Cowpea Severe Mosaic Virus in Five Legumes in Central Brazil

M. T. LIN, Associate Professor, and J. R. N. ANJOS, Former Graduate Student, Departamento de Biologia Vegetal, Universidade de Brasilia, 70910 Brasilia, DF, Brazil; and G. P. RIOS, Plant Pathologist, Centro Nacional de Pesquisa de Arroz e Feijão, C. P. 179, 74000 Goiânia, Goias, Brazil

ABSTRACT

Lin, M. T., Anjos, J. R. N., and Rios, G. P. 1982. Cowpea severe mosaic virus in five legumes in central Brazil. Plant Disease 66:67-70.

Calopogonium mucunoides, Centrosema pubescens, and *Vigna radiata* var. *radiata* were infected with serotype I of cowpea severe mosaic virus; *Crotalaria juncea* showing chlorotic mottling and leaf distortion was infected with serotype II of the virus. *C. juncea* with chlorotic leaf spots and *Vigna unguiculata* subsp. *sesquipedalis* were doubly infected with serotypes I and II.

Additional key word: epidemiology

Cowpea severe mosaic virus (CPSMV) (8), a beetle-transmitted, isometric virus previously known as severe subgroup or Arkansas serogroup of cowpea mosaic virus, is the most frequently found virus in mosaic-diseased cowpeas (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) in central Brazil (12,15). The CPSMV isolates of central Brazil have been separated into serotypes I and II (14). Although CPSMV is reported to be seedborne in cowpeas in other countries (8), the Brazilian isolates are apparently not seed-transmitted (4,5). However, many cowpea plants in the fields become infected with this virus as early as 2 wk after planting in central Brazil. This suggests that there are sources of primary inoculum of CPSMV in the vicinity of these fields.

Results of a survey of possible natural hosts of CPSMV in central Brazil are reported here, including identification of five legume species as natural hosts of CPSMV, serotyping of CPSMV isolates, and separation of serotypes from mixed infections. The nomenclature system for *Vigna* and *Phaseolus* spp. used in this paper is the one recognized by the Agricultural Research Service of the U.S. Department of Agriculture (9). Preliminary reports of this work have been published (13,16).

MATERIALS AND METHODS

Legumes with viruslike symptoms were collected in and near cowpea plantings in the state of Goias and the Federal District. Leguminous weeds were also collected from home gardens and lawns. Each sample was divided in two parts to be used for serology and host reaction. CPSMV was identified in gel diffusion tests with antisera to legume comoviruses including CPSMV as described (12,15). CPSMV isolates were serotyped by comparison with CPSMV isolates G-BE and G-12, representing serotypes I and II, respectively (14). Crude saps containing the respective virus isolates were used in double-diffusion tests with antisera diluted at 1:16. Well-defined, curved precipitin bands appeared after overnight incubation in a moist chamber, and spurs were clearly visible. Cross-absorbed antisera of serotypes I and II, which react only with their respective homologous antigens (14), were also used in the typing. For the host reaction study, each field sample was mechanically inoculated onto at least four seedlings of *Chenopodium quinoa* Willd.; *C. amaranticolor* Coste & Reyn.; cowpea cultivars Serido, Pitiuba, and IPEAN VII; soybean (*Glycine max* (L.) Merr.) cultivar IAC-2; and bean (*Phaseolus vulgaris* L.) cultivar Rico 23 (14). Inoculated plants were kept in a screenhouse and observed for symptom development.

Accepted for publication 5 October 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/82/01006704/$03.00/0

RESULTS

CPSMV was detected serologically in the crude sap of the following legumes: *Calopogonium mucunoides* Desvaux, with severe mosaic and blistering in leaves (Fig. 1); *Centrosema pubescens* Benth., with interveinal chlorosis and mosaic in leaves (Fig. 2); *Crotalaria juncea* L., with chlorotic mottling and leaf distortion (Fig. 3) or with chlorotic spots (Fig. 4); *Vigna radiata* (L.) Wilczek var. *radiata* Verdc., with mild mosaic and necrotic spots (Fig. 5); and *V. unguiculata* (L.) Walp. subsp. *sesquipedalis* (L.) Verdc., with chlorotic mosaic and severe leaf distortion (Fig. 6).

