Tobacco Streak, Broad Bean Wilt, Cucumber Mosaic, and Alfalfa Mosaic Viruses Associated with Ring Spot of *Ajuga reptans* in Australia

D. D. SHUKLA and K. H. GOUGH, Division of Protein Chemistry, Commonwealth Scientific and Industrial Research Organization, Parkville (Melbourne), Australia

ABSTRACT

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Ajuga reptans plants growning at three locations in and around Melbourne displaying ring spot, line pattern, scattered yellow spots, and yellow leaf margins were infected singly by tobacco streak (Ajuga-TSV), broad bean wilt (Ajuga I-BBWV), and cucumber mosaic (CMV) viruses. Plants at a fourth location showed a very mild chlorotic mottle, faint ring spot symptoms, and contained alfalfa mosaic (AMV) and BBWV (Ajuga II-BBWV) simultaneously. Ajuga-TSV was seed-transmitted in *Chenopodium amaranticolor*, *C. quinoa*, and *Vigna unguiculata* at rates of 48, 74, and 100%, respectively. The isolate was distinct from the *Ageratum houstonianum* isolate from Queensland in agar gel diffusion and cross-protection tests. It apparently is a new strain of TSV occurring in Australia. Ajuga I-BBWV was serotype I of the virus, whereas Ajuga II-BBWV is possibly a third serotype of BBWV not recorded previously. This is the first report of AMV and CMV in *A. reptans* in Australia and of *A. reptans* as a natural host of TSV and BBWV.

Additional key words: protein A-immune electron microscopy

Ajuga reptans L. (creeping bugleweed or carpet bugle) is a perennial ornamental plant often grown in rock gardens or borders. In summer of 1978, leaves of several plants in a planting in Melbourne showed ring spot, line pattern, scattered yellow spots, and yellow margins (Fig. 1A–D). Repeated isolations from the plants yielded a strain of tobacco streak virus (TSV). During 1979, several other plantings of *A. reptans* in and around Melbourne were surveyed for viruslike symptoms. Plants at two other locations displayed symptoms very similar to the one described (Fig. 2A–D) but were infected with strains of broad bean wilt (BBWV) and cucumber mosaic (CMV) viruses, respectively. Plants at a fourth location showed a very faint ring spot, mild chlorotic mottle symptoms, and contained strains of alfalfa mosaic virus (AMV) and BBWV simultaneously. This paper reports the isolation and identification of these viruses from *A. reptans*.

MATERIALS AND METHODS

Leaves from *A. reptans* that showed symptoms were collected at different locations, ground in 0.01 M phosphate buffer, pH 7.0, and inoculated onto Carborundum-dusted leaves of indicator plants. Plants were maintained in an insectproof glasshouse at 20–25 C. Cross-protection, insect transmission, agar gel double diffusion, and Protein A-immune electron microscopy (Protein A-IEM) were performed as described in previous papers (10,11,15). The sap and antiserum dilutions used for Protein A-IEM were 1:100 and 1:50, respectively.

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RESULTS

Tobacco streak virus. The TSV isolates induced pinpoint chlorotic and necrotic local lesions followed by systemic mottling and crinkling of leaves in *Chenspodium amaranticolor*; large necrotic local lesions and tip necrosis in *C. quinoa*; necrotic local lesions and systemic mottling in *Cucumis sativus*; local necrotic rings spreading into veins and systemic mottling in *Gomphrena globosa*; systemic veinclearing, necrotic half-rings, and necrotic spots followed by recovery in *Nicotiana clevelandii*; local necrotic concentric rings and severe systemic necrotic rings and spots followed by recovery in *N. tabaccum* L. 'Turkish' and 'Xanthi'; and pink local necrotic rings followed by systemic mottling in *Vigna unguiculata*. *Phaseolus vulgaris* and *Vicia faba* developed small pink and black necrotic local lesions, respectively, but were not infected systemically. No symptoms were observed on *N. glutinosa*, *Petunia hybrida*, and *Pisum sativum*; however, *N. glutinosa* proved to be infected locally and *P. hybrida* was infected systemically in back-transmission tests. The virus could not be recovered from *P. sativum*.

