Chapter 3 Virus Diseases of Tropical Vegetable Crops and Their Management

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Abstract Viruses have long been known to be prevalent in plants in tropical and sub-tropical developing countries, particularly in staple crops such as cassava, rice, coconut and pulses. The need to address a wider range of vegetable crops was identified by the IPM-Innovation Lab. To meet these needs, a team of virologists was organized to work across countries and regions with IPM specialists to document virus disease problems in priority crops; mainly tomato and peppers, melons and various gourds and cucurbits, locally preferred vegetables such as eggplant (brinjal), okra (bhendi) and yardlong bean, and fruits (passion fruit, tree tomato). These crops constitute important sources of income and food sources, for many farmers, and were observed to be infected by a wide diversity of viruses. Demands for increased production, increased uniformity of vegetables grown for domestic and export markets, changes in production practices leading to scale up of production of seeds and seedlings, changes related to intensification and global climate change, and greater crop uniformity across regions, appear to be associated with crop losses due to viruses. This chapter summarizes more than two decades of research results to identify problems, and describes progress to enhance local capacity for in-country diagnosis and implementation of integrated disease management practices.

Keywords Virus management • Virus diagnosis • Resistance • Seed-borne • Virusvector • Host-free period • Roguing • Clean seed and seedling • Capacity building

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Viral Disease Research in the IPM Collaborative Research Support Program (CRSP) and IPM Innovation Lab (IL)

Early participatory appraisals by the IPM CRSP host countries of the four initial regional sites in Phase I (1993–1998) recognized diseases believed to be caused by viruses or virus-like agents as farmers' problems in target vegetable crops. In Jamaica, the lead country of the Caribbean Site, an aphid-transmitted virus of a nontraditional export crop, hot pepper (Capsicum chinense, var. Scotch Bonnet), was identified as Tobacco etch virus (TEV; Genus Potyvirus), (McGlashan et al. 1993) which could not be controlled by insecticides to kill aphid vectors. Therefore, unsustainable production practices were associated not only with insect pests, but also with viruses that were transmitted by the insect pests. Furthermore, insecticides destroyed natural enemies of a broad mite pest whose feeding damage resembled symptoms caused by TEV. Expertise on viruses was brought into the project from Virginia Tech faculty late in Phase I. In Phase II (1998–2004), research on TEV and vector identification, and strategies for management of this virus disease were conducted in collaboration with the Caribbean Agricultural Research and Development Institute (CARDI) (McDonald et al. 2003a, b, 2004). Near the end of Phase II, special funding to begin using biotechnology to develop virus resistant papaya and tomato was awarded to the Central America Region collaborators: University of Georgia (UGA) and University of Arizona (UA) virologists and host country institutions, University del Valle de Guatemala (UVG), Fundación Hondureña de Investigación Agrícola (FHIA), and Escuela Agrícola Panamericana Zamorano. A request from the West Africa Region for assistance with severe tomato virus problems in Mali was addressed by adding expertise from University of California-Davis (UC-Davis).

In Phase III of the IPM-CRSP (2004–2009), the sub-award category of Global Themes to work across all Regional Sites was opened for proposals and two awards were funded. The first, "Collaborative Assessment and Management of Insect Transmitted Viruses – ITV", was led by Virginia Tech and focused on aphid- and whitefly-borne viruses in the Caribbean (Dominican Republic, Jamaica), Central America (Guatemala, Honduras), and West Africa (Burkina Faso, Mali). The second, "Integrated Management of Thrips-borne Tospoviruses in Vegetable Cropping Systems in South Asia and Southeast Asia Regions", was led by Washington State University (WSU). An additional global theme, The International Plant Diagnostic Network, collaborated with ITV scientists in diagnosis of viral diseases, thus extending expertise on viruses into several regional sites.

In Phase IV (2009–2014), the two global themes merged to form the International Plant Virus Disease Network (IPVDN), "Toward the Effective Integrated Pest Management of Plant Disease Caused by Viruses in Developing Countries: Detection and Diagnosis, Capacity Building and Training, and Formulation of IPM Packages". The IPVDN was led by Virginia Tech, with US virologists from UA, UGA, UC Davis, and WSU participating in various regions and activities. At its

peak, the IPVDN initiated and led activities in 19 countries of the 6 regional sites of the IPM-CRSP. The scope of the project allowed the US collaborating virologists a uniquely global view of viruses in tropical vegetable and specialty crops, and provided access to IPM researchers for inclusion of virus management in IPM packages. The project also enabled host country scientists the opportunity to meet and network with the team in workshops, and to meet other scientists within their region and across regions at sponsored workshops and at national and international scientific meetings.

The need for additional research on the TEV-induced losses in yield and marketable pepper fruit and similar problems in other crops were recognized and integrated into other programs by assembling US virologists that could work as a team, on common virus problems, across several crops and countries.

Diagnostic Approaches and Their Changes with Molecular Technology Development

Identification of viruses in samples of a crop, such as tomato, were accomplished using a battery of available immunological methods such as ELISA (enzyme linked immunosorbent assay) kits for common RNA viruses known to infect that crop. Agdia, Inc. (Elkhart, IN, USA) was often the commercial source of the tests, particularly for countries in Central America, South America and the Caribbean. Agdia's Immunostrip® Test was also widely used for RNA viruses (Fig. 3.1). For DNA viruses the methods – hybridization of radioactively labeled nucleic acid probes to "squash blots" of infected plant parts onto nylon were employed.

The two decades of virus research in the IPM CRSP/IL coincided with unprecedented advances in molecular and bioinformatic technologies for viral nucleic acid detection and sequence analysis to identify the viruses that infect plants and the classification of isolates and strains. Trans-border movement of samples to collaborators' labs for the purpose of making identifications required permits from USDA-APHIS. Later, the IPVDN team was at the forefront of adopting nitrocellulose membranes for tissue blot immunnoassays (Chang et al. 2011), and also FTA® cards (Leke et al. 2007; Naidu et al. 2013) and absorption strips and for trapping viral nucleic acids of high integrity. Begomovirus genome segments and satellites could be amplified and sequenced, then cloned and agroinoculated to observe their ability to induce virus symptoms. Bacterially-synthesized immunogenic coat proteins facilitated production of virus- and genus-specific antibodies for ELISA detection of begomoviruses and potyviruses. A broader range of immunodiagnostic reagents and services became available commercially. The challenge was to perform diagnostic procedures in host countries with limited laboratory capacity and supplies, and few trained scientists.



Fig. 3.1 Workshop participants practicing virus diagnostic techniques: (**a**) Tissue blot immunoassays at Tamil Nadu Agricultural University, Coimbatore, India. (**b**, **c**) Agdia immunostrips in the field at Nepalgunj, Nepal. (**d**) Workshop participants practicing ELISA. NARC, Kathmandu, Nepal

Virus Identification: A Critical Precursor to Successful IPM

Developing and implementing IPM approaches for the management of virus diseases in a crop first requires identification of the viruses and vectors or other means of transmission. Based on the name of the virus genus and the species, virologists use published and on-line information to predict the plant species that it might infect, its means of natural dissemination by insect vectors, and the likelihood of seed and contact transmission. The immediate challenges were assembling information about what viruses were present. A crop-specific baseline listing of viruses reported to infect solanaceous and cucurbitaceous vegetable crops was developed from published and web-based reports, and updated as new and emerging viruses were documented.

