) was detected in tomato and pepper in the western and north central areas. In potato from the western highlands, *Potato Virus Y* (PVY), *Potato Virus* X (PVX), *Potato virus S* (PVS), and PLRV as well as the zebra chip Liberibacter pathogen were detected.

Detection of Specific Potyviruses in Guatemala (E+T)

Palmieri, David Castañeda

Since *Potyvirus* members are constantly found in many places of Guatemala and in different crops, we need to start implementing a method for detecting potyviruses by ELISA and detecting specific potyviruses present. This knowledge could help in developing methods or strategies for preventing these virus infections and study the virus-vector relationship. Two pairs of PCR primers were tested for amplification of sequences encoding conserved portions of the NIb protein or the capsid protein. The NIb primer pair amplified a 350 bp fragment and CN48/oligo-dT primer pair amplified a 700 bp fragment.

Samples were collected from different crops and from different localities, including Salamá, Panajachel, Sololá, and Santa Lucía Cotzumalguapa, Escuintla. The samples were subjected to ELISA for general potyvirus and to RT-PCR with both pairs of primers. Our conclusions were that the CN48/Oligo-dT primer pair was very inconsistent in the amplification and was influenced by the crop. Results with the NIb primer pair were consistent in the amplification and adequate sequences were obtained from the products. The NIb primers could be used for different crops, with amplification obtained with similar conditions.

We also noticed that the ELISA test for potyviruses was very consistent with dicotyledonous crops, but it was not able to detect potyviruses from monocotyledons like sorghum. The RT-PCR showed high specificity (86%) and a low sensitivity (11.11%). After sequencing, two viruses were detected, *Bean yellow mosaic virus* (BYMV) in pea and *Johnson grass mosaic virus* (JGMV) in sorghum, that had not been detected previously in Guatemala. We believe that better sequences could be obtained if the products of the RT-PCR were cloned before sequencing. The isolated nucleic acids degraded readily, and clones were more stable and yielded better sequences. We plan to try other primers to see if they perform better using other regions of the potyvirus genome and will distinguish between potyviruses.

Detection of relative prevalence of viruses in solanaceous crops and weeds in Honduras by commercial PCR analysis (E+T)

Rivera, Melgar

Two surveying trips were conducted, and a total of 203 crop samples were collected and sent to AgDia, Inc., (Elkhart, IN, USA) for analysis. All the samples were collected in the six poorest departments in southwestern-western Honduras. This is the region where the USAID-funded ACCESO project is being conducted. As a collaborative activity, logistic and financial collaboration was provided to FHIA by ACCESO to implement this activity. Most samples were from *Solanaceaem,* and all were tested initially by PCR against a battery of twelve groups (genera or families) of viruses. Partial results of the general analyses of the first samples are available and show that 83.5% of the samples were positive for one or more virus groups, with *Begomovirus* being the predominant and detected in 73.8% of the samples. Other groups detected were *Carlavirus* (5.8%), *Closteroviridae* (2.9%), *Nepovirus* (2.0%), *Potexvirus* (3.9%), *Potyviridae* (2.9%), *Tobamovirus* (1.0%), and *Tospovirus* (1.0%). No members of the *Bromoviridae*,

Curtovirus, *Ilarvirus*, or *Tombusvirus* genera were detected. These results differ from the virus groups traditionally found in horticultural areas such as Comayagua, where the incidence of potyviruses has been higher, followed by begomoviruses and then tobamoviruses. As many as six of eight weed samples (75%) tested positive to one or more virus groups.

Distribution and diversity of whitefly vectors and begomoviruses in Guatemala over time (E+T)

Brown, Palmieri

Over the last decade of IPM CRSP activities, collections from different locations in Guatemala have been made for begomovirus detection and whitefly speciation and haplotyping.

Brown's lab reviewed all of the begomovirus core coat protein sequences from the study and initiated work to edit and analyze the sequence data (phylogenetic), so that redundant sequences will be removed from the tree to make it small enough to read. At the same time the data points were recorded for each crop and year based on the phylogenetic analysis, which allows us to identify the virus based on shared sequence homology with a reference species or strain. This also permits recognition of a new virus using the same analysis, verified by BLASTn matching using the NCBI database that contains sequences to all known begomoviruses (and therefore can alert to a new species or variant for which a sequence may have recently been submitted/since the reference sequence database was assembled).

