Recovery Plan

Cowpea mild mottle virus
Carlavirus: Betaflexiviridae*; order *Tymovirales

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Among legume crops in the U.S. soybean production occupies about 75,000,000 acres valued at approximately 37 billion dollars in 2012) (http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/related-data-statistics.aspx.). Other legume crops such as common bean (fresh and dry), cowpea, garbanzo bean (chickpeas), and peanut account for variable, but substantial acreages of legumes devoted to commercial (traditional and organic) production in many US states (http://www.nass.usda.gov/).

This report addresses the exotic seed borne virus, *Cowpea mild mottle virus* (CPMMV) (genus, *Carlavirus*; family, *Betaflexiviridae*; order *Tymovirales)* that infects a wide range of cultivated legumes and thereby poses a threat to US soybean production. The virus causes severe mosaic and/or necrotic symptoms in leaves, stems, and pods in many bean (*Phaselous* species), cowpea (*Vigna* species), and soybean (*Glycine max*) varieties grown in the Americas. CPMMV is believed to have been introduced first into South America from western Africa where it is presumed to be endemic, from where it spread into the Caribbean region.

Initially reported to infect cowpea in east and then west African countries the virus spread largely unnoticed to India and southeast Asia, and most recently, to the Americas and Caribbean region. The first detection in the Americas was in soybean crops in Brazil and Argentina where losses ranged from 10-100%. The virus has subsequently spread to Puerto Rico, and possibly also to Mexico, on infected seed from South America and perhaps Africa as well. In Puerto Rico soybean and bean seed are routinely planted in winter nurseries for legume breeding and commercial seed production efforts. In the U.S. mainland, an isolate has been detected using Next-Generation Sequencing (Illumina) in DNA extracts of the whitefly vector *Bemisia tabaci* (Genn.) in Florida during 2014.

The virus is transmitted in a non-persistent manner by members of the whitefly vector sibling species group, *B. tabaci* (Genn.), and importantly, through the seed of some legumes. Experimentally, CPMMV is readily mechanically transmissible through plant sap. The virus has an extremely broad host range among leguminous and non-leguminous hosts.

Currently, no monitoring is carried out to study the potential for, or to detect actual, introductions

of CPPMV into the USA. Because it has not been found yet in the US mainland, no specific expertise exists to detect the virus visually, and no molecular detection assays are available. To avoid a disastrous introduction and subsequent spread, there is a need to develop training materials, and to train diagnosticians and legume growers who may encounter this disease on beans, cowpea, and soybean in particular.

Also, efforts should be pursued to fully characterize the strain(s) occurring in Puerto Rico, and more recently, in Florida, and elsewhere (potentially) in legume growing areas of the USA, particularly where the whitefly vector prevails. If introduced into the U.S. mainland, CPMMV has great potential to spread through seed, on infected ornamental or vegetable transplants, and by the viruliferous whitefly, itself if previously associated with a virus-infected host. Further, nursery and vegetable seedlings destined for commercial and home gardener markets are now frequently produced in states far from where they are eventually planted, and so this route of spread could be quite significant, particularly when plants are transported within and between states in mild climatic conditions that produce substantial acreages of susceptible legume species, including common bean, cowpea, garbanzo bean, and peanut, and in some instances, soybean.

The introduction of the virus, which is most likely to occur through the accidental importation of infected seed from already infected locales, could pose a high risk to legume crop species. Thus, phytosanitary measures are recommended to avoid the introduction and spread throughout the US mainland, and for eradication in Puerto Rico, where it already occurs in winter soybean nurseries, and perhaps elsewhere by now. The distribution and incidence of CPMMV at the time of detection would guide response recommendations, which may include quarantine and/or eradication programs aimed at preventing further spread of the viral pathogen.

Cowpea mild mottle virus
genus, Carlavirus; family, Flexiviridae*; order *Tymovirales

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I. Introduction

First reports and economic damage. Initial and subsequent reports of the extent of damage and economic importance of CPMMV to legume crops have been inconsistent. For example, the virus was reported to have little effect on the growth and yield of infected cowpea crops in Ghana (Brunt & Kenten, 1973), Nigeria (Anno-Nyako, 1984), and Papua New Guinea (Philemon, 1987), of mung bean and French

bean in Tanzania (Mink and Keswani, 1987), and of French bean and soybean in Brazil (Costa et al., 1983). In contrast, CPPMV was responsible for 64-80% yield loss in groundnut (peanut) in Kenya (Bock et al., 1976, 1977). Elsewhere, in addition to annual losses in legume production resulting from CPMMV infection throughout western and eastern Africa, crop damage has been associated with whitefly outbreaks in Indonesia, resulting in 11-56% crop loss (Akin 2003; Time et al. 2010; Taiwo, 2003).

The causal virus. *Cowpea mild mottle virus* (CPMMV) is a single-stranded RNA virus encapsidated in a slightly curved,or 'flexuous' filamentous particle of 650 nm in size (Figure 1) that resembles those associated with members of the genus, *Carlavirus*. The virus forms inclusion bodies having a feather-like appearance in infected cells that differs from inclusion bodies observed for aphid-transmitted viruses (Figure 2) (Brunt et al. 1983).

The single-stranded RNA genome sequence of the type isolate of CPMMV is ~8,000 nucleotides (nt) in size, not taking into account the 3' terminal polyA tail (Menzel et al., 2010). The capsid protein monomer is ~32-36 kDa in size. The genome encodes six ORFs with an arrangement like members of the genus, *Carlavirus* (family, *Flexiviridae*; order *Tymovirales).* The CPMMV genome has been shown to undergo recombination, with the events detected thus far, occurring primarily in the polymerase gene and less frequently in other regions of the genome (Zanardo et al., 2014a).