Solanaceous vegetables. Viruses from eight viral genera and one viroid genus are known to infect one or more of the solanaceous crops grown widely in the IPM IL countries e.g. tomato, pepper and eggplant (Fig. 3.2). This illustrates the complexity of identifying the diverse viruses in these crops. All three crops are infected by viruses classified in two genera, *Tospovirus* and *Begomovirus*. Tospoviruses are transmitted by several species of thrips, such as *Thrips tabaci*, *Thrips palmi*,



Fig. 3.2 Viruses and viroids in IPM-IL countries infecting the solonaceous vegetables tomato, pepper, and eggplant, grouped by means of transmission, vector, and viral genus. Refer to acronyms in Tables 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6 for full name of each virus and viroid

Frankliniella fusca, Frankliniella occidentalis, and Frankliniella schultzei (Riley et al. 2011). It is likely that more naturally infecting viruses have been detected in tomato than in any other single crop plant. Just among the begomoviruses, there are 82 recognized begomovirus names that begin with the word "tomato", 40 beginning with Tomato leaf curl, and 10 with Tomato yellow leaf curl (Brown et al. 2015). Many of these viruses also infect pepper, even though pepper has its own suite of viruses (Kenyon et al. 2014). Fewer viruses have been described in eggplant, because it is not as widely grown outside of Asia and it's viral diseases not been as widely investigated. Begomoviruses are transmitted by whitefly vectors, mainly the whitefly *Bemisia tabaci* (sibling species), also known as the sweet potato, tobacco, or silverleaf whitefly (Gilbertson et al. 2015). Whitefly-transmitted viruses are not sap transmissible, but cloned DNA of geminiviruses and associated satellite DNA is infectious by agroinoculation. Whiteflies, including Trialeurodes vaporarium, are also vectors of viruses of the *Torradovirus* genus (Verbeek et al. 2014), known to infect only tomato, and of the *Ipomovirus* genus. An ipomovirus, *Eggplant mild leaf* mottle virus, is not included in Fig. 3.2 because it has not been detected in any of the IPM-IL countries. Similarly, a flea beetle-transmitted virus, *Eggplant mosaic virus* (Genus Tymovirus) is not included.

Aphids are vectors of viruses in the *Potyvirus* and *Cucumovirus* genera that infect these three solonaceous crops, and have a non-persistent/stylet-borne relationship with little vector species specificity. There are over 170 viruses classified as potyviruses, challenging the 288 begomoviruses for the genus with the greatest number of viruses (Brown et al. 2015). Seven distinct potyviruses, plus two

identified only to genus were identified in solonaceous crops in most countries of the IPM-IL. The potyvirus *Eggplant green mottle virus* (EGMV), which may be a strain of PVY, is included in Fig. 3.2, although it was isolated from eggplant in Nigeria (Ladipo et al. 1988a, b; Sadeghi et al. 2008). Genetic resistance to potyviruses has been widely used in non-pungent *Capsicum annuum* peppers, which possess a complexity of R genes that match with virus pathotypes. *Cucumber mosaic virus* (CMV), a globally distributed virus and the type species of the *Cucumovirus* genus, has a wide host range and is seed-transmitted in pepper and other host plant species. Several strains of CMV are recognized, and are found naturally infecting these three solanaceous species. *Eggplant mottle virus*, (genus *Tymovirus*), the only virus described on solanaceous crops that is transmitted by beetles – a flea beetle (*Epitrix* sp.) – has not yet been described in developing countries.

Viruses of the genera *Tobamovirus* and *Potexvirus*, and viroids (small naked RNA) of the *Pospoviroid* genus, are contact-transmitted and seed-borne, but have no known biological vector (Fig. 3.2). These pathogens, and most aphid transmitted viruses, can be transmitted by rubbing sap extracts or purified virus onto other plants. This process is used to demonstrate that the isolate can reproduce symptoms observed in the field, and is thus the causal agent of the disease.

Cucurbitaceous Vegetables Viruses from nine genera have been associated with diseases of cucurbit vegetables (Fig. 3.3), six of which are the same as those infecting solanaceous vegetables (Fig. 3.2). Only three viruses, the tospovirus



Fig. 3.3 Viruses in IPM-IL countries infecting cucurbitacious vegetables, grouped by means of transmission, vector, and viral genus. Hosts include cucumber, melon, pumpkin, squash, gourd (ash, bitter, bottle, snake, wax, etc.). TSWV Tomato spotted wilt virus, MYSV Melon yellow spot virus, LuYMV Luffa yellow mosaic virus, MCLCuV Melon chlorotic leaf curl virus, PuYMV Pumpkin yellow vein mosaic virus, SLCuCNV Squash leaf curl China virus, SLCuPV Squash leaf curl Philippines virus, ToLCNDV Tomato leaf curl New Delhi virus, CYSDV Cucurbit yellow stunting disorder virus, CABYV Cucurbit aphid-borne yellows virus, PRSV Papaya ringspot virus, WMV Watermelon mosaic virus, ZYMV Zucchini yellow mosaic virus, MNSV Melon necrotic spot virus

Tomato spotted wilt virus (TSWV), the begomovirus Tomato leaf curl New Delhi virus (ToLCNDV), and Cucumber mosaic virus (CMV) infect crop plant species in both host plant families. The three potyviruses, Papaya ringspot virus (PRSV), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus (ZYMV) in Fig. 3.3 are frequently detected in squash and other cucurbits in West Africa and Asia regions, including in plantings being grown for hybrid seed production, and are distinct from all of the potyviruses in Fig. 3.2. None of the IPM IL diagnostic efforts reported detecting the fungal transmitted virus, which is also seed-borne.

The viruses and viroids that are seed-borne have increased in prevalence and importance with the global changes in production practices hybridization, grafting, production of seedlings in glass and plastic houses, and global distribution of seed by multi-national companies appear to be factors associated with this increase. Production of tomato and pepper in greenhouses protects them from aerial vectors, namely whitefly and aphids, but provides a favorable environment for thrips. Plant manipulation requiring contact spreads virus to adjacent plants. Tobamoviruses can survive and spread in circulating irrigation water in greenhouses. Seed transmitted viruses are increasingly being monitored by phytosanitary and biosecurity agencies (Rodoni 2009). In workshops held by the IPM IL, the onus has been placed on the developing country that produces hybrid seed to assure that the virus is absent in seed lots. However, IPM IL scientists observed that some of these countries have little capacity to conduct these diagnostic tests (Anggrani and Hidayat 2014).

Documenting Viruses Associated with Diseases of Vegetables Crops

The main crops targeted by the IPM IL were those that were grown in temperate climates as well as sub-tropical and tropical countries, mainly tomato, chili and other hot peppers, cucumber, pumpkin, squash and other cucurbits. In later stages of the project, crops extended to include crops of native preference for consumption such as eggplant, okra and various gourds (bitter, bottle, ridge, snake) in India, passion fruit in Kenya and Uganda, tree tomato in Ecuador, and the tropical legumes yardlong bean in Indonesia and country bean in Bangladesh. Common weedy plants in ecosystems surrounding diseased fields were also examined and found to be reservoir hosts for the virus found on adjacent crops (Leke et al. 2012a; Melgarejo et al. 2014).

Many of the viruses in Figs. 3.2 and 3.3 were detected by IPM IL collaborating scientists at all sites. Comprehensive lists of viruses and viroids, the hosts from which they were isolated in the regions and countries in which the IPM IL had active efforts and projects are shown in Tables 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6. The tables include information on viruses detected in weeds and additional crops, including passion fruit, cotton, okra, legumes (yardlong and country bean, mungbean, common bean, cowpea), potato, tree tomato, and Chinese cabbage. Viruses in sweet potato grown in Central America were examined, but are not included. Each

listing is from an identification conducted by US and Host Country scientists working with or trained by the Virus and Diagnostic Global Themes of the IPM IL and associated projects of the Regional Programs. Much of the information has appeared in Annual Reports of the IPM CRSP and IPM IL over a span of years (www.oired. vt.edu/ipmil) and reported at scientific meetings as oral presentations or posters.

Diagnoses were generally from samples of symptomatic plants of priority crops and not from a survey of a crop within a country. Some systematic surveys were conducted in countries where funds were available from Host Country Missions or other sources. The listing of viruses in annual reports may only be indicative of a positive ELISA test for some RNA viruses. Membrane-based sample collection was used extensively for tissue blot immunoassay (Chang et al. 2011) and nucleic acid binding on FTA cards (Bagewadi et al. 2014; Leke et al. 2007; Naidu et al. 2013), as well as on nitrocellulose membranes (Martinez et al. 2014). Identification of begomoviruses was based on DNA sequence of part or all of the genome, and in some cases was followed up by biological tests. The genusspecific immunological tests were widely used, especially for Potyviruses and Tospoviruses, and provided results from which management approaches could be designed and validated, regardless of the species. However, exact identification to virus and/or strain required additional sequence analysis of viral RNA. At least two methods of identification, often including nucleotide sequencing, were used, for validation of any virus having a peer-reviewed publication cited in the tables. These reports range from finding a common virus in a new host as e.g. CMV in eggplant (Table 3.1), to extending a virus to a new host or geographic area, occurrence of seed-borne viruses (Table 3.2), and describing a new virus genus, such as Torradovirus (Table 3.5).

