

Recovery Plan for *Cowpea mild mottle virus*, a seedborne carla-like virus

Judith K. Brown
School of Plant Sciences
University of Arizona, Tucson

and

Jose Carlos Verle Rodrigues
University of Puerto Rico, San Juan

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Cowpea mild mottle virus (CpMMV)

Origin (endemism): Africa: Kenya (1957), Ghana (1973)*

Host: groundnut *Arachis hypogaea* L (1957, 1997, Sudan) cowpea *Vigna unguiculata* L. (1973)

Distribution: now, worldwide in all legume growing locales (27+ documented) but **importance in soybean** unrealized until recently.

Transmission

- Whitefly-transmitted in non-persistent manner; *Bemisia tabaci* (Genn.) sibling species group (Muniyappa, 1983) AAP 10 min, IAP 5 min
- Mechanically transmissible, experimentally
- Seed borne to varying extents in different species and varieties of same species; particularly severe in certain soybean varieties.

*often cited as the first report because the disease went largely unnoticed until then

Likely multiple strains, but largely uninvestigated

Synonyms:

Groundnut crinkle (Dubern and Dollet, 1981)

Psophocarpus necrotic mosaic (Fortuner et al., 1979)

Voandzeia mosaic (Fauquet and Thouvenel, 1987) in the Cote d'Ivoire, tomato pale chlorosis in Israel (Cohen & Antignus, 1982)

Tomato fuzzy vein in Nigeria (Brunt & Phillips, 1981)

Bean angular mosaic virus in Brazil (Costa et al., 1983; Gaspar et al., 1985) shown to be serologically most closely related to CPMMV = proposed, distinct strains

Isolates from solanaceous hosts in Jordan and Israel, although very similar to West African and Indian legume isolates, considered to be distinct strains – more information needed to confirm and differentiate (Menzel et al., 2010).

Wide variation in virulence of CPMMV isolates from other countries also reported (Anno-Nyako, 1984, 1986, 1987; Siviprasad and Sreenivasulu, 1996).

Take home message: Multiple strains, variable virulence, host range, other soybean infecting isolates among the most severe

Symptoms

Soybean: stem necrosis, death of plant



Angular leaf spot Phaseolus (Brazil)



Mosaic in bean, usually caused by BCMV



CpMMV in soybean – field inoculated by whitefly



‘Carlavirus-like’ - caveats

- Although CPMMV is classified as a carlavirus (Adams et al., 2005; Naidu et al., 1998) – it is serologically unrelated to 20 or more aphid-transmitted carlaviruses (Brunt and Kenten, 1973; Veerisetty and Brakke, 1977; Adams and Barbara, 1982; Brunt et al., 1982; Gaspar et al., 1985).
- May not be surprising because it differs from other carlaviruses in that it is transmitted by the whitefly *Bemisia tabaci* (Genn.) sibling species group.
- Further it produces brush-like inclusions (Brunt et al., 1983; Thongmeearkom et al. 1984; Gaspar and Costa, 1993a) that are **unique among the carlaviruses described thus far** (all aphid-transmitted).
- And, unlike the majority of aphid-transmitted carlaviruses, and CpMMV is seed-transmitted.

Expansion of CpMMV distribution

- Edwardson and Christie (1986) reported CpMMV could successfully infect forty-six plant species in ten botanical families, mainly Leguminosae.
- CpMMV was identified in soybean crops in Argentina and Brazil during 2001-02, initially with losses as high as 85% in Bahia State, Brazil (2400 acres).
- Since then CpMMV has become the primary virus of important affecting the soybean crop (Almeida et al. 2003, 2005); infection of the most susceptible varieties results in $> 85\%$.
- About the same time, losses associated with CpMMV infection occurred following unprecedented whitefly outbreaks in Indonesia; losses estimated between 11-56% (Akin 2003).

Breeding program in Brazil currently addressing this new threat, with some success

Almeida et al. (2003) screened 174 Brazilian soybean varieties by mechanical inoculation w/ severity rating scale:

26 % were resistant

32 % susceptible

41 % variably susceptible

Selected genetic materials are now in use as sources of resistance for Brazilian production conditions

In addition, authors reported thirteen biological distinct virus isolates, now incorporated into resistance trials.

Latest developments

Rosario et al. (2014) reported a CPMMV isolate harbored by a field-caught whitefly (Next Gen sequencing) in Florida, then native and cultivated plants - more closely related to Brazilian and Puerto Rico isolate (partial seq), versus 1973 Ghana isolate, at 98–99% AA identity

Zanardo et al. (2014) – replicase gene from a CpMMV isolate from soybean in Brazil shares 60-61% nt sequence identity with the Ghana isolate.

Also, evidence of recombination within CpMMV isolates, mainly in the polymerase gene

Offshore discovery – Puerto Rico

- In Puerto Rico soybean is grown in winter nurseries to advance breeding programs by several seed companies.
- However, recently, winter nursery seed increases constrained by a virus-like disease: dwarfing, shoot die back, and stem necrosis of soybean nurseries, making the damage intolerable.
- Symptoms of leaf vein and stem necrosis and plant stunting were observed in soybean experimental plots in the municipalities of Juana Diaz, Santa Isabel, Isabella and Guayanilla, in Puerto Rico.



Observations in a winter nursery in Santa Isabel showed 100% incidence and total yield loss in some soybean lines, **underscoring the potential of damage in soybean** if it were to infect the US mainland soybean crop.

