A New Green-Sterile Disease of Guar in South Africa

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ABSTRACT

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Guar (*Cyamopsis tetragonoloba*) in South Africa showed symptoms of small necrotic lesions on the leaves and reduced inflorescences. About half the seeds from infected pods were distorted and did not germinate. These symptoms are unlike any other viral diseases reported in guar, and the disease has been named the green-sterile disease. A flexuous viral particle (750 × 15 nm) has consistently been isolated from infected plants and seed. This viral particle is transmissible to *Glycine max, Lycopersicon esculentum, Phaseolus vulgaris, Vicia faba,* and *Vigna unguiculata* by mechanical inoculation. Symptoms on indicator plants were mainly localized or systemic red veins and chlorosis, whereas guar showed necrotic lesions after secondary mechanical inoculation. Nonpersistent aphid transmission was successful in some cases. Serological tests indicated a strong relationship to the potyviruses, namely, bean common mosaic virus, bean yellow mosaic virus, soybean mosaic virus, and potato virus Y. Cross-reactivity with guar symptomless virus antiserum was also established.

Guar (*Cyamopsis tetragonoloba* (L.) Taub.), thought to have originated in North Africa, is grown in the United States, India, Pakistan, and Africa for livestock feedstuff (13). Guar was introduced into South Africa in the late 1940s and is grown by the rural farmers in KaNgwane, Lebowa, Gazankulu, Venda, and the Lowveld. Recently, disease symptoms were observed on guar plants in fields in KaNgwane near the Eastern Transvaal. This disease has been named "green-sterile" and has not been reported elsewhere. Other reported viral diseases of guar include tobacco ringspot virus (6), the guar symptomless virus (GSV) isolated from seed (10), and two unidentified viruses—a lethal virus of *C. psoralioides* DC. (5) and a mosaic virus.

Preliminary investigations in South Africa failed to reveal a bacterial or fungal pathogen. Consequently, the aim of this research was to establish the causal agent of the guar green-sterile disease.

MATERIALS AND METHODS

Plant material. Seeds were provided by Chemserve Steinhall (Pty) Limited. Healthy (showing no symptoms) and infected guar plants were collected from fields in KaNgwane in the Eastern Transvaal. Plant material was collected for four consecutive years at the end of the growing season, when the guar plants were showing green-sterile symptoms. Cultivars used for crude and pure sap preparations were HSB-130, ZDPS, G-120, Lewis, SL-100, and TX-79-2741. Indicator plants used to determine host range (Table 1) were grown in cabinets at 25 or 30 C, 70% relative humidity, and a light intensity of 1,500 lx. A day/night cycle of 12 hr was maintained.

Distorted, necrotic seeds did not germinate, but healthy-appearing seeds from infected pods were allowed to germinate to see if symptoms developed. Both the seed coat and embryo of seeds from pods collected from green-sterile plants were tested for virus with homologous guar potyvirus and bean common mosaic virus (BCMV) antisera by indirect ELISA.

Mechanical inoculation. Infected guar leaves showing symptoms of sterility were macerated in 0.01 M phosphate buffer (w/v), pH 7.0, and Celite (100 μg/ml). After filtration through cheesecloth, the inoculum was gently rubbed over the surface of primary leaves of 10- to 14-day-old indicator and healthy guar plants. After 30 min, the leaves were rinsed in distilled water, and the plants were incubated at 25 or 30 C. As a control, healthy indicator plants were rubbed with distilled water and Celite or with sap from healthy guar leaves. Virus preparations, purified according to the method of Jafarpour et al (14), were also used for mechanical inoculation of the following plants: guar cvs. ZDPS and TX-79-2741 and *Phaseolus vulgaris* L. cvs. Top Crop, Wintergreen, and Contender. Contender plants showing symptoms from primary inoculations were used to mechanically inoculate guar and several indicator hosts in secondary and tertiary serial mechanical transmissions.