Virus species	Acronym	Host	References
India			
Begomoviruses			
Ageratum enation mosaic virus	AEV	Ageratum (weed)	
Cotton leaf curl Bangalore virus	CLCuBV	Crotons sparsiflorus (weed)	Annual Report (2014)
Malvastrum yellow vein virus	MYVV	Malvastrum coromandelinum (weed)	Annual Report (2014)
Bhendi yellow vein mosaic virus	BYVMV	Okra	Annual Report (2006)
Bitter gourd yellow vein virus ^a	ToLCuNDV-IN	Bittergourd, ash gourd, bottle gourd	Annual Report (2013)
Squash leaf curl China virus	SLCCNV	Bittergourd, wax gourd	Annual Report (2013)
Squash leaf curl Philippines virus	SLCuPV	Bittergourd	Annual Report (2010)
Pepper leafroll virus ^a	PeLRV	Pepper, tomato	

 Table 3.1 Begomoviruses and RNA viruses in the South Asia and Central Asia Regions

Table 3.1 (continued)

Virus species	Acronym	Host	References
Tomato leaf curl Karnataka virus ¹	ToLCKV	Tomato, cucurbit	
Tomato leaf curl New Delhi virus	ToLCuNDV	Eggplant	Pratap et al. (2011)
Tomato yellow leaf curl virus	TYLCV	Tomato, pepper	
RNA viruses			
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Cucumber, eggplant	Annual Report (2011)
Ilarvirus: Tobacco streak virus	TSV	Okra	Annual Report (2008)
Potyvirus : Zucchini yellow mosaic virus	ZYMV	Snake gourd	Nagendran et al. (2014a)
Papaya ringspot virus	PRSV	Cucurbit	Annual Report (2014)
Potyvirus genus		Pumpkin, tomato	
Tobamovirus : Cucumber green mottle mosaic virus	CGMMV	Snake gourd	Nagendran et al. (2014b)
Tospovirus : Capsicum chlorosis virus	CaCV	Pepper	Kunkalikar et al. (2007)
Peanut bud necrosis virus	PBNV	Tomato	Annual Report (2006)
Watermelon bud necrosis virus	WBNV	Melon	Annual Report (2007)
Nepal			
Begomoviruses			
Ageratum enation mosaic virus	AEV	Ageratum (weed)	Annual Report (2014)
Bhendi yellow vein mosaic virus	BYVMV	Okra	Annual Report (2014)
Tomato leaf curl virus	ToLCV	Tomato, potato	
Tomato leaf curl New Delhi virus	ToLCNDV	Tomato	Annual Report (2014)
Potyvirus : Chili veinal mottle virus	ChVMV	Tomato, pepper	Annual Report (2010)
Bean common mosaic virus	BCMV	Yardlong bean, cowpea	
Papaya ringspot virus	PRSV	Papaya	Annual Report (2010)
Zucchini yellow mosaic virus	ZYMV	Pumpkin, squash, gourds	Annual Report (2010)

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Virus species	Acronym	Host	References
Bangladesh			
Begomoviruses			
Tomato leaf curl virus	ToLCV	Tomato, pepper	Annual Report (2007)
Tomato leaf curl Joydebpur virus	ToLCJV	Pepper	Annual Report (2013)
Tomato leaf curl New Delhi virus	ToLCNDV	Tomato, cucurbit, pepper	Annual Report (2010)
Bhendi yellow vein mosaic virus	BYVMV	Okra	Annual Report (2007)
Mungbean yellow mosaic India virus	MYMIV	Mungbean, yardlong bean	Annual Report (2014)
Pumpkin yellow vein mosaic virus ^a	PYVMV	Bitter gourd, pumpkin	Annual Report (2014)
RNA viruses			
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Eggplant, cucumber	Bagewadi et al. (2014)
Potyvirus : Bean common mosaic virus	BCMV	Yardlong bean	Annual Report (2012)
Papaya ringspot virus	PRSV	Ash gourd, bottle gourd	Annual Report (2011)
Watermelon mosaic virus	WMV	Sponge gourd, ridge gourd	Annual Report (2010)
Zucchini yellow mosaic virus	ZYMV	Squash, snake gourd	Annual Report (2011)
Tobamovirus : Cucumber green mottle mosaic virus	CGMMV	Cucumber	Annual Report (2007)
Tajikistan			-
Potyvirus : Bean yellow mosaic virus	BYMV	Bean	Annual Report (2013)
Potato virus Y – NTN	PVY	Potato	Alabi et al. (2012)
Tospovirus : Iris yellow spot virus	IYSV	Onion	Annual Report (2014)

Table 3.1 (continued)

^aVirus names not italicized are not on the ICTV list of approved virus names (Brown et al. 2015)

Table 3.2 Begomoviruses and RNA viruses in the Southeast Asia Region

Virus species	Acronym	Host	References
Indonesia			
Begomoviruses			
Mungbean yellow mosaic virus	MYMV	Melon, yardlong bean	Annual Report (2014)
Pepper yellow leaf curl Indonesia virus	PYLCIsV	Pepper, tomato	De Barro et al. (2008) and Trisno et al. (2009)
Tomato leaf curl New Delhi virus	ToLCNDV	Tomato	
Tomato yellow leaf curl Kanchanaburi virus	TYLCKaV	Eggplant, tomato	Kintasari et al. (2013)
RNA viruses			
Ilarvirus: Tobacco streak virus	TSV	Okra	
Polerovirus:			
Cucurbit aphid-borne yellows virus	CABYV	Cucumber	
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Yardlong bean, cucumber, tomato, potato	Damayanti et al. (2010)
Potyvirus : Bean common mosaic virus	BCMV	Yardlong bean	Damayanti et al. (2010)
Chili veinal mottle virus	ChVMV	Tomato	Annual Report (2014)
Potato virus Y	PVY	Potato	Damayanti et al. (2014)
Potyvirus genus		Yardlong bean	
Turnip mosaic virus	TuMV	Chinese cabbage	
Comovirus : Squash mosaic virus	SqMV	Cucumber, zucchini	Annual Report (2011)
Tobamovirus : <i>Tomato mosaic</i> <i>virus</i>	ToMV	Tomato	Annual Report (2014)
Carlavirus: Potato virus S	PVS	Potato	Annual Report (2014)
Potexvirus : Potato virus X	PVX	Potato	Annual Report (2013)
Cambodia			
Begomoviruses			
Luffa yellow mosaic virus	LYMV	Cucumber, Ridge gourd, Ribbed gourd	Annual Report (2010)
Tomato leaf curl virus	TLCV	Cucurbits	Annual Report (2014)
Tomato leaf curl New Delhi virus	ToLCNDV	Cucurbit	
Tomato yellow leaf curl virus	TYLCV	Tomato, eggplant	Annual Report (2014)
RNA viruses			
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Cucumber	
Philippines			
RNA viruses			
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Virus species	Acronym	Host	References
Ghana			
Begomoviruses			
Sida yellow leaf curl virus	SiYLCV	Sida (weed)	
Tomato leaf curl Ghana virus	ToLCGV	Tomato	Annual Report (2013)
Tomato severe rugose virus	ToSRV	Tomato	
Viroids			
Pospiviroid : Potato spindle tuber viroid	PSTVd	Tomato	
Tomato apical stunt viroid	PASVd	Tomato	Annual Report (2013)
Mali			
Begomoviruses			
Sida yellow leaf curl virus	SiYLCV	Sida (weed)	Annual Report (2014)
Okra yellow crinkle virus	OYCrV	Okra	Annual Report (2007)
Cotton leaf curl Gezira virus	CLCuGeV	Cotton, okra	Annual Report (2007)
Tomato leaf curl Ghana virus	ToLCGhV	Tomato	
Tomato leaf curl Kumasi virus	ToLCKuV	Tomato	
Tomato severe leaf curl virus	ToSLCV	Pepper	
Tomato severe rugose virus	ToSRV	Tomato	
Tomato yellow leaf crumple virus ^a	ToLCrV	Tomato, pepper	Zhou et al. (2008)
Tomato yellow leaf curl virus	TYLCV	Tomato	Noussourou et al. (2008)
Tomato yellow leaf curl Mali virus	TYLCMLV	Tomato	Chen et al. (2009) and Zhou et al. (2008)
RNA viruses			
<i>Crinivirus</i> : Cucurbit yellow stunting disorder virus	CYSDV	Cucurbit	Annual Report (2008)
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Pepper	Annual Report (2010)
Potyvirus : Zucchini yellow mosaic virus	ZYMV	Squash	Annual Report (2009)
Pepper veinal mottle virus	PVMV	Pepper	Annual Report (2010)
Viroids			
Pospiviroid : Columnea latent viroid	CLVd	Tomato	Batuman and Gilbertson (2013)
Guinea, Senegal			·
RNA viruses			
<i>Cucumovirus</i> : <i>Cucumber</i> <i>mosaic virus</i>	CMV	Tomato	Annual Report (2011)
Burkina Faso			,
Begomoviruses			
Cotton leaf curl Gezira virus	CLCuGeV	Cotton	
Pepper yellow vein Mali virus	PeYVMLV	Pepper, tomato	Sattar et al. (2014)
Tomato leaf curl Mali virus	ToLCMLV	Tomato	Sattar et al. (2014)
Tomato yellow leaf curl Mali virus	TYLCMLV	Tomato	Sattar et al. (2014)