Similarly, the *Bemisia tabaci* and other whitefly cytochrome c oxidase I (COI) sequences were edited to correct for sequencing error and prepared for phylogenetic analysis. These data will yield an affiliation with a particular sister clade and/or as an outgroup if *B. tabaci* aligns with a whitefly species other than *B. tabaci*. There are a large number of sequences in our database from long-term studies, and so the procedure used (above) for the redundant haplotypes will be used for the virus sequences; the number of samples of a given haplotype, host, and geographic location will be tallied by year. An interesting trend is that *Trialeurodes vaporariorum*, the greenhouse whitefly, was seen to overtake previous *B. tabaci* niches in some locations. Another trend is that the B biotype of *B. tabaci*, which had displaced the local haplotypes in some areas, has been displaced in some of those locations by the local haplotypes. Both of these trends appear to be related to changes in local weather-climate factors (anecdotally occurring as warming trends are reported elsewhere). Our hope is that this long-term data set will reveal some interesting patterns in whitefly vector and virus diversity and distribution. If our 'warming' hypothesis is supported, this will be the only data set of its kind (as far as we are aware) for the Americas.

In Brown's lab, re-optimization of PCR for haplotyping whiteflies using the COI as a molecular marker was undertaken. Results have been erratic during the past year. With the inclusion of non-proof reading Taq polymerase, results were obtained for the PCR assay for the first eight months; again the assay failed to work. We are exploring the source of the proteinase K, which the Brown lab has found in the past to vary, depending on the source of the recombinant protease. The earliest assays used a protease isolated from culture microorganisms; recent preparations are known to be produced using recombinant methods, and different companies provide enzymes that do not necessarily work in the type of lysis procedure we previously employed. In the Brown lab we have abandoned the lysis method and now isolate total DNA from all whiteflies for COI-PCR amplification. It may be that the Del Valle lab will need to do the same.

Developing/optimizing the diagnostics for sweet potato viruses in Honduras (E+T)

Brown, Rivera, Melgar

The purpose of this activity is to identify viruses present in Honduras that pose a threat to sweetpotato, a locally-promising crop for export to North America and Europe. A total of 37 samples were collected from eight sites in five provinces and were locally analyzed using a NCM-ELISA test kit developed by the International Potato Center (CIP-Perú) and provided by Dr. Luis Salazar. This kit is designed for detection of ten viruses known to infect sweet potato worldwide. Two distinct viruses were identified: the aphid-transmitted potyvirus *Sweet potato feathery mottle virus* (SPFMV) and less frequently the whitefly-transmitted crinivirus *Sweet potato chlorotic stunt virus* (SPCSV). However, only 23 of the samples tested positive to any of the ten viruses. The development of RT-PCR is in progress to identify the prevalent strains of SPCSV, and to detect other RNA viruses that may occur in the crop. To date no begomoviruses have been identified using PCR in sweet potato samples from Honduras.

Cuttings purchased from the United States and other sources are certified virus-free. This leads to the hypothesis that endemic viruses, reaching 50% or greater disease incidence, are transmitted to sweet potato plants shortly after planting to as late as mid-season. Studies are underway to study the epidemiology of sweet potato viruses to determine their sources; this will aid in reducing disease incidence through vector control and/or clean cutting if cutting sources are found to be contaminated (uncertified commercial seed and seed saved from previous crops). Management currently emphasizes use of clean seed while discouraging planting of cuttings from the previous crop. Even so, purchasing seed from external sources creates a prohibitive expense for many farmers, and a locally run clean-seed program is needed.

Developing/optimizing diagnostic methods and host country capacity for detecting sweet potato viruses (E+T)

Brown, Palmieri

Work has been initiated to develop diagnostic capacities needed for the management of sweet potato virus diseases through the reduction of viruses in cuttings, enabling production of a local supply of clean cuttings and increasing knowledge of endemic viruses infecting this valuable crop in Central America.