Aphid transmission. Transmission studies were performed with *Myzus persicae* (Sulzer), a common vector of potyviruses. Aphids were reared on healthy Chinese cabbage plants in insect cages. The plants were kept at temperatures between 15 and 20 C and continuous illumination. Nonpersistent transmission was tested by brushing virus-free aphids into a petri dish and starving them for 1 hr in a cool, shaded place. This was followed by an acquisition access period of 2 min on infected TX-79-2741 plants. At least 10 aphids per plant were then transferred to guar and to the following healthy indicator plants: *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn., *Pisum sativum* L., *Nicotiana glutinosa* L., *N. tabacum* L., *N. clevelandii* A. Gray, *Glycine max* (L.) Merr., and *P. vulgaris* cvs. Bountiful, Double White Stettler, The Prince, Wintergreen, and Top Crop. The aphids were allowed a transmission feed of 1 hr. The aphids were then killed and the test plants allowed to grow for symptom observation. Tests of persistent aphid transmission were made in the same way as for nonpersistent virus transfer except that no starvation period was required and the acquisition and inoculation feeds were longer (24 hr). In a parallel control test, the aphids were fed on noninfected guar leaves and transferred to indicator plants for the relevant acquisition and transmission access times.

Biological properties. To determine longevity in vitro, sap from infected TX-79-2741 plants was maintained at room temperature and mechanical inoculations were made every 24 hr to Top Crop plants at 30 C.

Sap (2-ml samples) from infected TX-79-2741 plants was subjected for 10 min to a temperature ranging from 20 to 80 C, with 5 C intervals. Each heat-treated sample was mechanically inoculated onto Top Crop plants to determine thermal inactivation.

Virus purification. Three extraction methods (1,9,14) were attempted from infected TX-79-2741 and ZDPS plants and from mechanically inoculated Contender and Top Crop plants. The method of Jafarpour et al (14) gave the highest yield of virus and was used in isolations

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in three subsequent seasons. Healthy and infected leaves (150 g) were homogenized in a blender in 450 ml of cold 0.5 M phosphate buffer, pH 7.2. Crude extracts were filtered through two layers of cheesecloth and clarified by shaking for 10 min with an equal volume of chloroform. The suspension was centrifuged at 8,000 rpm in a Beckman J-14 rotor for 25 min, and the virus was precipitated from the supernatant with polyethylene glycol 6000 (8%, w/v) and NaCl (0.5%, w/v). The extract was stirred for 30 min and allowed to incubate at 4 C overnight. The precipitate was collected by centrifugation at 8,000 rpm for 30 min, and the pellet was resuspended in one-tenth the original volume of 0.025 M phosphate buffer, pH 7.2. This was followed by two cycles of differential centrifugation, a low speed for 30 min followed by 27,000 rpm for 2 hr in a Beckman Ti rotor. Pellets were resuspended in 0.1–0.5 ml of 0.025 M potassium phosphate buffer, pH 7.2.

Serology. The final virus preparation obtained after purification of infected guar plants was used to raise antisera to the guar virus. The method described by Vaitukaitus (19) was used; 500 μl of sample was combined with an equal volume of Freund's complete adjuvant, the resulting emulsion was divided, and two New Zealand white rabbits were each injected with 500 μl of emulsion. The rabbits were shaved of fur along the back and proximal limbs, and each received 10 × 50 μl intradermal injections. After 8 wk the rabbits were bled from both the central ear artery and the heart, obtaining approximately 100 ml of blood from each rabbit. The serum was obtained from the blood by centrifugation at 3,000 rpm for 10 min.

Viral antibodies were extracted from the rabbit serum by ethacridine (Rivanol) precipitation as described by Hardie and van Regenmortel (11), followed by protein A-sepharose affinity chromatography as described by Miller and Stone (15). The IgG fraction was adjusted to a concentration of 1 mg/ml, as determined by an absorbance of 1.4 at 280 nm. The antiserum was then stored at 4 C with 0.02% NaN_3.