 Table 3.3
 Begomoviruses, RNA viruses and Viroids in the West Africa Region

Tabl	e 3.3	(continued)	
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Virus species	Acronym	Host	References
RNA viruses			
Potyvirus : Cowpea aphid- borne mosaic virus	CABMV	Cowpea	Annual Report (2008)
Pepper veinal mottle virus	PVMV	Pepper	Annual Report (2008)
Papaya ringspot virus	PRSV	Cucurbit	Annual Report (2008)
Cameroon		- ·	·
Begomoviruses		Ageratum	Leke et al. (2012a)
Cotton leaf curl Gezira virus	CLCuGeV	Cotton	Annual Report (2007)
Okra yellow crinkle virus	OYCrV	Okra	Annual Report (2007)
Okra leaf curl virus			Leke et al. (2012b)
Tomato leaf curl Ghana virus	ToLCGV	Tomato	Annual Report (2008)
Tomato yellow leaf curl virus	TYLCV	Tomato	Annual Report (2009)
Tomato yellow leaf curl Mali virus	TYLCMLV	Tomato	
RNA viruses			
Potyvirus : Cowpea aphid- borne mosaic virus	CABMV	Cowpea	
Passion fruit woodiness virus	PWV	Passionfruit	
Potato virus Y	PVYb	Tomato	
Tobamovirus : Tobacco mosaic virus	TMV	Tomato	

^aVirus names not italicized are not on the ICTV list of approved virus names (Brown et al. 2015)

Virus species	Acronym	Host	References
Kenya			
Begomoviruses			
Tomato yellow leaf curl virus	TYLCV	Tomato	Annual Report (2008)
RNA viruses			
Potyvirus : Passion fruit woodiness virus	PWV	Passion fruit	Annual Report (2006)
Cowpea aphid-borne mosaic virus	CABMV	Passion fruit, cowpea	Annual Report (2009)
Potato virus Y	PVY	Tomato	Chikh Ali et al. (2015)
Uganda			
Begomoviruses			
RNA viruses			
Cucumovirus: Cucumber mosaic virus	CMV	Tomato	Annual Report (2012)
Tobamovirus : <i>Tobacco mosaic</i> <i>virus</i>	TMV	Tomato	Annual Report (2012)

 Table 3.4
 Begomoviruses and RNA virus in the East Africa Region

Virus species	Acronym	Host	References
Tomato mosaic virus	ToMV	Tomato	Annual Report (2011)
Cucumber green mottle mosaic virus	CGMMV	Cucumber	
Potyvirus : Passion fruit woodiness virus	PWV	Passion fruit	Otipa et al. (2013)
Uganda passiflora virus ^a	UgPV	Passion fruit	Ochwo-Ssemakula et al. (2012)
Potyvirus genus		Tomato	
Tospovirus : Tomato spotted wilt virus	TSWV	Tomato	Annual Report (2011)
Impatiens necrotic spot virus	INSV	Tomato	Annual Report (2008)
Tanzania			
Begomoviruses			
Tomato yellow leaf curl virus	TYLCV	Tomato	Annual Report (2011)
RNA viruses			
Potyvirus : Cowpea aphid-borne mosaic virus	CPABMV	Cowpea	

 Table 3.4 (continued)

^aVirus names not italicized are not on the ICTV list of approved virus names (Brown et al. 2015)

Table 3.5 Begomoviruses and RNA viruses in the LAC Region: Guatemala and Hondu
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Virus species	Acronym	Host	References
Guatemala			
Begomoviruses			
Pepper golden mosaic virus	PepGMV	Pepper, tomato	Palmieri et al. (2008) and Annual Report (2006)
Pepper huasteco yellow vein virus	PHYVV	Pepper, tomato	Palmieri et al. (2008) and Annual Report (2006)
Tomato leaf deformation virus	ToLDeV	Tomato	
Tomato mosaic Havana virus	ToMHV	Tomato	Palmieri et al. (2008) and Annual Report (2009)
Tomato severe leaf curl virus	ToSLCV	Tomato	Palmieri et al. (2008) and Annual Report (2006)
Tomato yellow leaf curl virus	TYLCV	Tomato, tomatillo, pepper	Salati et al. (2010)
RNA viruses			
<i>Crinivirus</i> : Cucurbit yellow stunting disorder virus	CYSDV		
<i>Torradovirus</i> : <i>Tomato</i> <i>chocolate spot virus</i>	TChSV	Tomato	Batuman et al. (2010)
Tospovirus : Tomato spotted wilt virus	TSWV	Pepper, tomato	Palmieri et al. (2008) and Annual Report (2012)
Potexvirus: Potato virus X	PVX	Potato	Annual Report (2011)
Potyvirus : Bean common mosaic virus	BCMV	Bean	Annual Report (2013)

Table 3.5 (continued)

Virus species	Acronym	Host	References
Potato virus A	PVA	Potato	
Potato virus Y	PVY	Potato, tomato	Annual Report (2012)
Tobacco etch virus	TEV	Tomato	
<i>Fabavirus</i> : Broad bean wilt virus	BBWV	Bean	Annual Report (2014)
Sobemovirus: Southern bean mosaic virus	SBMV	Bean	
Alfamovirus : Alfalfa mosaic virus	AMV	Tomato	
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Tomato	
<i>Tobamovirus</i> : <i>Tobacco</i> <i>mosaic virus</i>	TMV	Bean, tomato	Annual Report (2006)
Tomato mosaic virus	ToMV	Tomato	Annual Report (2012)
Polerovirus : Potato leafroll virus	PLRV	Potato	
Carlavirus: Potato virus S	PVS	Potato	Annual Report (2011)
Potato virus M	PVM	Potato	
Honduras			1
Begomoviruses			
Bean golden yellow mosaic virus	BGYMV	Phaseolus bean	
Melon chlorotic leaf curl virus	MCLCuV	Melon, watermelon	
Pepper huasteco yellow vein virus	PHYVV	Pepper, tomato	
Pepper golden mosaic virus	PGMV	Pepper, tomato	
Tomato golden mottle virus	ToGMoV	Tomato	Annual Report (2009)
Tomato leaf curl virus	ToLCV	Tomato	
Tomato leaf deformation virus	ToLDeV	Tomato, pepper	
Tomato mosaic Havana virus	ToMHV	Tomato, pepper	Annual Report (2010)
Tomato mottle virus	TMoV	Tomato	
Tomato severe leaf curl virus	ToSLCV	Tomato, Pepper	Annual Report (2009), (2010)
Tomato severe rugose virus	ToSRV	Tomato, pepper	
Tomato yellow leaf curl virus	TYLCV	Tomato, pepper	
RNA viruses			
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Pepper	Annual Report (2006)
Polerovirus : Potato leaf roll virus	PLRV	Potato	