Historical overview. *Cowpea mild mottle virus* (CPMMV) was first identified in Ghana during 1973 when it was detected in symptomatic cowpea (*Vigna unguiculata* L.) plants. The virus was found to be seed-borne to varying extents in three leguminous hosts examined, ranging from nearly 100% in soybean (*Glycine max* Merr.) and cowpea, to 1-4% incidence in common bean (*Phaseolus vulgaris* L.) (Bock et al.1975). Plants developing from CPMMV-infected seed were primarily asymptomatic or symptoms were inconspicuous, therefore, the virus was considered to be of only minor importance (Brunt and Kenten, 1973). Later, it was recognized that the disease of groundnut referred to as 'Ngomeni mottle' (Storey and Ryland, 1957) was caused by CPMMV, thus apparently the virus has been present in legume crops in Africa for a longer period of time than was initially realized. Also, Outbreaks of CPMMV were reported in peanut (groundnut) *(Arachis hvpogaea* L.*)* in Kenya and Tanzania during 1974-77 (Bock et al, 1975, 1977), and thereafter the disease was recognized as an impending, persistent threat to peanut production there (Bock and Waiigai, 1984). Soon after, CPMMV was reported in India and throughout Southeast Asia where it infected peanut and soybean crops (Iizuka et al., 1984; Iwaki et al., 1986). During 1992-94 the first outbreak of CPMMV disease occurred in Sudan, causing heavy losses (10-100%) in the irrigated peanut crop, grown along the Blue River and the White Nile River, however, rain-fed crops were found to be unaffected. Early-stage infected peanut plants produced no pods whereas, those infected during or after flowering experienced losses as great as 60%. The outbreak in Sudan was associated with heavy whitefly vector infestations and represented the first report of the virus having reached major economic importance in peanut (El-Hassan et al., 1997). Although CPMMV now occurs in at least 27 countries and on all continents where legumes are grown, the distribution and damage caused by the virus has not been determined in a comprehensive manner, suggesting that it probably occurs more widespread and in previously unreported locations than is currently known.

Current status in soybean in the Americas. Soybean (*Glycine max* Merr.) is one of the most important grain legume crops grown throughout the world for human consumption, being used for animal feed, oil production, confectionaries, and human consumption, among other purposes. Weeds, insect pests, and diseases are the major biotic stresses that limit soybean production. In recent years attempts have been made to develop stress tolerant/resistant and high yielding soybean varieties by several biotechnology companies. In addition to infecting legume crops in Africa and elsewhere worldwide where CPMMV occurs naturally, or has been introduced, most recently, CPMMV has become the primary yield-limiting virus infecting the soybean crop in Argentina and Brazil, since it was first identified in soybean in 2000-01 in Goias State (Almeida et al. 203; 2005). However, it should be noted that the first detection of CPMMV in Brazil was made in common bean (Costa et al., 1983), suggesting that the virus could have been introduced there on infected bean seed, although it is not possible to rule out the possibility that the initial introduction was by way of infected soybean seed. No trace-back has been carried out to identify the prospective source(s) of the infected seed, but if accomplished, could provide valuable epidemiological information relevant to breeding programs and diagnostics development.

In Puerto Rico, soybean is cultivated in winter nurseries to advance breeding programs by the United States Department of Agriculture, U.S. universities, and several seed companies. However, this effort has become constrained by widespread CPMMV infection of soybean plantings, placing a major limitation on the use of this valuable location for breeding objectives and for increasing seed during the winter months. During 2010 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 one winter nursery in Santa Isabel, 100 percent incidence was observed in certain of the soybean lines, indicating the extreme potential of this disease to undermine the present and future cultivation of soybean in Puerto Rico either in winter nurseries or during other times of the year (Rodrigues et al., 2014).

During 2010, 100% infection by CPMMV was documented in a number of soybean lines planted at Juana Diaz, Puerto Rico by the Illinois Crop Improvement Association. At the same time, virus infection were observed in experimental soybean field blocks located at the Agricultural Experimental Station in Juana Diaz, Puerto Rico. Preliminary observations indicated that owing to the magnitude of the losses, the outbreak was likely associated with infections that occurred during early stages of growth, and most probably, was seed borne.

II. CPMMV Disease Symptoms

The symptoms associated with CPMMV include foliar chlorosis, and at times, foliar necrosis, overall stunting of soybean plants (Figures 3-5). Infection by CPMMV causes similar symptoms but with varying severity and extent of yield loss in bambara groundnut, groundnut (peanut), soybean, and winged bean in Cote d'lvoire, Ghana, India, and Indonesia (Fauquet et al., 1979; Thouvenel et al., 1982; Fauquet and Thouvenel, 1987; Offei and Albrechtsen, 2005). The basis for the wide range of responses by plant hosts to CPPMV infection has not been determined. The differential symptom development observed in the same host species is thought to be due to differences in virulence between the viral isolates themselves, to the differential susceptibility among the different host plant species and their respective cultivars, and/or to widely variable environmental conditions that prevail in the different

CPMMV affected locales (Rosario et al., 2014; Jeyanandarajah and Brunt, 1993; Zanardo et al., 2014b). For example, CpMMV isolates from Israel and Ghana infect members of the Solanaceae (Antignus and Cohen, 1987; Brunt and Kenten, 1993), while the Florida and Brazilian, Florida, Iran, and Thailand isolates of CpMMV do not (Almeida et al., 2005; Iwaki et al., 1982; Tavasoli et al, 2009). Despite these differences, CpMMV isolates thus far have not been distinguished by electron microscopy or serologically, suggesting that host range and possibly RNA sequence divergence will provide the most robust differentiation of isolates, with extent of genome divergence potentially serving as one of the most important means of virus identification, together with biological characteristics.

In Puerto Rico the soybean isolates of CPMMV cause dwarfing, shoot die back, and stem necrosis. The major outbreaks there have been in soybean nurseries grown for seed production, making the extensive damage intolerable. Thus far, 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, Puerto Rico. Observations in a 2005 winter nursery in Santa Isabel showed 100 percent incidence in some soybean lines, indicating the potential of damage of this disease in soybeans. Symptoms were associated with flower dropping and poor development of the pods. Seed harvested from infected plants showed severe deformation and reduced weight, compared to seeds from healthy, virus-free plants (Rodrigues et al, 2008).

III. Biology, Spread, and Risk

Host range. Natural hosts include *Canavalia ensiformis*, groundnuts (*Arachis hypogaea*), *Phaseolus lunatus, P. vulgaris, Psophocarpus tetragonolobus*, soyabeans (*Glycine max*), tomatoes (*Lycopersicon esculentum*), *Vigna mungo,* probably eggplant *Solanum melongena,* the cowpea species: cv. Blackeye (*Vigna unguiculata*), *Vicia faba* and *Vigna subterranean*, and bean, *Vigna unguiculata* subsp. *Sesquipedalis*. The virus also occurs in various weeds (Fabaceae), including *Stylosanthes* and *Tephrosia* spp. Many more hosts can be artificially inoculated (Brito et al., 2012, Rodrigues et. al., 2014).