The samples of *Calopogonium mucunoides, Crotalaria juncea,* and *V. radiata* var. *radiata* were collected in the vicinity of cowpea fields with a high incidence of CPSMV in the state of Goias. More than 50% of the plants of these three species had symptoms, and most had holes in the leaves similar to those made by chewing insects. Plants of *Centrosema pubescens*, a common weed in lawns, were collected in front of the National Congress buildings in Brasilia, the Federal District. About 20% of the *C. pubescens* plants had symptoms, but none had holes in leaves. A single *V. unguiculata* subsp. *sesquipedalis* plant was found in a home garden in Goiânia, the state of Goias. The plant was at the stage of forming pods, and many holes were observed in the leaves.

The CPSMV isolates from *Calopogonium mucunoides, Centrosema pubescens,* and *V. radiata* var. *radiata* formed distinct precipitin lines that fused completely with that of serotype I but spurred with that of serotype II (Fig. 7). When tested against the absorbed antisera, these isolates reacted only with serotype I, forming a line that fused completely with that of serotype I (Fig. 7). Therefore, these isolates were identified as the serotype I of CPSMV.

Results with crude sap from *Crotalaria juncea* plants showing chlorotic mottling and leaf distortion (Fig. 3) indicated that these plants were infected with serotype II (Fig. 8). Crude sap from *C. juncea* showing chlorotic spots (Fig. 4) and *V. unguiculata* subsp. *sesquipedalis* gave homologous reactions to serotypes I and II (Fig. 9). When crude sap from these plants was replaced with a mixture of serotypes I and II, the gel diffusion patterns were the same as shown in Fig. 9. When crude saps from these *C. juncea* and *V. unguiculata* subsp. *sesquipedalis* plants were inoculated onto IAC-2 soybean, which is susceptible to serotype I but immune to serotype II (14), only serotype I was detected and isolated from soybeans inoculated with the sap from *V. unguiculata* subsp. *sesquipedalis*. However, both serotypes were detected in soybeans inoculated with *C. juncea* sap.

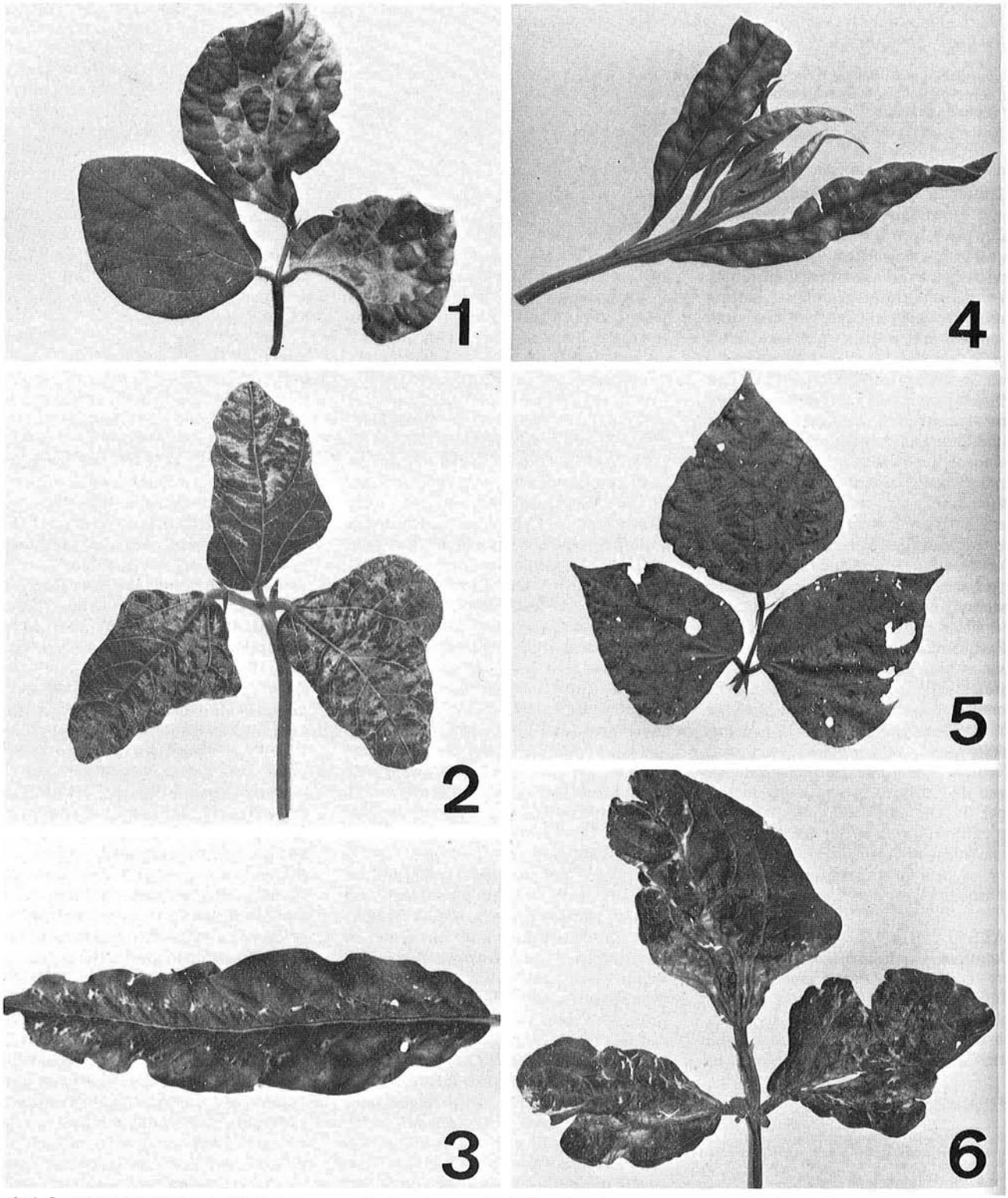
Serotypes I and II in *V. unguiculata* subsp. *sesquipedalis* were separated by the following procedures. An equal volume of the cross-absorbed antiserum of serotype I was added to 2 ml of sap of *V. unguiculata* subsp. *sesquipedalis*. The mixture was incubated in a water bath at 37 C for 1 hr, and the precipitates were removed by low-speed centrifugation. The supernatant was inoculated onto