Antisera to the following viruses were kindly donated: BCMV and tobacco ringspot virus (TRSV) by A. Brunt, Glasshouse Crops Research Institute, Littlehampton, England; bean yellow mosaic virus (BYMV) by A. Brunt and by G. Loebenstein, Volcani Center, Bet Dagan, Israel; and potato virus Y (PVY), soybean mosaic virus (SMV), and southern bean mosaic virus (SBMV) by G. Pietersen, Rietondale Research Centre, Pretoria, South Africa. Antiserum to GSV was provided by J. Vetten, Plant Virus Institute, Braunschweig, Germany, who had worked on GSV with D. E. Lesemann.

Before routine enzyme-linked immunosorbent assays (ELISAs) were done, the optimal dilutions for antiserum, sample, and conjugate were established. Optimal concentrations were taken as the lowest concentration giving a high absorbance reading at A_{405} for positive infected samples and low readings for healthy controls. The indirect ELISA method was used to detect viral antigen. Antiserum for the indirect ELISAs was further purified by protein A-sepharose affinity chromatography (15) and host-adsorbed with healthy guar sap. Serological methods also included $F(ab')_2$ ELISAs (3), plate-trapped antigen ELISAs (18), and indirect ELISAs for virus detection in aphids (16). Both titer-plate and dot-blot immunobinding assays (12) were performed.

RESULTS

Field symptoms. Infected plants formed smaller leaves and had fewer, often sterile, inflorescences along the stem (Fig. 1) than did healthy plants (Fig. 2). The distribution of diseased guar in the field appeared to be random or sporadic. About 50% of the seeds from infected pods were distorted and discolored (Fig. 3), while the rest appeared normal in color and size. The sterile plants sometimes had petioles and elongated stems that remained green long after healthy plants had senesced. The distorted, shriveled seeds from infected pods did not germinate, whereas the healthy-appearing seeds from infected pods germinated without the appearance of symptoms.

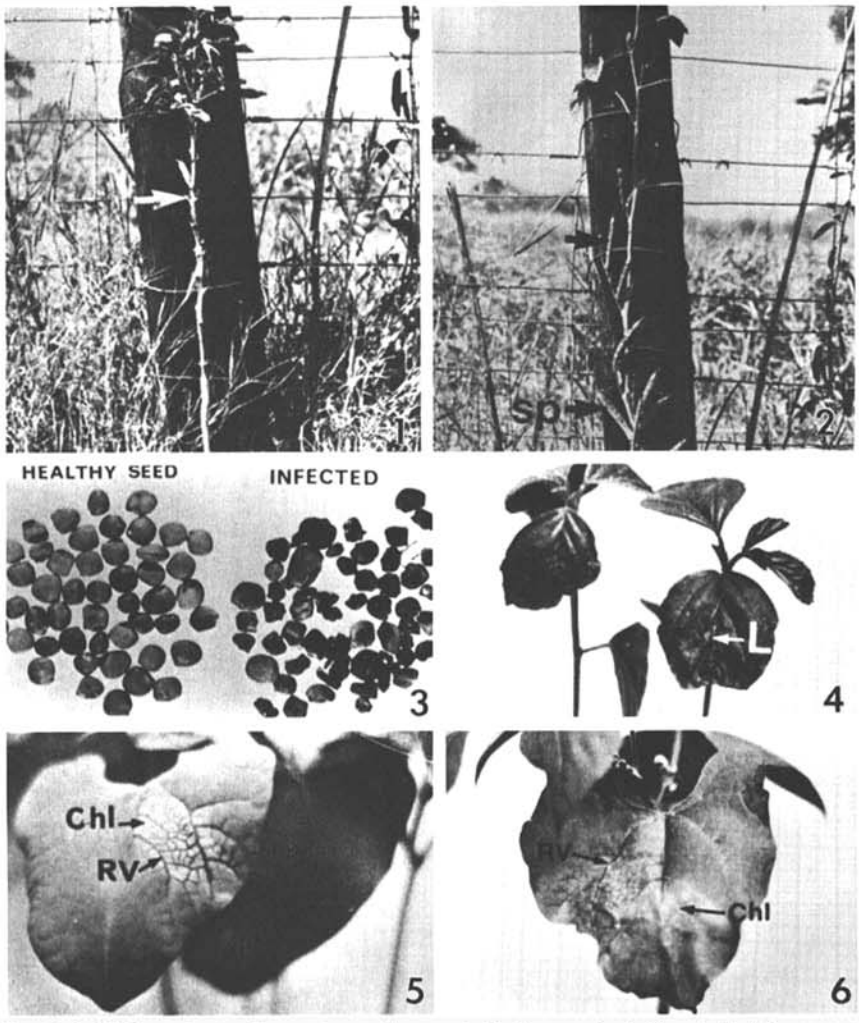
Symptoms on test plants. Symptoms appeared on several *P. vulgaris* cultivars at 25 C after primary sap inoculations with infected leaf extracts of guar cvs. TX-79-2741 and ZDPS (Table 1). Symptoms were also observed on *Lycopersicon esculentum* Miller, *G. max*, *Vigna unguiculata* (L.) Walp., and *Vicia faba* L. Symptoms were not observed on *P. vulgaris* cvs. Peru 0251, Sanilac, Pinto, and Canadian Wonder or on other indicator hosts, including *C.*