Vector transmission. The whitefly *B. tabaci (Genn.)* is reported to be the only insect vector of CPMMV. The whitefly vector transmits the virus in a non-persistent manner (Muniyappa, 1983), and therefore is non-circulative and unlikely to be transovarially transmitted. The first report of experimental whitefly transmission was from Japan with an isolate present there ((Iwaki et al., 1982). Also, *B. tabaci* was shown to transmit isolates of CPMMV from Israel (Cohen and Antignus, 1982), Brazil (Costa et al., 1983), India (Muniyappa and Reddy, 1983; Yadav et al., 2013), Nigeria (Rossel and Thottappilly, 1985; Anno-Nyako, 1986; Thottappilly and Rossel, 1992), Indonesia (Saleh et al., 1989), and Jordan (Monsour et al., 1998). These reports corroborate the association of whitefly with the disease outbreak in Sudan peanut crops during 1992-94 (El-Hassan et al., 1997). In Brazil the B biotype of *B. tabaci* was identified as the vector of CPMMV there (Marubayashi et al., 2010). CPMMV was successfully transmitted by grafting, mechanical inoculation, and by the B biotype (J.K. Brown, unpublished results) of the whitefly *B. tabaci* in Puerto Rico (Rodrigues et al. 2008, Rodrigues et al. 2014)

Seed transmission. The Ghanaian isolate, obtained from a seed-infected cowpea seedling in a vegetable garden, was subsequently shown to be seed-transmitted to unspecified levels in cowpea, soybean and French bean (Brunt and Kenten, 1973). CPMMV is also seed-transmitted in other plant species and other

countries. Several preintroductions of cowpea lines tested from Botswana, India, and Kenya were shown to harbor CPMMV (Gillaspie et a., 1995). However, reported levels of seed transmissibility of CPMMV isolates in different host species and countries are contradictory. Thus the virus was reported to be seed-borne to a level of 1-3% in cowpea in India (Nain et al., 1994), to 0.9% in soybean in Thailand (Iwaki et al., 1982), to 0.05-1.66% in 25 soybean cultivars in India, to 8 of 27 soybean cultivars in India (Yadav et al., 2013), to 6-21% in bambara groundnut in the Cote d'Ivoire (Fauquet and Thouvenel, 1987) and to unreported levels in soybeans in the Cote d'Ivoire (Fauquet and Thouvenel, 1987) and in cowpea in India (Mali et al., 1989), Burkina Faso and Swaziland (Hampton et al., 1992). A severe strain of CPMMV was shown to be seed-borne in groundnut in India (Sivaprasad et al., 1990). Thus far, no seed transmission has been demonstrated for the Puerto Rican CPMMV isolate, however, soybean seeds from virus-infected plants were shriveled and smaller compared to seeds from virus symptom free plants (Fig. 1). The symptoms that developed following graft-inoculation (Fig 2B), whitefly-mediated transmission (Fig. 2 A), and mechanical inoculation (Fig. 2C) were like those observed in the field-infected plants.

Epidemiology. The epidemiology of CPMMV has yet to be investigated in a comprehensive manner. The relative importance of infection in weeds and seed transmission as primary sources of inoculum for crop infection especially needs to be studied further, as does the importance of seed transmission in the accidental dissemination of virus from currently infected locales, some of which are endemic and others resulting from exotic introductions. Perennial weed species naturally infected by CPMMV are possibly important sources of infection for both tomatoes and leguminous crops in Jordan, Kenya, Nigeria and India (Bock et al., 1976; Anno-Nyako, 1984; Muniyappa and Reddy, 1983; Monsour et al., 1998), Similarly, seed transmission is known to occur in a large number of diverse legume crops. Therefore, it seems likely that plants that become infected through the seed would seem likely to serve as primary sites of infection for further spread by whiteflies within and to adjacent susceptible crops and weeds. The seed borne nature of the virus in cowpea and common bean, however not confirmed in soybean, suggests that the potential is great for inter-regional and international dissemination of CPMMV.

IV. Identification of CPPMV Strains, Detection, and Monitoring

Taxonomy and identification. CPMMV is a species in the genus, *Carlavirus (family, Betaflexiviridae*; *order Tymovirales (*Adams et al., 2005). Viruses (or diseases) such as *Groundnut crinkle virus* (Dubern and Dollet, 1981), *Psophocarpus necrotic mosaic* (Fortuner et al., 1979) and *Voandzeia mosaic virus* (Fauquet and Thouvenel, 1987) in Cote d'Ivoire, Tomato pale chlorosis disease in Israel (Cohen & Antignus, 1982), Tomato fuzzy vein disease in Nigeria (Brunt & Phillips, 1981) and *Bean angular mosaic virus* in Brazil (Costa et al., 1983; Gaspar et al., 1985) were shown to be serologically most closely related to CPMMV, and are so have been grouped under the same species name of CPMMV. Virus isolates from solanaceous host species in Jordan and Israel, although seemingly similar serologically and with respect to biological characteristics to West African and Indian legume isolates, have been considered as distinct strains, however, additional information is needed to clarify the identity and taxonomy of this group of isolates/strains (Menzel et al., 2010). Differences in the virulence of CPMMV isolates occurring elsewhere in additional countries also have been noted (Anno-Nyako, 1984, 1986, 1987; Siviprasad and Sreenivasulu, 1996).

Although CPMMV is classified in the *genus, Carlavirus* (Adams et al., 2005; King et al., 2011; Naidu et al., 1998), it has been shown to be serologically unrelated to 20 or more aphid-

transmitted carlaviruses (Brunt and Kenten, 1973; Adams and Barbara, 1982; Brunt et al., 1982; Gaspar and Costa, 1993). This may not be surprising because CPMMV also differs from all other known carlaviruses that are transmitted by the whitefly *Bemisia tabaci* (Genn.) sibling species group (Brown, 2010). Further it produces brush-like inclusions (Brunt et al., 1983; Thongmeearkom et al. 1984; Gaspar and Costa, 1993a) that are unique among carlaviruses described thus far. And unlike the majority of aphid-transmitted carlaviruses, CMMV is seed-transmitted. Thus, there remains some controversy as to whether CPMMV is a carlavirus, or a member of a new genus that is closely related to carlaviruses (King et al., 2011).