During 2010, 100% infection was observed in soybeans lines planted by the Illinois Crop Improvement Association at Juana Diaz, Puerto Rico, and in experimental soybean field blocks at the Agric. Exp. Station, Juana Diaz.

Preliminary observations indicated that the disease resulted from the initial infection coming from seed and then from secondary spread following whitefly-mediated inoculation.

Necrotic reaction of soybean in PR to field infection



Phase II symptoms-death

Stem, veinal, and pod necrosis, & death



Pod
necrosis,
death



Mock-inoculated soybean control



Closeup
Veinal
necrosis

Soybean – Symptom I
Juana Diaz



Soybean – Symptom II
Juana Diaz



Beans
Juana Diaz



Differential variety responses: mechanical inoculation



Mechanical inoculation – necrosis at spreading edges of inoculation site

Phase I – local symptoms



Phase II - systemic symptoms



Soybean seed is so debilitated, difficult to germinate



Summary

- The vulnerability of the legume crop (particularly soybean and at least susceptible *Phaseolus* spp) to infection by CpMMV is of particular concern because it is both seed and whitefly vector borne.
- Even a very low level of seed transmission followed by whitefly transmission could lead to the rapid and widespread distribution of CpMMV if left unchecked.
- The most proximal isolates to the US mainland occur in the Caribbean region. CpMMV was found in Florida (whitefly, so host is not known).
- Seed is transported from PR to many locations in the US and elsewhere for breeding efforts.
- Appears to represent a time-bomb scenario: immediate recourse is highly recommended.

Mitigation and management

Integrated approaches should be used to management of the disease, which may include control of the vector using insecticides, controlling weeds and other alternate hosts (once known).

The lack of understanding of viral genomic diversity and biological characteristics of the different isolates has hindered diagnostics development to ensure virus detection when it is present.

Efforts are needed to understand the ecology, molecular epidemiology, host-plant genetics, and risk posed by CpMMV to soybean and other legumes.

Without such knowledge, developing and sustaining durable virus-resistant varieties would seem to represent a difficult undertaking.

Immediate Research Recommendations

- Assess CpMMV genomic variability in Puerto Rico strains in relation to elsewhere (Next Gen Seq)
- Based on results, develop molecular diagnostics to discern strains based on variability.
- Establish panel of isolates or strains and characterize them biologically (experimental & natural host range).
- Screen ‘strategic’ soybean and other legume germplasm to determine risk: infectivity, % seed transmission (seed grow outs and molecular diagnostics).
- Screen strategic soybean and other key legume germplasm panels for resistance to genomic variants (panel)
- Develop program to assure movement of clean seed in and out of Puerto Rico, Brazil, and other locations used for breeding.
- This could be done at the new Center for Excellence in Quarantine & Invasive Species, UPR-San Juan.



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Thank you

Historical overview

- *Cowpea mild mottle virus* (CpMMV) was first identified in symptomatic cowpea (*Vigna unguiculata* L.) in Ghana during 1973 – considered of minor importance (Brunt and Kenten, 1973).
- Bock et al. (1976) reported CpMMV in peanut (groundnut) (*Arachis hypogaea* L.) in Kenya, and concluded with further investigation that the disease had been recognized there previously, but as 'Ngomeni mottle' (Storey & Ryland, 1957).
- CpMMV was thereafter reported in 27 countries + all continents where legumes are grown - exact distribution and extent of damage undetermined – more widespread than realized?
- CpMMV was first reported in Brazil in 1982 - losses associated with infection of *Phaseolus* – losses were considered to be minimal (Costa et al., 1983).

Conflicting reports...

- Reported to have little effect on the growth and yield of infected plants in cowpea crops in Ghana (Brunt & Kenten, 1973), Nigeria (Anno-Nyako, 1984), Papua New Guinea (Philemon, 1987); mung bean and French bean in Tanzania (Mink and Keswani, 1987); French bean and **soybean** in Brazil (Costa et al., 1983).
- In contrast – yield loss of 64-80% in groundnut in Kenya (Bock et al., 1976, 1977); conspicuous leaf chlorosis and plant stunting but unquantified yield losses of infected groundnut, soybean, bambara groundnut and winged bean in Cote d'Ivoire, Ghana, India, Indonesia (Fauquet et al., 1979; Thouvenel et al., 1982; Fauquet and Thouvenel, 1987; Offei and Albrechtsen, 2005).
- The causes of the wide range of host responses to infection have yet to be determined - probably due to:
 - differences in virulence of the virus isolates**
 - differential susceptibility of the cultivars of host species**
 - widely different environmental conditions**

Encodes six ORFs arranged like other known carlaviruses: 3' untranslated region of 120 nt shares 78-92% identity (Badge et al., 1996; Gaspar et al, 2008; Naidu et al., 1998) with seven isolates (partial sequences) (ICTV website; NCBI GenBank db).

Based on predicted amino acid sequences of carlaviruses (Menzel et al., 2010) CpMMV shares 46-59% identity with 8 aphid-borne carlaviruses = thereby **falling below the 80%** species demarcation cutoff (Adams et al., 2005) confirming species distinct species status.

Recently discovered CpMMV isolate from soybean in India shares only **75-79% nt** identity with the seven extant isolates (Yadav et al., 2013) = distinct strain?