A typical isolate of the virus had a thermal inactivation point between 60 and 70 C, a dilution end point between 10^{-4} and 10^{-5}, and a longevity in vitro of 48 hr. The isolate could not be transmitted from systemically infected Turkish tobacco to *N. clevelandii* by *Myzus persicae* by using short acquisition and transmission times.

In agar gel double diffusion tests, the isolate reacted positively with an antiserum to TSV (4) but not with antisera to other ilarviruses, namely apple mosaic, prune dwarf, prunus necrotic ringspot, and tulare apple mosaic viruses. The isolate did not react with antisera to AMV, BBWV, and CMV. Systemically infected leaves of *C. quinoa* were used as the source of antigen. No virus particles were seen in the electron microscope in leaf-dip preparations from *A. reptans* and several indicator hosts; however, numerous isometric particles with diameters ranging from 25 to 35 nm were seen when sap from systemically infected *C. quinoa* leaves was subjected to Protein A-IEM.

When seeds from TSV-infected *C. amaranticolor*, *C. quinoa*, *G. globosa*, and *V. unguiculata* were tested for transmission by using the criteria of symptoms in seedlings, back-transmission in *C. quinoa*, and immunodiffusion, the transmission rate was 48% in *C. amaranticolor*, 74% in *C. quinoa*, and 100% in *V. unguiculata*. The virus was not transmitted through the seeds of *G. globosa*.

Ajuga-TSV was compared with an isolate of the virus from *Ageratum houstonianum* Mill. (5) on the basis of symptoms in some indicator hosts, cross-protection tests, immunodiffusion, and electrophoretic mobility. Both isolates induced similar symptoms in *C. quinoa*, *C. sativus*, *N. rustica* L., and *N. tabaccum* 'Xanthi'; Ajuga-TSV produced more severe symptoms than did Ageratum-TSV. For cross-protection tests, Xanthi tobacco plants that recovered after infection with Ajuga-TSV were reinocu-

lated with Ageratum-TSV and vice versa; healthy Xanthi plants of the same age were used as controls. Three of five plants infected with Ageratum-TSV and challenged with Ajuga-TSV developed local symptoms, and one also produced typical systemic symptoms. Two of five plants infected with Ajuga-TSV and challenged with Ageratum-TSV also developed local symptoms; however, neither of them produced visible systemic symptoms. All control plants developed typical local and systemic TSV symptoms. In immunodiffusion tests, Ajuga-TSV reacted with TSV antiserum (4) up to a dilution 1:80, Ageratum-TSV only up to 1:20, and the former formed spurs against the latter when the isolates were placed in adjacent wells. In immunoelectrophoresis experiments, however, both isolates migrated toward the anode and had the same electrophoretic mobility.

Broad bean wilt virus. The Ajuga isolates of BBWV from a single infection (Ajuga I-BBWV) induced typical symptoms in indicator hosts (12,15). They reacted positively in immunodiffusion tests with antisera to BBWV serotypes I (homologous titer, 1:1,024) (16) and II (homologous titer, 1:128) (17) up to dilutions of 1:1,024 and 1:16, respectively, when systemically infected leaves of *C. quinoa* were used as the source of antigens. They did not react with antisera to AMV, CMV, and ilarviruses. Numerous isometric particles about 30 nm in diameter were seen when a typical isolate was subjected to Protein A-IEM by using the serotype I antiserum.

In immunodiffusion tests with the type strain (BB-BBWV serotype I) and a periwinkle isolate (PW-BBWV serotype II) of BBWV (15,17), using antisera to serotype I, Ajuga I-BBWV produced confluent precipitin lines with BB-BBWV and formed spurs against PW-BBWV. In reciprocal tests with antiserum to serotype II, the precipitin lines produced by PW-BBWV spurred over Ajuga I-BBWV precipitin lines and Ajuga I-BBWV and BB-BBWV formed confluent lines between themselves.

Cucumber mosaic virus. The CMV isolates from *A. reptans* induced typical symptoms of the virus in indicator hosts (14,15) and reacted positively with an antiserum to the Q strain of CMV (3) in Protein A-IEM and immunodiffusion. They did not react with antisera to AMV, BBWV, and ilarviruses.