Also, distinguishing species, strains, of variants has been confounded by evidence for variation between isolates otherwise identified as CPMMV. For example, CPMMV isolates originating from solanaceous hosts in Jordan and Israel appeared to be very similar to each other, but were distinct from selected legume isolates from India and West Africa. An immune-electron microscopy decoration study indicated that a legume isolate of CPMMV from Brazil was serologically, distantly related to two other legume isolates but strikingly dissimilar to a Jordanian isolate. Moreover, CPMMV isolates from Jordan and Israel differed from the legume isolates by inducing only banded or non-banded virion aggregates but no brush-like virion aggregates, as was reported to be characteristic of legume isolates of CPMMV from Africa, Thailand and Brazil. Under the conditions of the present study brush-like inclusions were also found with Indian and West African isolates (Mansoor et al., 1998).

The 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 to which partial CPMMV sequences are available in Genbank (ICTV website; NCBI GenBank database). A recently discovered CPMMV isolate infecting soybean in India shared only 75-79% nt identity with the other seven known isolates (Yadav et al., 2013).

A comparison of the predicted amino acid sequences of carlaviruses (Menzel et al., 2010) indicated that CPMMV shares 46-59% identity with 8 aphid-borne carlaviruses, thereby falling below the 80% cutoff for species demarcation (Adams et al., 2005), adding support for the recognition of CPMMV as a distinct carlavirus species. Recently, Rosario et al. (2014) reported a CPMMV isolate from Florida, which is more closely related to isolates from South America (Brazil) and the Caribbean (Puerto Rico) than to the Ghana isolate that shares 98-99% amino acid identity with these isolates. Zanardo et al. (2014b) reported that the replicase gene of the CPMMV isolate occurring in soybean in Brazil shared only 60-61% nt sequence identity with that of the Ghanan isolate.

Detection and monitoring. The ability to formally detect and monitor CPMMV in the US mainland is non-existent due primarily to its only recent introduction into the Caribbean region, and knowledge that soybean and other legumes grown there in winter nurseries have often been found to be infected by the virus. In Puerto Rico, a molecular diagnostic assay has been implemented, however, the ability to differentiate strains and optimize detection in different host species have not been addressed for these isolates (J.C. Rodrigues, unpublished results).

CPMMV detection has been primarily been accomplished using serological and then molecular methods. Monsour et al. (1998) demonstrated biological and serological differences between CMMV isolates from leguminous and solanaceous hosts. A polyclonal antibody raised to the

coat protein of a Brazilian isolate of CPMMV (overexpressed as a fusion protein in *E. coli*) was demonstrated to detect the virus using an enzyme-linked immunosorbent assay (ELISA) and by Western blot (Carvalho et al. 2013). Tavassoli et al (2009) developed ELISA and reverse-transcriptase polymerase chain reaction (RT-PCR) approaches to detect CPMMV isolates in soybean. Gaspar et al., (2008) developed a degenerate primer that facilitated amplification of a portion of the 3' terminus of three distinct carlaviruses. Most recently, RT-PCR amplification of a fragment of the coat protein gene was developed by a commercial source (AGDIA, Inc., Elkhart, IN, USA) with primers based on Maroon and Zavriev (2002).

V. Response (portions of this section are modeled or excerpted after the Red Leaf Blotch of Soybean Recovery Plan; see Hartman et al. 2009).

Currently no response to this potential crisis situation in legume species grown in Puerto Rico, Mexico, and the US mainland have been organized or mounted. The discovery of CPMMV in Puerto Rico is quite recent (within the last 7 years).

In general, once the detection of a high risk and/or select agent pathogen is confirmed by a USDA, APHIS, PPQ recognized laboratory, APHIS, in cooperation with the State Department of Agriculture, is responsible for the 'response'. The response involves deployment of teams of experts and survey personnel to the site of the initial detection to conduct investigations and initiate delimiting surveys. Actions may be include: (1) regulatory measures to quarantine infested or potentially infested production areas to prevent infected material from being transported outside the zone, and (2) control measures which may include host removal and destruction, and/or ensuring adherence to required sanitary practices. APHIS imposes quarantines and regulatory requirements to reduce or negate importation and interstate movement of quarantine-significant diseases or regulated articles, and works in conjunction with the respective state(s) to impose them together with state regulatory actions that can restrict intrastate movement.

After the results of a delimiting survey are known, if the disease (viral pathogen) is limited in distributed in commercial and non-commercial plant hosts, options for eradication include destruction of the crop and protective insecticide applications to kill the whitefly vector in the vicinity. If the infection is widely distributed, it is likely that the entire crop in all infected locations would be destroyed. Such measures, or others, will be determined by USDA-APHIS.

VI. USDA Pathogen Permits and Regulations (portions of this section are modeled or excerpted after the Red Leaf Blotch of Soybean Recovery Plan; Hartman et al. 2009).

Until now, no permit or specific regulations are currently in place, regarding detection, responding to and recovering from the introduction and spread of CPMMV in the U.S.

Permit and registration requirements for plant diseases and laboratories are regulated under two authorities: the Plant Protection Act of 2000 (codified at 7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (codified at 7 CFR Part 331). Laboratories receiving suspect CPMMV-infected plant materials are required to have PPQ permits. Laboratories possessing,

using, or transferring high risk and select agents must be registered; however, diagnostic laboratories that identify select agents or toxins are exempt from this requirement as long as they complete an APHIS/CDC Form 4 and destroy the culture within 7 days.

The permit requirements of the Plant Protection Act apply to all pests of plants or plant products. This includes importation and interstate movement of pure cultures, arthropod vectors of plant pathogens, diagnostic samples, and infected plant material. The movement of infected plant material, regardless of the pest's quarantine status, requires that the receiving laboratory to have a permit. The importation and/or interstate movement of soil is similarly regulated when the intent is to isolate microbes that may be pests of plants or plant products. For guidance on the permitting of plant pests and soil samples, consult the PPQ permit website available at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services Customer Services at (301) 734-0841.