Virus identification in tree tomato in Ecuador (E+T)

Ochoa, Tolin

Tree tomato is an important cash fruit crop for small scale farmers in the highland valleys in Ecuador, and it is one of the crops targeted by the LAC Regional Project. Thus far the IPVDN has not been involved, even though viral diseases are recognized as important constraints of this crop, causing serious yield losses and significantly reducing harvesting period.

At the June 2012 planning meeting, Ochoa presented symptoms of mosaic, leaf distortion, blistering, veinal chlo-

rosis, and fruit spotting often seen on tree tomato. Viruses detected in the 1990s included *Tomato mosaic virus, Tomato ringspot virus* (ToRSV), *Potato virus X, Tomato spotted wilt virus*, *Potato leaf roll virus*, *Potato virus S, Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus*, the potyviruses *Potato virus Y* (PVY*), Tamarillo mosaic virus* (TaMV), and a potyvirus identified only to genus. This complex includes viruses that are only mechanically transmitted or have thrips or aphid vectors. Current results from INIAP using mechanical and aphid vector inoculations suggest that PVY and/or serologically related potyvirus(es) are causing most of the symptoms found in Ecuador. They also showed that PLRV caused slight leaf deformation and ToRSV was asymptomatic. Contribution of other viruses to the disease syndrome is unknown. Plans were made to document the potyviruses and other viruses associated with specific symptoms in tree tomato and to determine their ecology and epidemiology in order to develop management practices.

INIAP also reported that viral diseases are commonly observed in melon and watermelon but that their etiology is poorly understood. Initially, *Papaya ringspot virus* (PRSV), CMV, and a potyvirus have been detected by INIAP using ELISA tests. Collaboration between INIAP and CIBE-ESPOL has been initiated for 2012-2013 focusing on documentation of these viruses to enable initiation of virus management research.

Tomato leaf curl disease in Ecuador and Peru is caused by a New World monopartite begomovirus (E+T)

Gilbertson, Melgarejo

All characterized whitefly-transmitted geminiviruses with origins in the New World (NW) have bipartite genomes, and the disease symptoms induced by these viruses are typically leaf crumpling, downward curling along with light-green to yellow mottling, and mosaic. However, unusual leaf curl symptoms in tomato plants have been observed in Peru (note: the IPM CRSP only funds the Ecuador research, but the same problem exists in both countries) since the mid-1990s and in Ecuador since 2003. These symptoms were also associated with whiteflies, which suggested that a begomovirus may be the causal agent. PCR analyses of plants from Ecuador and Peru with these symptoms revealed the presence of a begomovirus DNA-A component, but we could not detect a DNA-B component associated with the disease.

Our results now show that tomato leaf curl disease (ToLCD) in Ecuador and Peru is caused by a monopartite begomovirus and that this virus is an indigenous NW monopartite begomovirus. A number of variants of a begomovirus DNA component, cloned from tomato plants with ToLCD in Peru, were infectious and induced stunted growth and leaf curl symptoms indistinguishable from those observed in the field. This fulfilled Koch's postulates for the disease, and established that the causal agent is a monopartite begomovirus. The genome organization of these DNA components is similar to DNA-A components of NW bipartite begomoviruses, and sequence comparisons revealed highest identities (92-99%) with the DNA-A component of *Tomato leaf deformation virus* (ToLDeV), a recently described begomovirus from Peru. Biological properties of this monopartite begomovirus causing ToLCD in Peru were shown to be similar to those of other monopartite begomoviruses, including lack of sap transmission, phloem limitation, and inability to cause disease symptoms in tomato varieties with the *Ty-1* gene. Furthermore, mutational analyses revealed functional homology of the capsid protein and C4 genes of this monopartite begomovirus with counterparts in other monopartite begomoviruses. DNA components, cloned from samples of ToLCD collected in Peru in 1998 and 2010 differed in virulence and were found to be associated with a highly divergent region of the genome, including part of the intergenic region and the entire C4 ORF. These results establish that ToLCD in Ecuador and Peru is caused by an emerging NW monopartite begomovirus. Based upon current taxonomic guidelines, the DNA components of this begomovirus represent isolates and strains of ToLDeV. ToLDeV is the first example of an indigenous NW monopartite begomovirus, and evidence is presented that it emerged through convergent evolution.