Alfalfa mosaic and broad bean wilt viruses in mixed infection. When leaf extracts from *A. reptans* plants that showed mild ring spot and mottle symptoms were inoculated onto indicator plants, typical symptoms of AMV developed (7,13). *C. quinoa*, which usually shows systemic tip necrosis after infection with BBWV isolates (15), produced only chlorotic and necrotic flecking. A typical isolate, however, reacted positively with antisera to AMV (2) and serotypes I and II of BBWV up to dilutions of 1:10, 1:16 and 1:10, respectively, in immunodiffusion tests. It did not react with antisera to CMV and ilarviruses. In Protein A-IEM using antisera to AMV plus BBWV serotype II, bacilliform particles of different lengths (typical of AMV) and isometric particles of about 30 nm diameter (typical of BBWV) were observed. No attempt was made to separate AMV and BBWV from the mixture.

The isolate containing AMV and BBWV simultaneously was referred to as Ajuga II-BBWV. When Ajuga II-BBWV was compared with Ajuga I-BBWV and PW-BBWV in immunodiffusion tests using antisera to serotype I and II of BBWV, Ajuga I-BBWV and PW-BBWV each formed spurs against Ajuga II-BBWV with both antisera.

Back-transmission experiments. When the viruses and strains obtained from the *A. reptans* plants were inoculated onto healthy plants of this species by using infected *A. reptans, C. quinoa*, and Xanthi tobacco as virus sources, typical symptoms of only the isolate that contained AMV and Ajuga II-BBWV in mixed infection developed. When the *A. reptans* plants were indexed on indicator hosts 2 mo after inoculation, typical symptoms of only CMV from Ajuga-CMV-inoculated plants and of AMV from AMV plus Ajuga II-BBWV-inoculated plants developed. The identity of these viruses in test plants was confirmed by immunodiffusion tests.