The Agricultural Bioterrorism Protection Act specifies requirements for possession, use, and transfer of organisms listed as select agents or toxins, such as the newly listed CPMMV. Once an unregistered diagnostic laboratory identifies a presumptive select agent or toxin, they must immediately notify the Agriculture Select Agent Program (ASAP), complete an APHIS/CDC Form 4 within 7 days, and either destroy or transfer the agent to a registered entity within 7 days (prior approval of the ASAP required). If a diagnostic laboratory held part of a screened sample (or culture) for voucher purposes, and the sample forwarded to the USDA Beltsville Laboratory was identified as positive for the select agent, then the USDA Beltsville Laboratory will notify immediately both the ASAP and the sending diagnostic laboratory that a select agent has been identified. The USDA Beltsville Laboratory will submit the APHIS/CDC Form 4 within seven days, and all unregistered labs will either destroy, or transfer the samples to a registered entity, within 7 days of the receipt of the results. The Agriculture Select Agent Program personnel must witness the destruction of the sample(s) or culture(s) within that time period. Clarification of these requirements and other information related to adherence to the select agent regulations is available at: http://www.aphis.usda.gov/programs/ag_selectagent/ and http://www.selectagents.gov, or call (301) 734-5960, Agriculture Select Agent Program.

VII. Economic Impact and Compensation

As indicated (see *Introduction*) yield losses due to CPMMV infection of legume crops, in general, of as much as 10-80% have been reported in Africa and India, whereas, in Argentina and Brazil, extreme yield losses, ranging from 10-100% were reported following the introduction of CPMMV, most probably on seed. Recent statistics from affected areas in sub-Saharan Africa where legumes are widely planted mostly in smaller acreages are partially available, and the disease has continued to persist (Taiwo, 2003; Time et al., 2010).

Among the potential hosts of CPMMV in the U.S. soybean is the crop of greatest concern, followed by common bean (dry and fresh marker), cowpea, garbanzo bean, and peanut, particularly when the respective crop is grown in mild climate locales where the whitefly vector is endemic and typically abundant during the warmest part of the growing season. Soybean in the US (2012) is planted to approximately 75,000,000 acres valued at about 37 billion dollars). (http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/related-data-statistics.aspx.), and in

some instances, is grown where the climate is mild. Common bean (fresh and dry), cowpea, and garbanzo beans (chickpeas) account for variable, but substantial acreages of legume production in the US (http://www.nass.usda.gov/), and also are at risk, particularly where the whitefly vector is endemic. However, all areas, regardless of the climate, are susceptible to the introduction of CPMMV through seed.

Compensation by the Risk Management Agency (RMA) for a loss caused by CPMMV would likely become available, if the producer can confirm that available control measures were taken. This would imply that certified clean seed is made available. If the insured does not provide evidence of procurement of clean seed, then it would seem unlikely that insurance coverage would be provided.

VIII. Mitigation and Disease Management

The vulnerability of the legume crop to infection by this whitefly-transmitted virus is of particular concern because it is both seed and whitefly vector borne, indicating that even a very low level of seed transmission followed by whitefly transmission could lead to rapid and widespread re-distribution and advanced spread of CPPMV in a relatively short time, if not detected early, and then left unmanaged. Any mitigation strategy that is considered and implemented must be coordinated among Federal, State and local regulatory officials because efforts by private individuals or industry are not expected to provide widespread disease control.

***Prevention and exclusion*.** CPMMV is not yet reported in soybean or any other legume or non-legume host in the mainland USA, but the risk for its introduction in legume growing areas seems quite high because seed that is increased offshore, or that is the product of breeding programs from infected locations (in Africa, Argentina, Brazil, perhaps Mexico, and Puerto Rico) likely makes it way to the US for spring-summer season breeding activities. Thus, phytosanitary regulations are needed. If CPMMV is discovered to already occur in the USA, it probably has high potential for rapid spread and could be difficult to control, at least at the outset, particularly in areas where the whitefly vector is abundant. Therefore, exclusion of this disease through port activities is an essential initial step in the mitigation and disease management strategy.

Diagnostics development. The lack of understanding of viral genomic diversity has hindered the development of diagnostics tests to ensure virus detection when it is present. Therefore, research efforts are needed to better understand the ecology, epidemiology, genetics, and population biology, and risks posed by CPMMV on a global basis. Without such knowledge, developing and sustaining durable virus-resistant varieties would seem to be a difficult undertaking.

Biological, chemical, and cultural control. Integrated approaches should be used to management of this aggressive viral pathogen, however, it should be noted that mitigation and management approaches that specifically address CPMMV have not been previously addressed for US crops. Based on knowledge of other seed-borne viruses, plant viruses transmitted in a non-persistent manner by the whitefly vector, *B. tabaci*, and efforts in Brazil to develop resistance in soybean, management practices can be extrapolated to apply to CPMMV outbreaks in the US, depending on the plant host species that are involved. As such, short-term approaches

would include control of the whitefly vector using appropriate insecticides (Belay et al. 2012) together with biological control using natural enemies when feasible. Medium-term approaches would reply on establishment and enforcement of clean seed testing programs for common bean, cowpea, garbanzo, and soybean seed to be planted in the US. In addition, once diagnostic tests are available to detect all introduced strains (and differentiate between other exotic strains, as is possible), removal or management of weeds and other alternate hosts (solanaceous hosts, ornamentals, and other minor crop or legume host species) identified as such, appropriate management strategies would be practiced within the outbreak zone. In the long term, once the virus has been introduced, virus-resistant varieties will be essential to protect major legume crops from becoming infected and/or serving as a virus source for other crops in the vicinity. An alternative plan may be needed for winter nursery screening and seed increases currently carried out in Puerto Rico and Mexico, in that seed will need to be rid of virus, and a routine diagnostic testing implementing, together with strict quarantine to prevent new introductions. It may be necessary to shift soybean and other seed programs to areas where WFs and those weeds are less problematic, however, it is difficult to envision where such a location exists that also would not eventually become contaminated due to exotic introductions if clean seed enforcement is not practiced in general. Otherwise, a comprehensive revision of seed phytosanitary protocols should be conducted to reduce the incidence of the virus in multiplication seed fields.

