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# plant disease

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## DISEASE NOTES

## Natural Infection of Yardlong Bean (*Vigna unguiculata* subsp. *sesquipedalis*) by Cowpea chlorotic mottle virus

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## ABSTRACT

Yardlong bean [*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.] is a distinctive subspecies of cowpea, with apparent origin in East Asia. Yardlong bean is characterized by extremely long and thin pods, and is considered one of the top ten Asian vegetables. It is now grown extensively in Asia, Europe, Oceania, and Americas. In 2012, yardlong bean plants showing severe yellowing were found in an experimental plot at the UCV Agronomy Faculty in Aragua State, Venezuela. The observed incidence of foliar yellowing symptoms, resembling those associated with the infection of a bromovirus, was 30%. The field samples with virus-like symptoms of yellowing were collected for further studies. Previously, we reported the presence of *Cowpea mild mottle virus* (CPMMV), a carlavirus with filamentous particles, in yardlong bean in the country (Brito et al. 2012), but electron microscope observations using the leaf-dip method excluded CPMMV, since symptomatic leaves contained icosahedral virus-like particles, ~26–28 nm in diameter. Leaf extracts from field samples were mechanically inoculated onto *V. unguiculata* cv. Tuy, *Vigna radiata*, *Phaseolus vulgaris* L. cv. Tacarigua, *Chenopodium quinoa* Willd., *C. amaranticolor* Coste et A. Reyn, *Gomphrena globosa* L., and *Glycine max* (L.) Merr. All the seven mechanically inoculated plant species were found susceptible to the virus. Among these, *C. amaranticolor*, *C. quinoa*, and *G. globosa* developed chlorotic local lesions 10 to 14 days post inoculation (dpi), while *V. radiata* developed local necrotic lesions on inoculated leaves. Seedlings of *V. unguiculata* and *G. max* were found systemically infected and showed mottle and mosaic symptoms on upper noninoculated leaves at 7 dpi. Extensive severe systemic chlorosis was developed in cowpea after 15 dpi. A virus isolate was

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propagated in *V. unguiculata* cv. Tuy, grown under greenhouse conditions, purified (de Carvalho et al. 2013), and tested by Western blot using polyclonal antisera specific to *Cowpea mosaic virus* (CPMV), *Southern bean mosaic virus* (SBMV), *Broad bean mottle virus* (BBMV), *Cucumber mosaic virus* (CMV), and *Cowpea chlorotic mottle virus* (CCMV). A conspicuous immunoreactive band with an approximate size of 19 kDa was obtained in purified virus sample against BBMV and CCMV antisera. Total RNA was extracted from 20 symptomatic plants and from purified virions, and tested by RT-PCR using degenerate primers Ilar1F5 (GCNGGWTGYGGDAARWCNAC) and Ilar1R7 (AMDGGWAYTYGTNYGTRTCACC), specific to the detection of members of the genus *Iilarvirus* and family *Bromoviridae* (Untiveros et al. 2010). A PCR fragment of the expected size (~300 bp) was amplified for all samples. Two amplicons were sequenced and the identical (100%) consensus sequences submitted to GenBank with Accession Nos. KJ810515 (from field yardlong bean) and KJ810516 (from purified virions). The deduced amino acid sequence contained 97 residues encoding part of the viral methyltransferase which was 93% identical to that of a CCMV methyltransferase (AAN37635.1). A nucleotide BLAST analysis of the sequence revealed 85% identity with CCMV strain T (AF325739). The level of sequence similarity with CCMV suggested that the virus from yardlong bean may be a distinct strain of this species. Phylogenetic analysis of the putative methyltransferase gene demonstrated that CCMV yardlong bean clustered separately from the known strains of CCMV. Because chrysomelids are known vectors of bromoviruses, virus-free *Andrector ruficornis* Olivier, *A. arcuatus* Olivier, and *Ledesmodina auricollis* Lèfevre adults were exposed to symptomatic cowpea leaves for a 48-h acquisition access period and then cage-confined with ten healthy *V. unguiculata* cv. Tuy and *P. vulgaris* cv. Tacarigua each for a 48-h inoculation access period. Symptoms were reproduced in all tested plants after a 21-day period and CCMV infection was confirmed by RT-PCR assay. To our knowledge, this is the first evidence of the presence of CCMV in yardlong bean, and the first report of CCMV transmission by chrysomelid beetle *L. auricollis*. This is an important information for the Venezuelan cowpea's market as CCMV-infected yardlong bean plants could act as virus sources for secondary spread by beetle vectors.

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Section: 

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