Periwinkle, A Latent Host for Broad Bean Wilt and Cucumber Mosaic Viruses in Australia

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ABSTRACT

SHUKLA, D. D., D. S. TEAKLE, and K. H. GOUGH. 1980. Periwinkle, a latent host for broad bean wilt and cucumber mosaic viruses in Australia. Plant Disease 64:802-803.

Cucumber mosaic and broad bean wilt (BBWV) viruses were isolated from symptomless periwinkle plants growing in and around Brisbane. Mechanical inoculation of periwinkle with the two viruses produced only latent infections. The periwinkle isolate of BBWV differed in host range, symptomatology, and antigenic properties, compared with the broad bean (type) strain of the virus. The periwinkle isolate is apparently a member of BBWV serotype II, which has not previously been reported from Australia. This is the first report of cucumber mosaic virus in periwinkle in Australia and of periwinkle as a natural host of BBWV.

Periwinkle (*Vinca rosea* L.) is a common ornamental plant in gardens in southeastern Queensland. Although it is occasionally planted, more often it grows from self-sown seed. A survey showed that it was an unsuspected, symptomless reservoir of broad bean wilt (BBWV) and cucumber mosaic (CMV) viruses. This article reports the isolation and identification of these viruses from periwinkle.

MATERIALS AND METHODS

Symptomless leaves and young shoots from periwinkle plants were collected from gardens in Brisbane, Beenleigh, and Nambour. The leaf tissues were ground either in 0.05 M phosphate buffer, pH 7.0, or in distilled water and inoculated onto Carborundum-dusted leaves of indicator plants. Test plants were maintained in a glasshouse at 20–25 C and observed as long as 6 wk for symptom development.

Ouchterlony double diffusion tests were done in 1% agarose-water gels. Six 4-mm wells were equally placed around a center well at an edge to edge distance of 6 mm. The CMV antisera were those previously produced against isolates from gerbera and gladiolus (1,5). BBWV antisera were those produced against serotype I and II (10) and the broad bean (BB;Type) strain (8) of the virus.

After the BBWV isolates were identified, parallel mechanical inoculations were made with one typical periwinkle (PW) isolate and the BB strain (8) to selected test species, using infected *Nicotiana clevelandii* plants as the source for both isolates. Back inoculations were made about 4 wk after inoculation to *Chenopodium amaranticolor* or *C. quinoa*. Physical properties in vitro were determined for the PW isolate by using systemically infected *C. quinoa* as source and *C. amaranticolor* as test plants. The isolate was also tested for transmissibility by *Myzus persicae*. Nonviruliferous aphids, maintained on broad leaf dock (*Rumex obtusifolius* L.), were starved for 15 min and then given brief feedings on systemically infected *C. amaranticolor* leaves. The aphids were then transferred to young, healthy broad bean plants.

RESULTS

CMV was isolated from periwinkle plants growing in Brisbane, Beenleigh, and Nambour. Most CMV isolates were identified by host range and symptomatology (2), but the identity of one typical isolate from Brisbane was confirmed using the CMV antisera. Young periwinkle plants grown from seed in the glasshouse remained symptomless after sap inoculation with the virus. However, the virus was recovered from top leaves of the inoculated plants 4 wk after inoculation.

BBWV was isolated from eight of 12 periwinkle plants at one site in Brisbane. The isolates produced chlorotic or necrotic local lesions followed by systemic necrosis in *C. amaranticolor* and *C. quinoa* and chlorotic or necrotic local lesions in *Phaseolus vulgaris* 'Bountiful' and *Vigna unguiculata* 'Blackeye.' No symptoms were produced in *Datura stramonium* and *N. tabacum* 'Xanthi.' Icosahedral particles about 30 nm in diameter were found in leaf dip preparations of infected *C. amaranticolor* with chlorotic local lesions. One typical isolate of the virus had a thermal inactivation point between 60 and 65 C, a dilution end point between 10^{-5} and 10^{-6}, and a longevity in vitro of 9 days at about 20 C. The isolate was successfully transmitted to two of five broad bean plants by *M. persicae* in a nonpersistent manner. It reacted with antiserum to the BB strain of BBWV (8).

Reactions of indicator plants in the initial isolation experiment indicated that two of the eight infected periwinkle plants also were infected with CMV. When PW-BBWV or PW-BBWV + CMV complex was mechanically inoculated onto young periwinkle plants in the glasshouse, no symptoms were produced by either inoculum. However, the viruses were recovered from top leaves of the inoculated plants in back inoculations to *C. amaranticolor* and Xanthi tobacco.