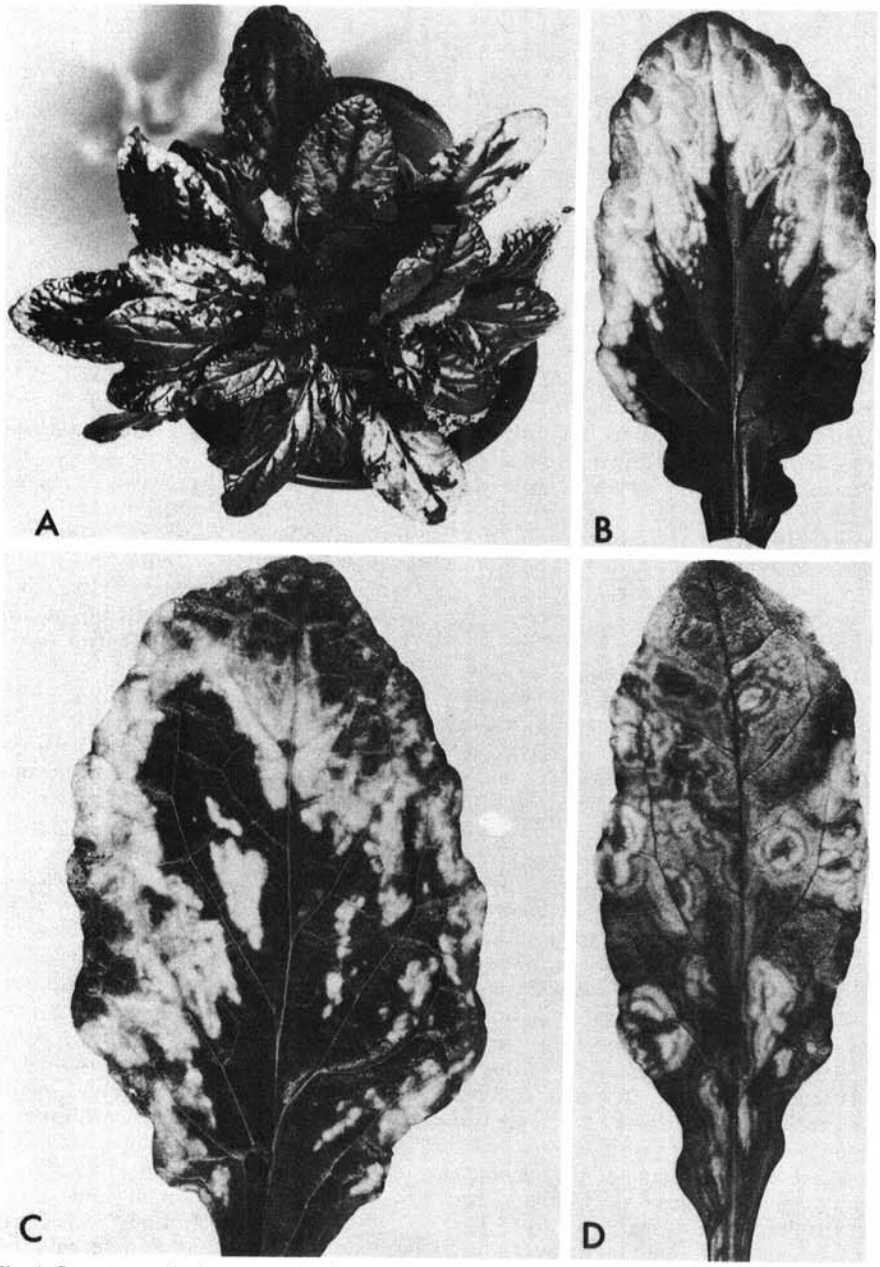

Fig. 1. Symptoms of tobacco streak virus **(A)** in a plant and **(B—D)** in leaves of *Ajuga reptans*.

DISCUSSION

It was surprising to find that *A. reptans* plants growing at three different locations and displaying similar ring spot symptoms contained different viruses,