Germplasm and CPMMV disease resistance. Sources of resistance to CPMMV in legumes are scarce. Brace (2012) reported that because the high incidence of CPMMV-like symptoms occurring in Puerto Rico and Mexico, allowing reliable phenotypic data to be collected and some inferences about the inheritance to CPMMV-like in soybean to be reached. Phenotypic segregation of F_2-derived lines fit a 3 susceptible to 1 resistant segregation ratio, and all F_1 plants evaluated became infected. Based on the segregation ratio and phenotype of F_1 plants, CPMMV-L resistance was controlled by a single recessive allele designated as rbc1. Molecular mapping further confirmed a single gene model by identifying only one significant region on Chromosome 18 (LG G). Location of the locus was narrowed to a 2.4 cM region flanked by the SSR markers, BARCSOYSSR_18_0456 and BARCSOYSSR_18_0458. Suryanto et al. (2014) suggested that soybean resistance for an Indonesian CPMMV isolated was additive and controlled by two duplicate, recessive epistasis genes.

While the main soybean and most other legume-growing states in the US are temperate, CPMMV could become a threat to fresh or dry bean production in at least some of those locations, even if only as a seasonal pathogen, when introduced through seed. If isolates not restricted to legumes are involved, vegetable and ornamental crops could also be affected. Although the epidemiology of CPMMV in temperate climates has not been studied, it seems likely that the virus could survive from season to season in the mild climate zones in the US, after an accidental introduction by seed or even the whitefly vector harboring the virus ingested from various species of infected plants (asymptomatic or symptomatic). Indeed, an isolate of CPMMV has already been identified in whiteflies from Florida (Rosario et al. 2014), however, the exact origin of this isolate has not been determined, nor is it known if CPMMV is more widely distributed in Florida or other Sunbelt states where whiteflies thrive, than thought.

Education. Education efforts to raise awareness of CPMMV symptoms, host range, and transmission modes i.e. by seed and *B. tabaci* are needed. Importers, plant breeding programs,

growers, diagnosticians, and cooperative extension experts must be made aware of the disease and trained to identify the virus, once diagnostics are available. Information on how to distinguish this virus from others that cause similar symptoms, particularly in legumes, will be critical for detection at ports of entry and in the field. These measures will be instrumental in providing additional information about the threat posed by CPMMV, and downstream management practices that are put into place to mitigate further introductions and/or spread from infected fields.

IX. Current Infrastructure, Needs and Experts

Infrastructure. On the mainland USA there is no current effort devoted to research or breeding for resistance to CPMMV. However, upon identification of CPMMV in local fields, research has been underway at the Agricultural Experimental Station (AES) System, University of Puerto Rico. The virus has been identified in two areas previously dedicated to growing *Phaselous* species and winter soybean seed multiplication nurseries located in both the southern and northern parts of the island. These AES were the first sites where the disease was observed in Puerto Rico. Collaborative studies were carried out by UPR-AES researchers and seed companies having mutual concerns about the potential threat and/or immediate impact of the disease on the soybean crop in USA and elsewhere, where the seed will be grown for research purposes (breeding, variety trials) and/or for commercial production. One option for furthering the research findings could involve a collaborative effort between the University of Puerto Rico and APHIS, utilizing the newly constructed, certified quarantine facility laboratory and greenhouse facilities (10,000 sq. ft.) at the AES in Rio Piedras.

CPMMV Experts: Because of the impact of the disease associated to soybean described in South America, efforts to characterization of the virus, vector relationship and breeding for resistance have been so far mostly conducted at research agencies and agricultural universities in Brazil.

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X. Research, Extension, and Educational Priorities for CPMMV in Legumes and Other Susceptible Plant Species

- Immediate efforts should be undertaken to determine if CPMMV is seed borne or potentially so (presence in seed lots produced offshore, infected areas) in imported soybean seed, common bean, cowpea, and other susceptible legumes.
- Education (outreach, pamphlets with symptoms on various hosts, distribution in the Americas) to alert producers, educators, diagnosticians, scientists, and regulators to recognize the symptoms of CPPMV disease in its numerous hosts (not only legumes), and its likely risks, including introduction and transmission through seed, and of spread by the whitefly vector in mild climate locales of the US mainland.
- Develop training materials for port of entry Safeguarding Specialists (USDA) and Department of Homeland Security personnel, and First Detectors.
- Knowledge of the identity and distribution of CPMMV isolates that may already occur in the US and/or be transported here on seed or other plant materials has become imperative in mild climate areas of the country where the whitefly vector is endemic, and where legume crops are grown from seed produced in CPMMV infected areas.
- A thorough understanding of viral genomic diversity and of the biological characteristics of those variants, including host range, is needed to develop comprehensive diagnostics tools to ensure that the virus is detected when it is present.
- Reliable molecular detection assays are needed that detect the apparent range of genomic variability represented by extant CPMMV isolates in the Americas, Caribbean, and other locations worldwide from where the US receives seed or other plants that could harbor the virus.
- Studies are needed to understand the ecology, molecular epidemiology, host-plant genetics, and overwintering capacity, and to assess the risk posed by CpMMV to soybean and other legume and non-legume host (particularly, Solanaceae) species grown in the mainland US.
- Screening for sources of resistance in soybean and other legume species of importance to the US, including common bean and peanut, is crucial to prepare for impending outbreaks. Solanaceous species such as pepper and tomato may likewise be deserving of attention if it is found that legume-infecting isolates also infect solanaceous crop hosts, given the broad host range reported for CPMMV thus far.
- Develop approaches to manage the disease, including a certified, clean seed program, effective control of the whitefly vector using insecticides, biological control, and/or biopesticides, and management of weeds and other alternate hosts (once known).