Incidence of viruses in solanaceous crops in IPM package development trials conducted in Guatemala by the LAC regional project (AR)

Palmieri, Tolin, Arevalo

In Guatemala some experiments were conducted on potato, tomato, and pepper at San Andrés Semetabaj, Sololá, and on tomato and pepper at Salamá, Baja Verapaz, to find out if the program with chemical treatment from the grower was more efficient than others that use fewer chemicals, are environmentally friendly, and are less expensive. In the two regions, three different programs were used to manage the crops, and all except potato were grown in macrotunnels. It is common practice for growers to use macrotunnels for 60-65 days after transplanting to protect crops from virus-transmitting insects (whiteflies, aphids, thrips) during early stages. After that the agribón was removed. Samples were collected from all plots and tested for several pests and diseases reported in previous years, including viruses. Details are reported by the LAC project. Only the virus results are discussed here.

The three options for crop management were replicates of: 1) grower practices, which used several chemical treatments, including foliar and drip irrigation-applied imidacloprid targeted to vector insects; 2) biological control; and 3) IPM system combining chemical, biological, and cultural practices that included sorghum strips between plots as trap or barrier crops to reduce vector pressure. (See the LAC section for more specifics.)

In the samples collected in the Salamá plots, 16 tomato and 6 pepper were positive for one or more viruses. The highest number of virus-infected tomato plants were detected in the chemical treatment option (9), followed by the IPM option (8). Tomato in the grower or chemical plot had potyvirus, begomovirus, and TSWV. In the biological treatment, TMV and potyvirus were detected. In the IPM treatment, only potyviruses were detected.

In samples collected in the Sololá experiments, eight potato, four tomato, and one pepper were positive for one or more viruses. At this location, four potato plants were infected in the biocontrol plot (potyvirus, PVX, PVS), three in the IPM plot (PVX, PVS, PVY), and one (PVX) in the chemical/grower plot. These viruses are commonly found in potato seed tubers. In tomato, only PVY and another potyvirus were detected in the biological treatment plot, and no viruses were detected in tomato in the chemical or IPM plots. The incidence of virus in pepper was also low. These experiments demonstrate the potential benefit of IPM practices in generally reducing virus incidence.

The identification of the viruses, particularly in potato, suggests that virus incidence may be reduced by using virus-free seed.

IPM package development: Management of the zebra-chip disease-psyllid complex of potato and other solanaceous crops in Guatemala and Honduras (AR)

Brown, Rivera, Espinoza, Palmieri

Zebra chip of potato and vein greening of tomato caused by the fastidious bacterium *Candidatus* Liberibacter *solanacearum* emerged as new diseases, and the pathogen and tomato/potato psyllid vector were identified for the first time during in about 2008 (U-AZ, FHIA, Univ-Del Valle). In Honduras, yellow sticky traps have been implemented for psyllid monitoring to learn more about the epidemiology primarily in potato and the biology and life history of the psyllid vector in the highland areas where potatoes are grown. Transmission studies are underway to define transmission parameters to better understand pathogen-vector relationships

Our collecting plan was to use in-field traps and leaf-turn counts, but this yielded few insects since they were not undertaken at the right time and frequency. The use of a leaf-blower/sucker apparatus is proposed to carry out this objective more efficiently. We also learned that, because of the high mountain locations of the affected potato fields, personnel are needed that reside in or near the sampling areas during the pre-dry, dry, and post-dry part of the season to provide consistent sampling timing and weekly collections from yellow sticky traps to monitor dispersal and work with FHIA, who will coordinate the efforts. Psyllid and plant samples have been sent to Brown's lab in Arizona for: detection of Liberibacter (qPCR and PCR, respectively) in plants and psyllids; and psyllid haplotyping (COI), to determine whether populations are of local origin or are migrating from other areas and if polymorphisms can be identified among populations. This information is need to target vector control as a strategy for disease management of this virus-like pathogen.