Serido cowpeas, from which only serotype II was detected and isolated. This method was also used to separate serotypes I and II in *Crotalaria juncea*.

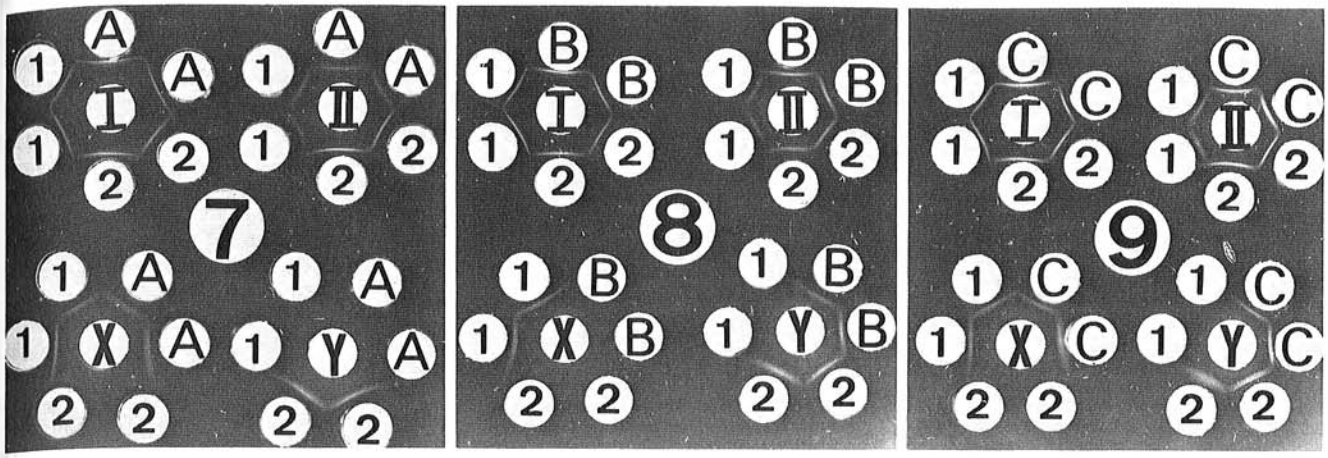
In the inoculation tests, serotype I from *V. radiata* var. *radiata*, *Crotalaria juncea*, and *V. unguiculata* subsp. *sesquipedalis* and serotype II from *V. unguiculata* subsp. *sesquipedalis* were similar in host reactions to their respective serotypes from cowpeas (14). However, serotype I from *Calopogonium mucunoides* did not infect *Chenopodium amaranticolor* or *Chenopodium quinoa*; it was able to infect cowpea (inducing mild mosaic) only after the virus had been maintained in IAC-2 soybeans. The isolate from *Centrosema pubescens* induced typical pinpoint necrotic lesions in *Chenopodium amaranticolor* only after the virus had been multiplied in cowpea or soybean. The two isolates of serotype II from different sources of *Crotalaria juncea* were different from the serotype II of *V. unguiculata* subsp. *sesquipedalis* in that they infected IAC-2 soybean systemically.