Table 1. Symptoms appearing at 25 or 30 C on indicator plants 10 days after mechanical inoculation with sap from infected guar (*Cyamopsis tetragonoloba* (L.) Taub.)

Indicator hosts Cultivars	Symptoms Inoculated leaves	Systemic (30 C)
Phaseolus vulgaris L.		
Monroe	Red vein necrosis, chlorosis (25 C)	...
Amanda	...	Leaf curl
Nep 2	Red vein necrosis, chlorosis, leaf curl (30 C)	...
Diacol Calima	Red vein necrosis, chlorosis (25 C)	...
Top Crop	Red vein necrosis, chlorosis (25 and 30 C)	...
The Prince	...	Leaf curl
Bountiful	Red vein necrosis, chlorosis (25 C)	Leaf curl
Double White Settler	...	Leaf curl
Wintergreen	Chlorosis, blistering (25 C)	Red vein necrosis, chlorosis
Contender	Red vein chlorosis, necrosis (25 and 30 C)	...
Seminole	Red vein necrosis, chlorosis (25 C)	Red vein necrosis, chlorosis
Golden Podded Wax	Red vein necrosis (25 C)	...
Lazy Housewife	...	Leaf curl
Genuine Cornfield	Red vein necrosis, chlorosis	...
Natal Round Yellow Sugar	...	Leaf curl, chlorosis
Vigna unguiculata (L.) Walp.		
Rhino	Chlorosis (25 C)	...
Blackeye	Red local lesions (25 C)	...
Vicia faba L.		
Blue Peter	...	Leaf curl, mosaic
Abundance	...	Vein necrosis, chlorosis, leaf curl
Lycopersicon esculentum Miller	...	Mosaic, leaf blistering
Glycine max (L.) Merr.	Chlorosis, mosaic (25 and 30 C)	Chlorosis
Guar		
TX-79-2741	...	...