PW-BBWV and BB-BBWV induced similar symptoms on *C. amaranticolor, C. quinoa, Gomphrena globosa, N. clevelandii, Petunia hybrida, P. vulgaris* 'Bountiful,' and *Vicia faba*. The only difference was that the BB strain produced more severe symptoms than the PW isolate did. The two isolates differed markedly, however, in their reactions on *N. glutinosa, N. tabacum*, and *V. unguiculata*. The BB strain induced many local and systemic chlorotic concentric rings that became necrotic on *N. glutinosa*, whereas the PW isolate produced only a few local necrotic rings without any systemic symptoms, but the virus was recovered from the top leaves. *N. tabacum* cultivars Turkish and Xanthi developed local necrotic and systemic chlorotic rings and patterns after inoculation with the BB strain, but both remained symptomless after inoculation with the PW isolate, which could be recovered only from inoculated leaves. Both isolates induced similar local lesions on *V. unguiculata* 'Blackeye'; however, the BB strain also infected this species systemically, causing pronounced mottling.

PW-BBWV and BB-BBWV were compared in immunodiffusion tests with equivalent volumes of expressed leaf sap from infected *C. quinoa* plants as antigen sources. The antibody titers obtained with the three antisera and the two isolates are presented in Table 1. When the isolates were placed in adjacent peripheral wells and when antiserum to serotype I or the BB strain was in the center well, the precipitin lines produced by the BB strain extended over the lines produced by the PW isolate. In reciprocal tests with antiserum to serotype II, the precipitin lines produced by the PW isolate spurred over the BB strain precipitin lines (Fig. 1).

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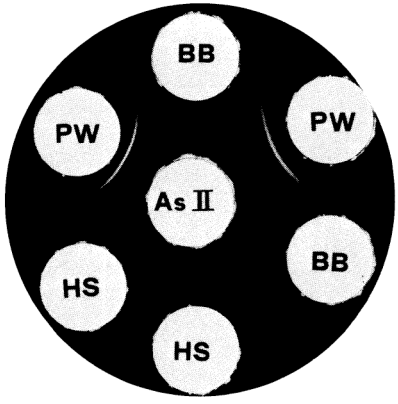

Fig. 1. Agar gel double diffusion test with antiserum to serotype II (As II, central well) and the antigens of periwinkle (PW) and broad bean (BB) strains of broad bean wilt virus (peripheral wells). HS = healthy sap. *Chenopodium quinoa* was the source of antigens.

Table 1. Reactivities between antisera and the periwinkle (PW) and broad bean (BB) strains of broad bean wilt virus in sap from infected plants

Antiserum	Homologous titer[a]	Antibody titer with antigens	
		BB	PW
BB strain	1:1024 (8)	1:1024	1:16
Serotype I	1:128 (10)	1:128	1:32
Serotype II	1:128 (10)	1:16	1:64

[a] Literature citations in parentheses.

DISCUSSION

Our results show that periwinkle is a host of CMV and BBWV in Queensland. Although the two viruses did not produce any visible symptoms in periwinkle, they cause serious diseases in many economically important crop plants around the world. Being perennial, periwinkle plants can serve as reservoirs for these aphid-transmissible viruses in Queensland. CMV has long been known to be present in Queensland (7) and also has been found in periwinkle in other countries (4), but this is the first isolation of the virus from this host in Queensland. This is also the first report of BBWV in periwinkle and of its presence in Queensland.

Our PW isolate differed from BB-BBWV (8) in host range, symptomatology, and antigenic properties. Although BBWV isolates with identical or different antigenic properties have previously been found to differ in host range and symptomatology, the one common property has been their ability to infect different *N. tabacum* cultivars systemically, with ringspot symptoms in most cases (3,6,9,10). In repeated inoculation tests, however, our PW isolate did not cause systemic infection in this plant species.

The BB strain of BBWV commonly found in some parts of Australia has been placed in serotype I of the virus (10). Although the antibody titer obtained with our PW isolate and antiserum to serotype II was 1:64 (Table 1), compared with a homologous titer of 1:128 (10), the precipitin lines produced by this isolate and the BB strain in the immunodiffusion plates indicated that the PW isolate is apparently more closely related serologically to serotype II than to serotype I. Others have found that antiserum to BB strain (8) does not react with serotype II isolates of the virus (3,10). In our experiments, however, this antiserum produced visible precipitin lines with the PW isolate up to a dilution of 1:16. BBWV serotype II had previously been found only in the United States and Argentina (3,10).

ACKNOWLEDGMENTS

We are grateful to R. G. Garrett for the aphid transmission facilities and for providing the BB strain of BBWV and its antiserum. We are also grateful to J. K. Uyemoto for the gift of antisera to the two serotypes of BBWV.

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