Virus species	Acronym	Host	References	
Potexvirus: Potato virus X	PVX	Potato	Annual Report (2010)	
Potyvirus: Potato virus Y	PVY	Tomato	Annual Report (2009)	
Papaya ringspot virus	PRSV	Cucurbit	Annual Report (2009)	
Pepper mottle virus	PeMoV	Pepper		
Tobacco etch virus	TEV	Pepper		
Watermelon mosaic virus	WMV	Melon		
Zucchini yellow mosaic virus	ZYMV	Melon		
Tobamovirus : Pepper mild mottle virus	PMMoV	Pepper	Annual Report (2009)	
Tobacco mosaic virus	TMV	Tomato, potato, eggplant	Annual Report (2006)	
Tomato mosaic virus	ToMV	Tomato	Annual Report (2007)	
Tospovirus : Tomato spotted wilt virus	TSWV	Pepper	Annual Report (2009)	

Table 3.6	Begomoviruses	and RNA	viruses in	the LAC	Region:	Dominican	Republic,	Jamaica,
and Ecuad	or							

Virus species	Acronym	Host	References
Dominican Republic			
Begomoviruses			
Jatropha yellow mosaic virus	JMV	Jatropha (weed)	Melgarejo et al. (2014)
Tomato yellow leaf curl virus	TYLCV	Tomato	Annual Report (2006)
Tomato yellow leaf curl virus -Is	TYLCV-Is	Tomato	Annual Report (2011)
Tomato yellow leaf curl virus -Mld	TYLCV- Mld	Tomato	Kon et al. (2014)
RNA viruses			
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Pepper	Annual Report (2007)
Potyvirus : Tobacco etch virus	TEV	Pepper	Annual Report (2007)
Tospovirus : Tomato spotted wilt virus	TSWV	Pepper	Martínez et al. (2014)
Tomato chlorotic spot virus	TCSV	Pepper	Batuman et al. (2013b)
Tobamovirus : Tobacco mosaic virus	TMV	Tomato	
Pepper mild mottle virus	PMMoV	Pepper	Annual Report (2008)

Table 3.6 (continued)

Virus species	Acronym	Host	References
Jamaica			
Begomoviruses			
Tomato yellow leaf curl virus	TYLCV	Tomato	Annual Report (2006)
RNA viruses			
Potyvirus : Tobacco etch virus	TEV	Hot pepper	Annual Report (2005)
Potato virus Y	PVY	Pepper	
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Pepper	
Ecuador			
Begomoviruses			
Pepper leafroll virus	PpLRV	Pepper, tomato	Annual Report (2015)
Tobacco yellow crinkle virus	TbYCV	Tomato, melon, passionfruit. papaya	Annual Report (2014)
Tomato leaf deformation virus	ToLDeV	Tomato	Annual Report (2012)
RNA viruses			
Potyvirus : Papaya ringspot virus	PRSV	Melon, watermelon	Annual Report (2012)
Peru tomato mosaic virus	PerTMV	Tree tomato	Annual Report (2013)
Potato virus Y	PVY	Tree tomato	Annual Report (2012)
Polerovirus : Potato leaf roll virus	PLRV	Tree tomato	Annual Report (2014)
<i>Cucumovirus</i> : Cucumber mosaic virus	CMV	Melon, cucumber	Annual Report (2014)
Tospovirus : Melon yellow spot virus	MYSV	Melon	Quito-Avila et al. (2014)

Management Through IPM Approaches

Developing and implementing IPM approaches for the management of arthropodtransmitted virus diseases first requires identifying the virus (es) and vector(s) involved and secondly, developing an understanding of the biology of these viruses and their vectors in cropping systems, e.g., the host-range of the viruses and vectors including potential weed hosts. Based upon this information, an IPM approach can be proposed that involves production of virus-free transplants or seeds, implementation of regional host-free periods, planting improved varieties (either early maturing [after the host-free or low-vector period] or disease resistant), and sanitation, i.e., emphasizing the importance of removing old crops after harvest and not planting



Fig. 3.4 Steps in a research program showing systems components of an IPM package to manage aphid-transmitted potyvirus in Jamaica (McDonald et al. 2003a, b, 2004)

new crops near old crops (for an example see: Salati et al. 2010; Gilbertson et al. 2011). Other strategies that may be used include roguing of infected plants (Karthikeyan et al. 2012), reflective mulches, and weed management in and around crops. Only as a last resort will management of insect vectors with insecticides be used. Some degree of success has recently been attained with the newer bio-rational insecticides. Clearly, for integrated management to be developed and applied effectively, a cropping systems approach taking into account multiple interacting factors is essential. The identification of TEV in pepper in Jamaica and the additional studies on the aphid-virus-host interactions by the IPM IL scientists (McDonald et al. 2003a, b, 2004; Tolin et al. 2007) was essential in designing a research program for an IPM approach for the management of this disease (Fig. 3.4).

A key component of IPM systems is also the development of resistant varieties, which can be achieved through various means, including plant breeding programs conducted by national and international organizations, and by private companies (e.g., seed companies). However, equally important is the establishment of field trials to screen germplasm in areas where virus pressure is high. This allows for the identification of varieties that are locally adapted, acceptable to farmers, and which are resistant/tolerant to viruses prevalent in a given area.

IPM Package

The IPM Innovation Lab has focused its efforts on designing and delivering an IPM package including a set of different components, often referred to as technologies. These will vary based on the location, crop, and time. Farmers can use one or several of the technologies in a packages depending on local conditions and availability of these components. An example of an IPM package is listed below (oired.vt.edu/ipmil)

- 3 Virus Diseases of Tropical Vegetable Crops and Their Management
- · Choose virus resistant or well-adapted varieties
- Use of disease-free seeds, seedlings, and planting material
- Grow healthy seedlings in plastic trays with coco-peat and *Trichoderma* under net covers
- Rogue symptomatic seedlings in the nursery and subsequent roguing of infected/ symptomatic plants in the field during the first 3–4 weeks after transplanting
- Remove and destroy weeds, volunteer crops, and crop residues that serve as a source of the virus or vectors
- Use appropriate crop density
- Control virus vectors by crop rotation, crop isolation, and barrier crops (Chavan 2015)
- · Use reflective mulches to deter vectors

Observations on Clean Seed and Seedling Production/Managing Virus Vectors

Clean seed and seedling programs have been highlighted in workshops as being essential to successful crop production. Seed-borne viruses often build up in seeds that are saved by growers from plants infected with a potyvirus, CMV, and the stable, mechanically-transmitted tobamoviruses TMV/ToMV and viroids in tomato. Aphids are the most common vectors of seed-borne viruses, mainly potyviruses and CMV (Fig. 3.2). The wide-spread prevalence of the tobamoviruses in tomato globally (Guatemala, Uganda, Bangladesh, Nepal) suggests an increase in seed-borne virus, reduced use of resistant varieties, or/and development of new strains of virus overcoming resistance in tomato. Seed-borne viruses in peppers, melons and other cucurbits, and beans, appear to have increased in farmer-saved seed. However, our Indonesian farmers found that commercially-sold lots of yardlong bean seeds were infected with BCMV and that only 60 % of emerging seedlings emerged (Damayanti et al. 2010). Researchers in Uganda selected seed from symptomless hot peppers to develop a line for farmers that had decreased virus levels and greater seedling vigor. From initial plants, viruses can be spread mechanically (through handling during transplant, pruning, and picking).

Seed transmission has not been demonstrated with viruses that are -transmitted by whiteflies and only rarely by thrips, and not in vegetable crops. Thrips may transmit seed-borne Ilarviruses such as *Tobacco streak virus* in okra in Indonesia (Table 3.2), but most likely only mechanically via pollen movement.