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Tables

Table 1. Host plants collected near soybean fields and screened with ELISA for *Carlavirus*. Santa Isabel, Puerto Rico. (Rodrigues et al. 2014)

Scientific name	Family	ELISA result	# plants tested	% Incidence
Ludwigia octovalvis (Jacq.)	Onagraceae	+	4	25
Solanum americanum Mill.	Solonaceae	+	4	25
Triantheme portulacastrom L.	Aizoaceae	+	4	25
Ipomea sp.	Convolvulaceae	+	4	25
Boerhavia erecta L.	Nyctaginaceae	+	4	25
Argemone Mexicana L.	Papaveraceae	+	4	50
Euphorbia heterophylla L.	Euphorbiaceae	–	4	0
Amaranthus dubius Mart.	Amaranthaceae	–	4	0
Malvaceae sp.	Malvaceae	–	4	0
Datura stramonium L.	Solonaceae	–	4	0
Cleome gynandra L.	Cleomaceae	–	4	0
Kallstromia maxima L.	Zygophyllaceae	–	4	0
Macroptilium lathyroides (L.) Urban	Fabaceae	–		0
Vigna unguiculata L.	Fabaceae	+	8	25
Lycopersicon esculentum L.	Solonaceae	–	4	0
Cucurbita sp.	Cucurbitaceae	–	4	0
Ipomoea batatas (L.) Lam.	Convolvulaceae	–	4	0
Glycine max (L.) Merr.	Fabaceae	+	8	25

Figures

Figure 1. Transmission electron micrograph of *Cowpea mild mottle virus* slightly flexuous rods ~ 650-700 nm x 13 nm diameter (Courtesy, Phil Jones, Rothamsted Experiment Station, UK).

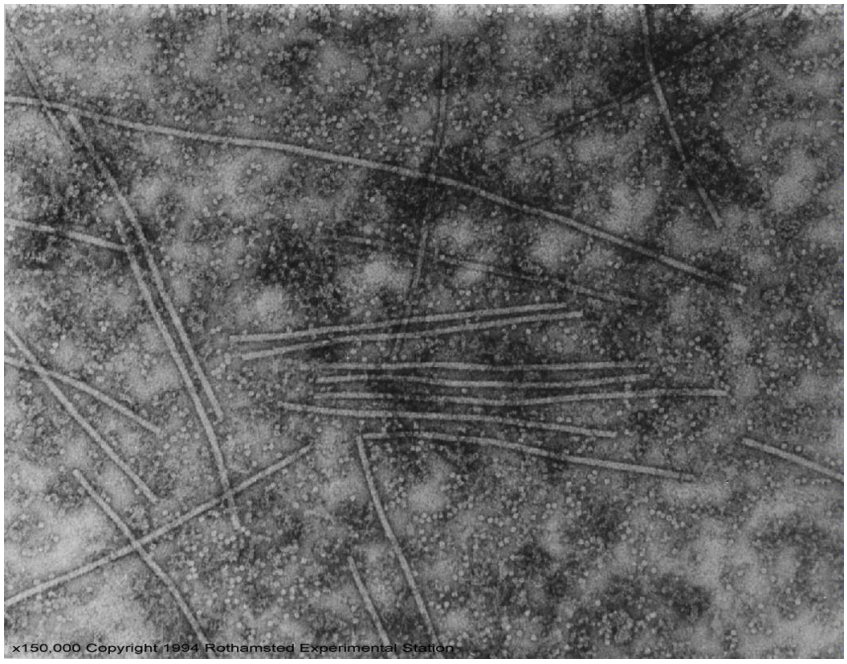

Figure 2. Feather- or brush-like inclusions in the cytoplasm of infected leaf cells. A similar type of inclusion body has been observed for other members of the genus, *Carlavirus*, but it differs somewhat from those associated with the aphid-transmitted carlaviruses (from Rodrigues et. al. 2014).

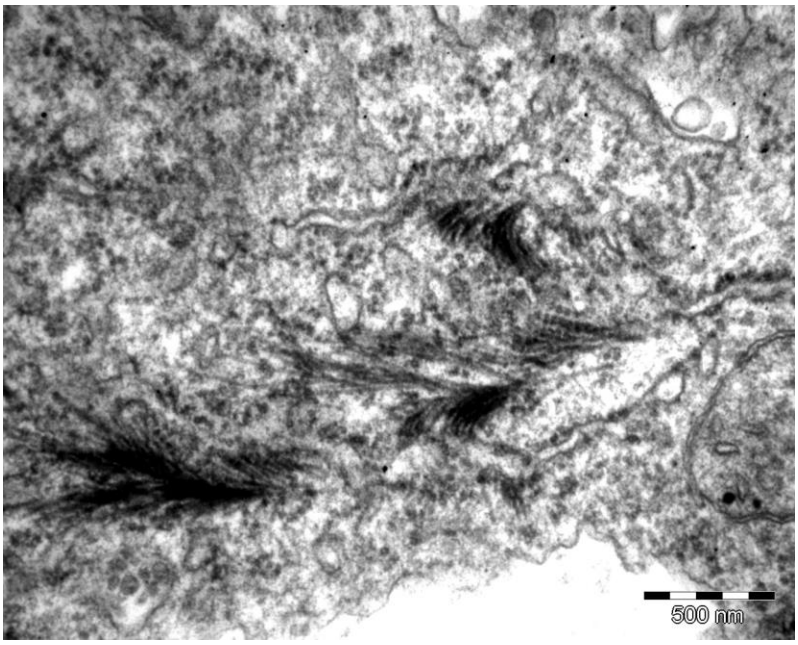

Figure 3. Field symptoms of *Cowpea mild mottle virus* (CPMMV) (A). Symptomatic pods and stem dieback (B). External symptoms in seeds collected from CPMMV-infected plants (top panel), and symptom-free seeds collected from healthy plants (lower panel).