The Brown lab has pursued the development of potato psyllid-specific mtCOI primers that will amplify the mtCOI. This will be applied to reinforce the haplotype differences (that separate two Arizona psyllid populations (Yuma, Wilcox) from Honduras psyllid) discovered during the pilot study; this suggests (albeit preliminary because of small sample size) that more than one haplotype occurs in Honduras. Additional samples are needed to corroborate this hypothesis and to cover two major locations in Honduras as well as psyllids from the Guatemalan highlands. PCR primers for the detection of *Ca*. Liberibacter and positive control DNA have been provided to the University del Valle in Guatemala lab to enable detection of the this emergent, fastidious bacterium in psyllids and/or tomato or other plants. This increases the capacity in Central America for pathogen detection and should enable accelerated monitoring needed to monitor disease management.

IPM package development: Host-free periods for management of whitefly-transmitted viruses in tomato in the Dominican Republic (AR)

Gilbertson

The implementation of the 3-month whitefly host-free period in the main processing tomato production areas of the Dominican Republic (DR) has been a key component in the successful IPM program for TYLCV. Although the DR is no longer an official part of the IPM-CRSP, continued monitoring has been supported through leveraged funding provided by Transagricola SA. Whiteflies were collected by in-country collaborators, and virus detected by PCR at the Gilbertson lab at UC-Davis. A reduction of TYLCV-positive whiteflies has indicated a high efficacy of the host-free period in the two major tomato-growing areas of the DR, the north (around Santiago) and the south (Azua Valley). This monitoring provides essential data of the efficacy of this management practice as it is applied in new areas.

Whitefly samples (30-50 insects/location) collected in September and October of 2011 had very little TYLCV detected (September: 1 positive/6 total - North, 2/17 - South; October: 0/6 - North, 3/17 - South), indicating that the 2011 host-free period was effective in reducing the level of the virus in whiteflies. Levels of TYLCV in whiteflies remained low in October, November, and December (0-1/6 - North and 1-4/17 - South). No virus-infected plants were observed in the field in October and November; however, in early December a small number of virus-infected plants were observed in the North and South. In early January 2012, the number of whiteflies in which TYLCV was detected began to increase (4/6 - North and 6/17 - South). By February 2012, all 6 whitefly samples from the North and all 17 whitefly samples from the South were strongly positive. The incidence of TYLCV-infected tomatoes in the field also increased substantially during this time. By this point, most tomato fields were well into the green fruit stage, when virus infection of plants has a greatly decreased impact on yield. Whiteflies collected in March and April 2012 from the North and South continued to have high incidences of TYLCV (4-5/6 - North and 14-16/17 - South). However, harvest was well underway by this time and, although the incidence of the virus had increased substantially, the virus had relatively little impact on yields because most of the plants were infected late.

Another factor that has minimized the impact of the TYLCV on the tomato harvest is the increased planting of TYLCV-tolerant and resistant varieties. As the harvest was being completed (May), the amount of TYLCV in whiteflies remained high (5/6 - North and 11/17 - South). In June, when the host-free period began to be implemented, the number of positive whiteflies began to decline (2/6 – North and 6/17 - South). This decline continued into the host-free period, with 0/6 -North and 2/17 - South in July and 0/6 positives - North and 1/17 positives - South in August.

Overall, these results indicated that the host-free period implemented in 2012 was again very effective in reducing the amount of TYLCV in whiteflies and that the virus inoculum pressure should be low heading into the 2012-13 growing season. Together with the increased planting of tolerant and resistant varieties, especially late in the season, the impact of TYLCV in the 2012-2013 growing season should be minimal. Our results also indicate that the host-free period is effective against the new TYLCV strain detected in the DR, TYLCV-Mld. Thus, the monitoring of TYLCV in whiteflies throughout the year continues to be an important part of the effective IPM program for this virus in the Dominican Republic, and it is essential for successful implementation in other locations globally.