Clean seedlings produced in bulk for transplant may be infected early via vectors from virus-infected abandoned fields, if not protected from the vectors, usually aphids, thrips, or whiteflies.

Understanding the virus-vector interactions is crucial in managing both the vector and the virus/virus disease. The best approach is an IPM approach based on cultural practices, biological control, and chemical control methods (Chavan 2015). This follows the same principles proposed by Zitter and Simons (1980) in controlling viruses by managing virus vectors, virus transmission by vectors, and cultural practices. Cultural practices include controlling the virus inoculum sources, vector, control, isolation from potential sources of virus and vector, isolation by time, barrier crops, interplanting, and adjusting plant density (Zitter and Simons 1980).

The success of chemical control of virus vectors depends on mode of transmission of the virus by the insect vector. Insecticides are more effective in controlling semi- and persistently-transmitted viruses and are not effective against non-persistently transmitted viruses (Lecoq and Katis 2014). Insecticides will actually increase the rate of transmission on non-persistently transmitted viruses as these pesticides increase the probing incidence and therefore potential for acquisition and transmission (Chavan 2015). Using mineral and vegetable oils can be helpful in managing virus vectors and reducing transmission of non-persistently transmitted viruses when virus inoculum pressure is low or moderate (Lecoq and Katis 2014). One adverse effect of mineral and vegetable oils is the potential phytotoxicity that depends on concentration, crop, and environmental factors (McDonald et al. 2004).

Insecticides should be used in combination with other control methods for efficient management of whiteflies and whitefly transmitted viruses. Neonicotinoids are among the most suitable chemical insecticides for controlling whitefly populations as they quickly reduce the population and therefore reduce their chance to transmit the virus. Insecticide resistance development and adverse effects on pollinators are serious concerns and a reason why several countries have banned or put forward plans to eliminate the use of neonicotinoids (Lapidot et al. 2014).

Megasari et al. (2014) reported that chitosan was effective in suppressing *Aphis cracivora* (a vector of BCMV) populations and feeding preference and lead to reduction in the BCMV titer, incidence and severity in yardlong beans in Indonesia. Chitosan (0.9%) as a foliar application was the most effective chemical for controlling both the vector and the disease (Dayamanti et al. 2010; Megasari et al. 2014).

As a result of limitations to effective management of insects by chemical insecticides the IPM concept is a necessity. Other IPM components used to manage virus vectors include the use of row covers (cloth, net, plastic), and screen/plastic houses or tunnels (Fig. 3.5). A limitation to smallholder farmers is the additional cost and the return on investment (in Nepal, a smallholder farmer will recover cost of a $20 \times 3m$ plastic tunnel after 1 year). Large growers in the LAC region have experienced virus diseases in moderately contained greenhouses with thrips-transmitted tospoviruses in pepper and tomato (Martínez et al. 2014; Batuman et al. 2013a) and with PMMoV in peppers.

Growing seedlings in plastic trays with coco-peat with or without *Trichoderma* and under net covers (Fig. 3.6) has been field tested in India and resulted in lower virus incidence in the nursery and in the field after transplanting compared to seed-lings raised in nursery beds in open field nurseries (Fig. 3.7). This technology was subsequently transferred and disseminated in Bangladesh, Nepal (Fig. 3.8), and other IPM IL host countries. Another successful technology developed and disseminated by this program is roguing infected/symptomatic seedlings in the nursery before transplanting and subsequent roguing of infected/symptomatic plants in the field after transplanting. This practice was especially successful in managing thrips-transmitted PBNV in tomato in India (Naidu 2012) because it reduced the inoculum source and lowered the spread and disease incidence in the field.



Fig. 3.5 *Left* – Growing cucurbit plants under row covers in Gazipur, Bangladesh. A fine mesh is used to reduce virus vectors and other insects from damaging the crop. *Middle* and *right*: Mesh house in Salama Valley, Guatemala (tomato) to exclude whitefly vectors of begomoviruses



Fig. 3.6 Commercial nursery seedling production in Coimbatore, Tamil Nadu, India. Seeds are planted in plastic trays, using coco-peat inoculated with *Trichoderma* as the growth medium, under net covers. Yellow sticky traps and roguing are also used

Planting infected seeds poses a threat to farmers and international trade (Lecoq and Katis 2014) and use of infected seeds has been a factor for rapid and long distance spread of several virus diseases, especially in cucurbits (Lecoq and Desbiez 2008). Commercial seed companies have used disinfection (Trisodium phosphate)



Fig. 3.7 Open field tomato nursery - Coimbatore, Tamil Nadu, India



Fig. 3.8 Farmers in Kathmandu Valley, Nepal raising their seedlings in plastic trays, using cocopeat inoculated with *Trichoderma* as the growth medium, under net covers. Yellow sticky traps and roguing are also used

and dry heat (72 °C for 24–72 h) without affecting seed germination. However these treatments are not applicable in small scale seed production or where farmers produce their own seeds (Lecoq and Katis 2014). Furthermore they are effective only for tobamoviruses that contaminate the surface of the seed, and not for the embryo infecting potyviruses and CMV.

Several cucurbit viruses such as ZYMV are seed transmitted and virus symptoms occur during early stages of seedling growth and development (Figs. 3.3 and 3.9). On several field visits to Bangladesh, India, and Nepal, IPM IL scientists observed more than 90% virus incidence in cucumber, pumpkin, and squash in the seedling stage or before flowering. The resulting virus disease can lead to severe yield loss and reduction in food and income. Saving seeds only from healthy plants or using certified hybrid seeds can be used as one IPM component, in addition to managing aphid vector populations.

Avoiding over lapping crops, especially in cucurbits and other crops that have a short growing cycle where farmers grow several consecutive crops, is crucial in reducing both the virus and vector (Lecoq and Katis 2014).

Relying on chemical pesticides for control of tomato pests is the most common control method used by farmers in Mali. However, this is not very effective against whiteflies (Nouhoheflin et al. 2007).



Fig. 3.9 Seed-borne viruses are a serious problem in cucurbit production. About 90% of the seedlings in this field in Nepal showed virus-like symptoms. Plants tested positive for ZYMV. *Left*: Naidu Rayapati (WSU) showing farmers how to recognize virus-like symptoms in the field. *Right*: close-up of mosaic symptoms on squash



Fig. 3.10 Continuous cropping provides an inoculum source of virus and vectors to infect newly planted crops. *Left*: new tomato crop planted adjacent to older crop where leaf curl disease was detected (Kathmandu Valley, Nepal); *Right*: Women farmers planting a field of tomato adjacent to a crop infected with a thrips-transmitted virus (Coimbatore, Tamil Nadu, India)

Host free periods have been the most successful strategy for management of whitefly-transmitted begomoviruses. This requires an area-wide approach and has been successful in managing TYLCV in the Dominican Republic and Mali and saving the tomato industry in these countries (Gilbertson 2011; Noussourou et al. 2008; Palmieri et al. 2008). Since these viruses persist in the vector but do not pass to the next generation, growing plants that are not hosts to the virus allows whitefly populations to be "cleansed" of virus, thus eliminating the source of virus for new tomato crops. Impact assessment studies reported by Nouhoheflin et al. (2010) showed that combining a host-free period and virus-tolerant seeds as a component of an IPM strategy resulted in \$4.8 million to \$21.6 million in benefits in Mali. The benefit was divided into one third for producers and two thirds for consumers. Similar strategies could be used on a smaller scale by crop rotation and by avoiding continuous or adjacent planting of the same crop to reduce exposure to a source of virus inoculum (Fig. 3.10).

Genetic and Induced Resistance

Varietal selection and breeding for genetic resistance, if available, is an important component of an IPM Package, but has not been an activity of the IPM IL other than informational. There have been several reports of applications of biologicals including *Trichoderma* and *Pseudomonas* to reduce symptom severity and decrease losses to virus disease. The mechanism is proposed to be induction of resistance to the virus, or an increase in tolerance. Research reported from India, Indonesia (Damayanti and Wiyono 2013) and Guatemala – Annual Report 2013- suggest these approaches should be considered for IPM Packages with crops having severe virus disease problems. Several reports suggest that chitosan induces systemic resistance against viruses. Noiket et al. (2014) reported that chitosan treatment improved seed germination and growth of the tomato variety Thai Sridathip 3 and reduced TYLCV symptoms. Chitosan formulation with *Pseudomonas* sp. reduced the severity of ToLCV in India (Mishra et al. 2014).