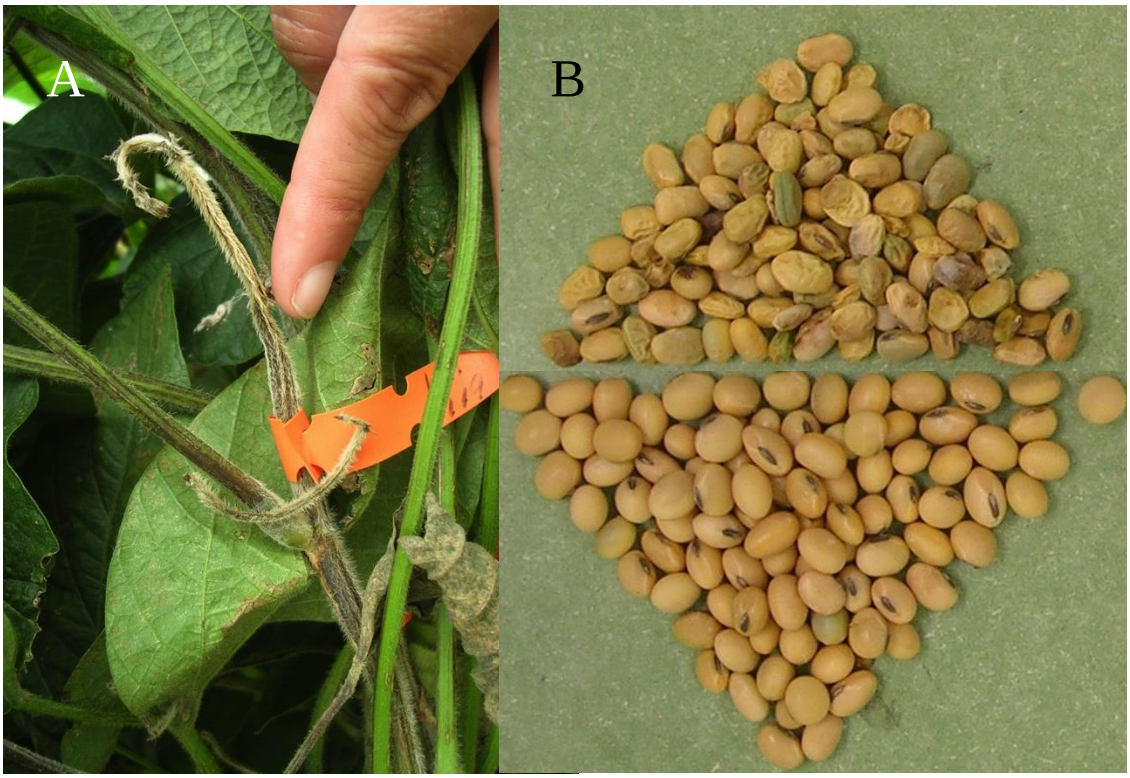

Figure 4. Transmission of the virus by graft-inocuation (A), whitefly transmission (B), and mechanical inoculation (C-D). Pods from mock-inoculated, healthy soybean plants (C), and pods from virus-infected, symptomatic soybean plants (D). Initial foliar symptoms developed 21 days post-inoculation, and symptoms were full-blown one week later in leaves, stems, and in pods, when present.

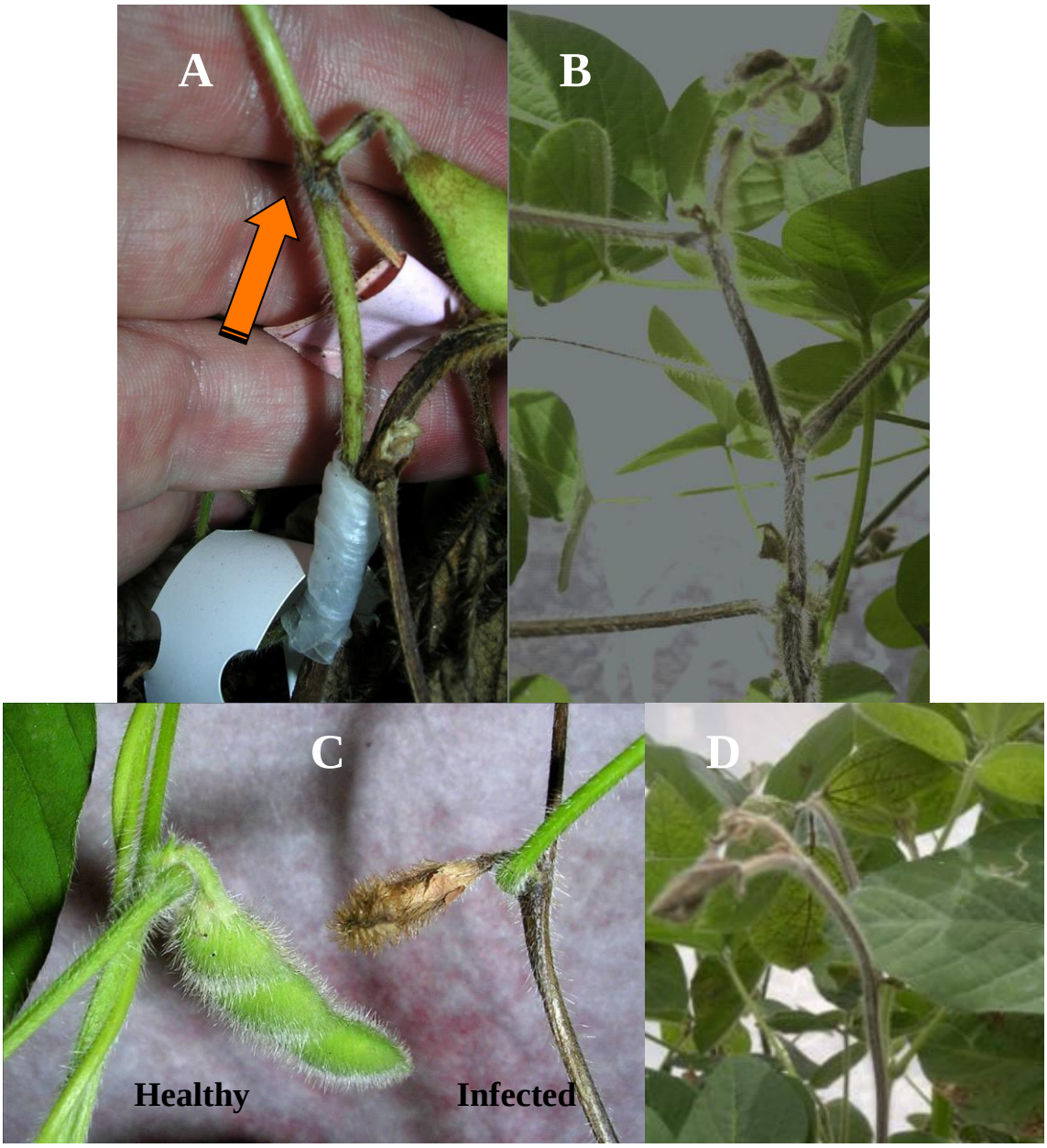

Figure 5. Mechanically-inoculated soybean leaves showing initial necrosis (A), and necrosis at the spreading edges of the site of inoculation (B).