DISCUSSION

Although CPSMV is reported to infect many legume species experimentally (1,4,6,7), reports of natural infection of legumes other than cowpea are relatively few. Dale (6) reported natural infection of



Figs. 1–6. Symptoms induced by cowpea severe mosaic virus in naturally infected *Calopogonium mucunoides* **(1)**, *Centrosema pubescens* **(2)**, *Crotalaria juncea* **(3 and 4)**, *Vigna radiata* var. *radiata* **(5)**, and *V. unguiculata* subsp. *sesquipedalis* **(6)**.



Figs. 7–9. Serotyping of the cowpea severe mosaic virus isolates from naturally infected legume hosts in agar gel plates. I and II = antisera to serotypes I and II, respectively; X and Y = cross-absorbed antisera of serotypes I and II, respectively. **(7)** A = crude sap of *Calopogonium mucunoides;* **(8)** B = crude sap of *Crotalaria juncea*, showing chlorotic mottling and leaf distortion; **(9)** C = crude sap of *Vigna unguiculata* subsp. *sesquipedalis*. 1 and 2 = crude saps containing serotypes I and II, respectively.

Phaseolus aureaus Roxb. (*Vigna radiata* var. *radiata*), *Phaseolus mungo* L. (*Vigna mungo* (L.) Hepper), *Crotalaria juncea, Cajanus indicus* Spreng., *Vigna sesquipedalis* (*V. unguiculata* subsp. *sesquipedalis*), and soybean by CPSMV in Trinidad. Natural infections of soybean have been recorded in the United States (17), Puerto Rico (18), and Brazil (3). *Phaseolus lathyroides* L. is a major reservoir of CPSMV in Puerto Rico (2) and in the state of Ceará, Brazil (11). Natural occurrence of CPSMV in winged bean (*Psorphocarpus tetragonolobus* DC.) in northern Brazil (10) and in hoary tick-clover (*Desmodium canescens* DC.) in Illinois (17) has been reported. The present study showed that five legume species were naturally infected with CPSMV in central Brazil.

We also identified the serotypes of CPSMV isolates. Serotype I was detected in all five species, and serotype II was found in three of them. Serotype I had previously been shown to be more common in this region (14). Mixed infections with both serotypes occurred in *Crotalaria juncea* and *V. unguiculata* subsp. *sesquipedalis*. Both serotypes in these hosts were apparently in more or less equal concentration, as judged by the intensity and form of the precipitin bands in agar gel diffusion tests (Fig. 9).

The CPSMV isolate from *Calopogonium mucunoides* was unable to infect *Chenopodium amaranticolor* and *Chenopodium quinoa*. The isolate from *Centrosema pubescens* also did not infect *Chenopodium amaranticolor* in the initial tests with inoculum prepared directly from field samples. *Chenopodium amaranticolor* is listed as a diagnostic plant for CPSMV (8). The failure of the *Centrosema pubescens* isolate to infect *Chenopodium amaranticolor* might be the result of low virus concentration or inhibitors in the sap of *Centrosema pubescens*, because inocula from systemically infected cowpea or soybean induced typical lesions in *Chenopodium amaranticolor*. The *Calopogonium mucunoides* isolate failed to infect *Chenopodium amaranticolor* and *Chenopodium quinoa* even after it had been multiplied in a systemic host. Furthermore, this isolate induced mild but never severe mosaic symptoms in the three cowpea cultivars tested. These properties distinguish this isolate from any other CPSMV isolate that we have studied in Brazil.

Since two serotype II isolates from *Crotalaria juncea* were able to infect IAC-2 soybean, the possibility of using this cultivar to differentiate these two serotypes (14) is eliminated. Nevertheless, this cultivar was a better diagnostic plant than *Chenopodium amaranticolor* in the initial isolation of CSPMV from *Calopogonium mucunoides* and *Centrosema pubescens*.

The transmission of CPSMV in nature depends mainly upon beetle vectors. The observation that the CPSMV-infected *Centrosema pubescens* plants had no holes in their leaves similar to those made by beetles is interesting. The possibility of seed transmission of CPSMV in this host will be investigated.

CPSMV causes the most economically important disease in cowpea in Brazil, and limited data are available on the epidemiology of this virus (11). The present finding that five common legumes are natural hosts of CPSMV in central Brazil contributes to the understanding of the epidemiology of this virus in this region. Further studies will be conducted to determine to what degree CPSMV-infected legumes may contribute to the spread of this virus to cowpea.