Training and Capacity Building

The IPVDN, in cooperation with the Diagnostics Global Theme and Regional Sites, has provided training in these identification methods for scientists from many of the collaborating IPM IL host countries and for degree-seeking graduate students. The following are some of the training activities conducted in the last phases of the project. In July 2008, a week-long phytopathological diagnosis workshop in Guatemala, organized by IPVN, included more than a day of virology for participants from Central American countries and Jamaica (Fig. 3.11). During Phase IV of the IPM IL (2009–2014), the IPVDN hosted 35 training programs with the participation of 1185 people, (85% men and 15% women). In total, we conducted 16 workshops, 13 specialized trainings, 3 farmer meetings, 3 conferences/seminars. These were tailored to the specific needs of scientists, technicians, extension agents, students, and farmers. The IPM CRSP/IPM IL completely or partially supported more than 30 scientists/technicians in their BS, MS, or PhD degrees, in which IPVDN scientists guided their thesis/dissertation research and other activities related to virus diagnostics, virus management and IPM.

In November 2010, IPVDN organized a workshop on "Management of Viral Diseases of Vegetable Crops" in Honduras. The purpose of this workshop was to train field extension agents on virus disease management and how to integrate this into an IPM approach. About 90 participants, including more than 45 extension agents of the projects EDA-MCA and USAID-RED, the most important providers of assistance to small vegetable growers in Honduras, attended this workshop. In addition, participants included 20 field extension workers of the development agencies/projects Visión Mundial (World Vision), FUNDERH, FHIA, Global Village (Aldea Global) and PROMIPAC/Zamorano, and 15 agents representing seed companies and local distribu-



Fig. 3.11 Workshop participants in the Disease Diagnostic Workshop, Guatemala from Guatemala, Honduras, El Salvador, Nicaragua and Jamaica

tors of other agricultural inputs. Another workshop organized by IPVDN in Honduras in 2010 focused on "Potato psyllid/*Ca*. Liberibacter solanacearum, a new bacterial-insect vector complex causing diseases of potato and tomato in the Americas." The objective of the workshop was to inform professionals that the causal agent for this disease was not a virus and the focus was on psyllid, not whitefly, vectors.

In July 10-13 2012, the IPM IL and USDA sponsored a plant virology symposium entitled "Management of Insect-transmitted Virus Diseases in Vegetables in the Tropics and Subtropics," held at Tamil Nadu Agricultural University, Coimbatore, India. The main purpose of this symposium was to review the current status of insect-transmitted virus disease management in the tropics and subtropics. Plant virologists and entomologists from the U.S., India, and IPM CRSP host countries including Bangladesh, Ghana, Honduras, Indonesia, Senegal, Uganda, and Tanzania discussed the current status of research, education, and extension relevant to the management of virus diseases. Collaborating scientists focused on building multidisciplinary global expertise to address insect-transmitted virus diseases impacting agricultural sustainability and food security in developing countries. Discussions focused on emerging and re-emerging virus diseases, especially those of vegetable crops in IPM CRSP host countries, and establishing a coordinated program on identification and management of virus diseases affecting cucurbits, eggplant, okra, pepper, and tomato. A plan for future collaborative research under an IPM CRSP global theme project, the International Plant Virus Disease Network was discussed.

Participants Focused on the Need for:

- Developing standard operating procedures (SOPs) for virus identification and protocols for collecting, shipping and processing samples and validation of diagnostic test results.
- · Developing local networks of experts for each IPM CRSP region
- Devising a reporting mechanism for recording incidence of plant virus diseases and evaluating host plant resistance
- Documenting the impact of virus diseases and benefits of their management to convince donors to fund R&D activities related to virus diseases in small-holder agriculture

In April 2014, the IPVDN conducted the "IPM Innovation Lab plant virus disease global theme workshop on seed-borne virus diseases of vegetable crops" in Nepal. Participants included representatives of the Department of Agriculture, Nepal Agricultural Research Council (NARC), USAID, International Development Enterprises (iDE), Knowledge-based Integrated Sustainable Agriculture and Nutrition project (KISAN), Center for Environmental and Agricultural Policy Research, Extension and Development (CEAPRED), Agrovets, Himalayan College of Agricultural Sciences and Technology (HICAST), and agricultural universities. Discussions covered the basic aspects of plant virus diseases, detection and diagnosis, epidemiology, and management. Round table discussions focused on virus diseases of cucurbits, tomato, okra, and pepper. A capacity building session tackled the needs in terms of formal education, training of practitioners, research, and extension. Participants were divided into four groups and each discussed the four categories and suggested needed strategies to build the capacity in plant virology in Nepal.

In June 2014, the IPM IL organized an "International Workshop on Seed-and-Seedling-borne Diseases of Vegetable Crops" in Hyderabad, India. This workshop was held in collaboration with the USAID mission in India and the Indian National Institute of Plant Health Management, Hyderabad. The main purpose of this workshop was to present results over time from the IPM-IL research programs, the current status of seed-borne diseases, and the role of the seed supply chain in their management.

In April 2015, the IPM Innovation Lab (IPM IL) organized a workshop on "Disease Diagnosis and Basic Plant Pathological Techniques for Early Career Scientists" that was conducted in collaboration with NARC and iDE, Nepal at the regional NARC station in Khajura, Banke (Fig. 3.12). The main objective of this workshop was to provide training on plant disease diagnosis and management with emphasis on bacterial, fungal, and viral diseases.

The participants (29 males; 4 females) of the workshop were drawn from NARC stations across Nepal, faculty and students from the Institute of Agriculture and Animal Sciences/Tribhuvan University, HICAST, Agriculture and Forestry University (AFU), and field officers from the IPM Innovation Lab-Nepal/CEAPRED. The participants studied the principles of identification, isolation, and purification of bacterial and fungal diseases, and investigated extraction and testing of botanicals against fungal diseases. A major focus was on plant virus diseases, especially those of importance to Nepal and South Asia. Topics covered basic virus



Fig. 3.12 Workshop participants, Disease Diagnosis and Basic Plant Pathological Techniques, for Early Career Scientists Khajura, Banke, Nepal

characteristics, epidemiology, and management. Special lectures and laboratory sessions addressed various aspects involved in the detection of plant viruses and the capacity to conduct these techniques in NARC. Participants received hands-on experience in identifying virus symptoms in the field, collecting samples for disease identification, and conducting serological techniques (ELISA, Immunostrips) for virus detection. This validated the fact that such diagnostic assays can be successfully conducted with minimal facilities to achieve intended results and impacts in NARC stations.

IPM IL scientists from the US contributed to the sections covering plant virus diseases and NARC scientists contributed to fungal and bacterial diseases sessions – this is an example of drawing on local expertise and supplementing it with expertise from the US to train the next generation of agricultural scientists and extension personnel for overall capacity building in Nepal. This workshop was the result of continued collaboration between IPM IL and NARC and highlights the exemplary partnership among IPM IL, NARC and NGOs like iDE and CEAPRED.

IPVDN Achievements

Highlights in Asia

 Bean common mosaic virus and Cucumber mosaic virus were associated with virus disease epidemics in yardlong beans in Indonesia. Both viruses are seedborne and aphid-transmitted. Transfer of this technology and diagnostic methods to Indonesian scientists helped to contain the disease in subsistence agriculture. These results were acknowledged in USAID EGAT Bureau for Economic Growth, Agriculture & Trade, vol 1.1, 2009 under the title "A team of scientists from the EGAT-managed Integrated Pest Management Collaborative Research Support Program (IPM CRSP) and Indonesia's Bogor Agricultural University identified a new virus disease that is devastating yardlong bean crops in Java, Indonesia."