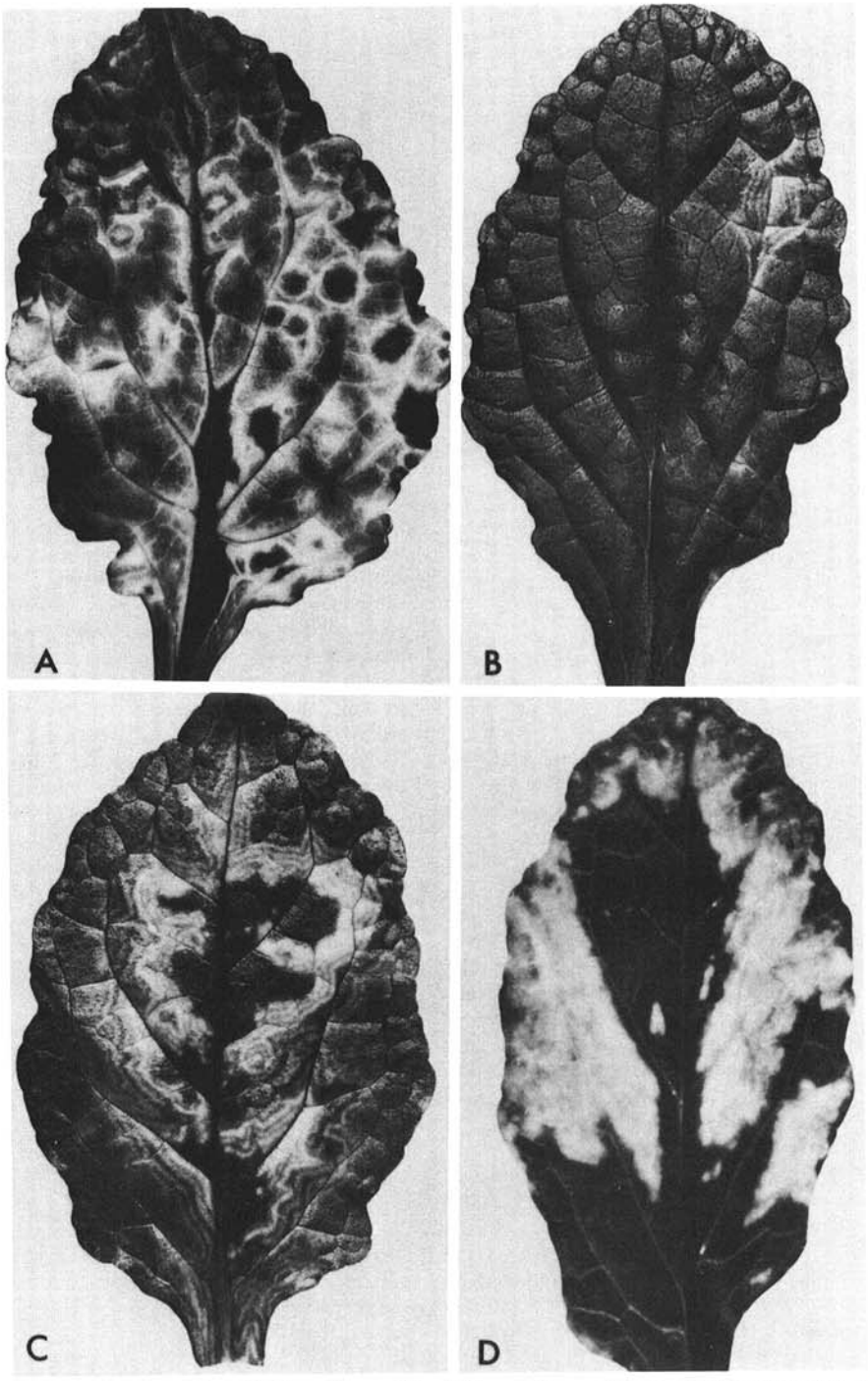

Fig. 2. Symptoms of **(A and B)** broad bean wilt and **(C and D)** cucumber mosaic viruses in leaves of *Ajuga reptans*.

namely TSV, BBWV, and CMV. Plants at the fourth location also exhibited ring spot, although a very mild one, and contained AMV and BBWV simultaneously. Because they are perennial, *A. reptans* plants can serve as potential reservoirs for these viruses in Victoria.

In Australia, the occurrence of TSV has been recorded only in Queensland. It was isolated from eight different plant species including dahlia, strawberry, tobacco, and *Ageratum houstonianum* (1,5,6). All of the Queensland isolates had identical antigenic properties in immunodiffusion tests (1,6). In contrast, Ajuga-TSV was distinct from the *Ageratum* isolate of the virus from Queensland (5) in gel diffusion and cross-protection tests, demonstrating that Ajuga-TSV is a new strain of the virus occurring in Australia.

A. reptans plants from two locations yielded BBWV. Ajuga I-BBWV was identical in antigenic properties to serotype I isolates of BBWV (17) commonly found in some parts of Australia, including Victoria (15). Ajuga II-BBWV, however, is possibly a third serotype of BBWV not recorded previously. It gave a very low titer with antisera to both the serotypes and when it was compared with serotype I and II isolates of BBWV in immunodiffusion tests using antisera to both the serotypes, the precipitin lines produced by the serotype I and II isolates spurred over the Ajuga II-BBWV isolate precipitin lines.

Although the AMV and CMV isolates from *A. reptans* were not compared with currently occurring strains of these viruses in Australia, they appeared to be the strains of AMV and CMV commonly found in this country on the basis of symptoms in indicator hosts.

Ajuga-TSV and Ajuga I-BBWV repeatedly failed to infect healthy *A. reptans* plants after mechanical inoculations. Although Ajuga-CMV multiplied systemically in its original host, it did not produce visible symptoms. Symptoms were produced only by the isolate containing AMV plus Ajuga II BBWV. The failure of Ajuga TSV and Ajuga I-BBWV to infect and of Ajuga-CMV to produce symptoms in *A. reptans* may be attributed to genetic differences in the host plants because the *A. reptans* plants used were not derived from the same stock as those found naturally infected.

The occurrence of CMV and AMV in *A. reptans* has already been reported from Denmark and the United States (8,9); however, this is the first report of *A. reptans* as a natural host of TSV and BBWV.

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