ACKNOWLEDGMENTS

The authors thank J. P. Fulton and H. A. Scott, University of Arkansas, for providing the antisera to legume comoviruses and A. Primo of CENARGEM/EMBRAPA, Brasilia, DF, and L. X. Filho, formerly University of Brasilia, for taxonomic confirmation of *Calopogonium mucunoides* and *Centrosema pubescens*, respectively. The technical assistance of J. M. de Sousa is appreciated.

LITERATURE CITED

1. Agrawal, H. O. 1964. Identification of cowpea mosaic virus isolates. Meded. Landbouwhogesch. Wageningen 64(5):1-55.
2. Alconero, R., and Santiago, A. 1973. *Phaseolus lathyroides* as a reservoir of cowpea mosaic virus in Puerto Rico. Phytopathology 63:120-123.
3. Anjos, J. R. N., and Lin, M. T. 1980. Queima do broto em soja causado por um membro do serotipo I do vírus do mosaico do caupi serogrupo Arkansas. (Abstr.) Fitopatol. Bras. 5:382-383.
4. Caner, J., Silberschmidt, K., and Flores, E. 1969. Ocorrência do vírus do mosaico da Vigna no Estado de São Paulo. O Biológico 35:13-16.
5. Costa, A. S., Oliveira, A. R., Kitajima, E. W., and Matsuoka, S. 1969. Occorência do mosaico do feijão macassar em São Paulo. Rev. Soc. Bras. Fitopatol. 3:56-57.
6. Dale, W. T. 1949. Observations on a virus disease of cowpea in Trinidad. Ann. Appl. Biol. 36:327-333.
7. Debrot, E., and Rojas, C. E. B. 1967. El virus del mosaico del frijol, *Vigna sinensis* Endl. (cowpea mosaic virus), en Venezuela. Agron. Trop. Maracay, Venez. 17:3-15.
8. de Jager, C. P. 1979. Cowpea severe mosaic virus. No. 207. Descriptions of Plant Viruses. Commonw. Mycol. Inst. Assoc. Appl. Biol. Kew, Surrey, England. 4 pp.
9. Gunn, C. R. 1973. Recent nomenclatural changes in *Phaseolus* L. and *Vigna* Savi. Crop Sci. 13:496.
10. Kitajima, E. W., Noda, H., Lin, M. T., and Costa, C. L. 1979. Um mosaico em feijão-de-asa (*Psorphocarpus tetragonolobus*) causado por um isolado do subgrupo severo do vírus do mosaico da Vigna. Fitopatol. Bras. 4:519-524.
11. Lima, J. A. A., and Nelson, M. R. 1977. Etiology and epidemiology of mosaic of cowpea in Ceará, Brazil. Plant Dis. Rep. 61:864-867.
12. Lin, M. T. 1979. Purification and serology of legume and corn viruses in Brazil. Fitopatol. Bras. 4:203-213.
13. Lin, M. T., Anjos, J. R. N., and Rios, G. P. 1980. Occorência natural do serotipo I do "cowpea mosaic virus-Arkansas serogroup" em *Centrosema pubescens* e *Calopogonium mucunoides* no Brasil Central. (Abstr.) Fitopatol. Bras. 5:418.
14. Lin, M. T., Anjos, J. R. N., and Rios, G. P. 1981. Serological grouping of cowpea severe mosaic virus isolates from Central Brazil. Phytopathology 71:435-438.
15. Lin, M. T., Kitajima, E. W., and Rios, G. P. 1981. Serological identification of several cowpea viruses in Central Brazil. Fitopatol. Bras. 6:73-85.
16. Lin, M. T., and Rios, G. P. 1980. Infecção natural e simultânea de *Vigna sesquipedalis* por serotipo

I e II do "cowpea mosaic virus-Arkansas serogroup." (Abstr.) Fitopatol. Bras. 5:420.
17. McLaughlin, M. R., Thongmeearkom, P., Goodman, R. M., Milbrath, G. M., Ries, S. M., and Royse, D. J. 1978. Isolation and beetle transmission of cowpea mosaic virus (severe subgroup) from *Desmodium canescens* and soybeans in Illinois. Plant Dis. Rep. 62:1069-1073.
18. Thongmeearkom, P., and Goodman, R. M. 1976. A severe disease of soybeans caused by an isolate of cowpea mosaic virus. (Abstr.) Proc. Am. Phytopathol. Soc. 3:209.