- In India, capacity building in plant virology was undertaken at the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Scientists at this institution are now able to identify the most prevalent plant viral diseases in their state, including discovery of newer, economically-important vegetable viruses that had not yet been reported in India.
- Farmer participatory IPM packages were implemented in Tamil Nadu for mitigating negative impacts of the thrips-transmitted *Peanut bud necrosis virus* (PBNV) on tomato production. Raising clean tomato seedlings and roguing of symptomatic seedlings during and soon after transplanting reduced virus incidence and gave economic benefits to resource-poor farmers who had previously incurred costs for spraying pesticides in unsuccessful efforts to control virus spread by thrips vectors. The IPM IL team worked collaboratively with farmers' groups and research and extension personnel in several tomato-growing regions to disseminate benefits of IPM as an affordable strategy to reduce crop losses due to PBNV and produce quality fruits for consumers (Naidu 2013).
- In Nepal, Bangladesh, Cambodia and Tajikistan, the information generated by science-based identification of viruses infecting vegetables is helping scientists learn if a virus is likely to be transmitted by seed, and whether aphids, whiteflies or thrips are the vectors. With accurate identification of viruses, NGOs and farmers can implement crop sanitation and disease management strategies benefiting poor farmers.
- Central Asian countries were found to have very little knowledge of viruses. Training
 was initiated by a scientist from Uzbekistan, but the program was later limited to
 Tajikistan. In conjunction with a training workshop to an eager audience, viruses were
 documented from potato, onion, and tomato, but no additional work was conducted.

Highlights in Africa

- A whitefly-transmitted begomovirus was identified as the cause of a leaf curl disease of tomato in Mali. A host-free period in which no tomato could be grown in an area for 2 months, following practices in place in Dominican Republic and Guatemala, restored tomato production in the Baguineda area.
- A complex of diverse begomoviruses and viroids have recently been identified as causes of severe leaf curl diseases and a stunting disease known locally as "rasta" in tomato in Ghana.
- Passion fruit woodiness in Uganda and Kenya is associated with potyviruses that are very closely related to *Cowpea aphid-borne mosaic virus* (Ochwo-SSemakula et al. 2012; Otipa et al. 2013). Detection methods by ELISA and PCR have been developed, and new laboratories constructed in which the tests can be performed. Management strategies have been targeted to virus-free seedlings, and educational workshops have been held for training of seedling producers (Ochwo-

Ssemakula et al. 2013). Interventions to decrease dissemination in the field by aphids are also under study.

- Tomato surveys in Uganda by ELISA detection show up to 60% virus infection, with major incidence of TMV and ToMV in farmers' fields (Arinaitwe et al. 2013). Capacity now exists in Tanzania for additional surveys in the region to document the prevalence of begomoviruses in tomato.
- Diagnostic workshops with the Diagnostics global theme (IPDN) have been held in East (Ghana) and West (Tanzania) Africa, and SOPs (Standard Operating Procedures) have been drafted for standardized detection of TYLCV and viruses associated with passion fruit, and other viruses.

Highlights in Latin America and Caribbean Region

- Capacity building for virus identification and management trained Universidad del Valle de Gutemala (UVG) personnel and students, joint workshops conducted by in-country and US collaborators. Three women scientists, two from Jamaica and one from Guatemala, earned graduate degrees studying viruses in the United States.
- Whitefly vector biology and diversity two species of whitefly, *Bemisia* and *Trialeuroides*, are vector species at different altitudes and regions of Guatemala. Vector population diversity was assessed in association with the begomoviruses transmitted. Thrips were shown to be important vectors of tospoviruses in greenhouse peppers in Dominican Republic and Guatemala.
- High incidence of PVX in potato and TMV in tomato in Western Highlands of Guatemala shows the need for clean seed and seedling programs and improved sanitation practices.
- Sweet potato virus diagnostic probes have been designed and validated to enable developing seed certification activities with sweet potato in the Central America region of their origin (Avelar and Brown 2014).
- Tree tomato viruses in Ecuador were identified as PVY and *Peru tobacco mosaic virus*, both aphid-transmitted potyviruses. In Ecuador, the first survey for cucurbit viruses identified the tospovirus MYSV, which is the first report of this virus in the New World.
- In Honduras, the non-viral cause of Zebra chip of potato was identified, together with its psyllid vector. Vector phenology patterns were studied, leading to recommendations for management practices (Rehman et al. 2010).

Highlights Across Countries and Regions

- Potato viruses identified by host country scientists in Guatemala and Indonesia were the same viruses, showing the need to virus-test seed and propagules, to aid subsistence farmers whose practice of using self-saved potato as seed has led to a build-up of viruses.
- Common diagnoses of TMV and ToMV in tomato in several countries (Guatemala, Uganda, Nepal, India), and the persistence of these viruses on seed and their highly contagious nature to spread by contact, suggests the need for sanitation practices in hybridization, grafting, transplanting, and cultivation in all countries where tomatoes are intensively grown in fields and in contained houses.

- Discovery of seed-borne viruses TSV in okra and CMV in eggplant that pose severe threats to major vegetables grown for food in Mali and Bangladesh, and grown in Central America as oriental vegetables.
- Understanding the taxonomic status of the whitefly *Bemisia tabaci* sibling species complex in relation to begomovirus-vector interactions, and the genetic diversity of vector haplotypes in relation to the pathogen genotype, for both the whitefly and psyllid vectors in the context of changing climate and agricultural systems of tropical countries.

Top Ten Recommendations

Based on IPM IL work, and our interactions with host country scientists, extension agents, plant protection specialists, and plant protection students, discussions with plant virologists, plant protection and IPM specialists at professional meetings, USAID workshops, and professional symposia we propose the following as general recommendations to strengthen the capacity of local scientists in identification of viruses and diagnostic capabilities.

Diagnostics

- 1. Conduct workshops and training in host counties in house
- 2. Support national centers in developing diagnostic labs and methods
- 3. Establish centers of excellence that help with regional training, testing, and in time help transition to individual country institutions
- 4. Need to update overall poor laboratory infrastructure, in terms of equipment, electrical power, and skilled personnel
- 5. Need to standardize testing
 - (a) Sampling and sample preservation
 - (b) Shipping
 - (c) Protocols
 - (d) Validation
- 6. Need quick tests for field work
- 7. Need to develop low cost tools, kits, tests
- 8. Progress has been made and we should be positive and should create new systems to continue going forward
 - (a) An increasing number of labs are doing good diagnostics work
- 9. Train extension workers, farmers, technicians on diagnostic methods, proper identification
 - (a) How to interact with diagnostic labs (public or commercial)
 - (b) Need effective communication

- 10. Every country needs to have the capability to do pest/disease diagnostics
 - (a) National level
 - (i) Time may be limiting to wait for results from regional labs/centers of excellence
 - (b) Need certified accredited labs

Capacity Building

- 1. Networking, in person and virtual, and up-to-date information on people, projects, and opportunities.
- 2. Journal access (reading and publishing).
- 3. Inter-Africa collaboration between institutes (international and national programs, universities, international centers)
- 4. Build South-South collaborations
 - (a) South-South: National partners should be at the core of the project
 - (b) EU model of institutional collaboration across a region.
- 5. Web resources for networking literature
- 6. Imposed mixing of people at conferences
- 7. South-south communication and collaboration, especially more within Africa
- 8. Establish a type of "community of practice" information and support for materials exchange (quarantine capacity)
- 9. International exchanges with incentives for students to return to their home countries
 - Follow-up grants
 - Policy changes
 - Study other successful models. (India, China salaries and funding, facilities)
- 10. Integration/synergy of projects on common themes.

A quarter century ago Bos (1992) noted that "In the developing countries, knowledge regarding viruses and their ecology and detectability remains essential". Our recommendations mirror those suggested by Bos (1992) regarding the survey/identification of viruses and diagnostic tools/methods, understanding the ecobiology of viruses, and developing comprehensive control strategies for virus diseases in developing countries. There still exists a pressing need to train a future generation of plant virologists and plant protection specialists, especially at resource poor institutions in developing countries. The IPM IL IPVDN scientists' major paradigm shift was to train the host country participants and build their capacity and confidence in disease diagnosis and virus identification and management.

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