quinoa, C. amaranticolor, Pisum sativum, N. clevelandii, N. glutinosa, N. tabacum cv. Samsun, *Datura stramonium* L., *Petunia* × *hybrida* Hort. Vilm.-Andr., and *Lupinus albus* L. No symptoms were observed on guar after primary inoculations. However, secondary and tertiary serial mechanical transmissions to guar, with extractions from *P. vulgaris* cv. Contender (showing red vein and chlorosis from primary inoculations with infected guar sap), resulted in formation of lesions and curl on guar leaves (Fig. 4). The most common bean symptoms were small necrotic red lesions appearing 5 days after inoculation, followed by hypersensitive red vein necrosis and chlorosis (Fig. 5). Symptoms were mostly restricted to inoculated leaves of *P. vulgaris* cvs. Nep 2, Monroe, Top Crop, Genuine Cornfield, and Contender. Symptoms occasionally spread systemically to one new leaf, such as in *P. vulgaris* cvs. Seminole, Wintergreen, Abundance, and Golden Podded Wax. In *P. vulgaris* cvs. The Prince, Double White Settler, Natal Round Yellow Sugar, Amanda, Bountiful, and Lazy Housewife, leaf curling and blistering were also observed at 30 C. Studies done over four consecutive years gave the same results with primary sap inoculations.

P. vulgaris cultivars showing symptoms at 25 C from primary mechanical inoculations were used for secondary and tertiary serial inoculations. Symptoms were mainly as observed before (Table 1) but were more severe in some cases, namely, *P. vulgaris* cv. Top Crop (Fig. 6), *G. max* (Fig. 7), and *Vigna unguiculata* (Fig. 8). *P. vulgaris* cvs. Top Crop and Contender mechanically inoculated with sap from serial transmissions to *P. vulgaris* cv. Contender (showing red veins and chlorosis) showed typical hypersensitive red vein necrosis as before but also occasionally showed yellow mosaic and blistering. *P. vulgaris* cvs. Top Crop, Wintergreen, and Contender mechanically inoculated with purified virus preparations (14) showed typical vein necrosis and chlorosis (Table 1). Two of the eight guar cv. TX-79-2471 plants showed necrotic lesions identical to those observed in the secondary and tertiary serial transmissions (Fig. 4).



Figs. 1–6. (1) Guar plant with symptoms of green-sterile diseases, including sterile inflorescences (arrow). **(2)** Healthy guar plant with seedpods (sp) in the axils (arrows). **(3)** Both healthy-appearing and distorted seeds from infected pods. **(4)** Lesion (L) on leaf of guar plant mechanically inoculated with sap from serial transmission to *Phaseolus vulgaris* cv. Contender. **(5)** Chlorosis (Chl) and red veins (RV) of leaf of *P. vulgaris* cv. Top Crop after primary mechanical inoculation at 30 C. **(6)** Red vein necrosis (RV), extreme chlorosis (Chl), and wilting of leaf of *P. vulgaris* cv. Top Crop after secondary serial passaging.

Aphid transmission. No symptoms were observed on indicator plants fed with aphids in tests of persistent transmission. With nonpersistent aphid transmission, leaf malformation and yellow mosaic were produced at 30 C on *G. max* and *P. vulgaris* cvs. Bountiful, Double White Settler, The Prince, and Top Crop. No symptoms were observed, however, on guar cvs. ZDPS and TX-79-2741, *C. quinoa, C. amaranticolor, Pisum sativum, N. glutinosa, N. tabacum*, and *N. clevelandii*. These symptoms of leaf curl and yellow mosaic were similar to those produced at 30 C but differed from the vein necrosis observed at 25 C.

Seed transmission. Healthy-appearing seeds from infected pods did not show any symptoms at germination despite containing virus, as was detected by ELISA with BCMV antiserum and antiserum raised against the potyvirus isolated from infected plants. Shriveled necrotic seeds from infected pods were also positive for BCMV and the potyvirus. Serological tests demonstrated that the potyvirus was present in seed coat and embryo tissue.

Biological properties. Inoculation of sap from infected guar cv. ZDPS onto *P. vulgaris* cv. Top Crop indicated that the longevity in vitro at 25 C was 2–3 days. The thermal denaturation point was 55–60 C and the dilution end point was 10^{-3}–10^{-4}. Red local lesions followed by red vein and chlorosis appeared 5 days after inoculation.

Virus purification. No virus particles were isolated from frozen (−20 C) infected guar leaves or indicator plants showing symptoms. The only successful isolations were obtained from fresh material collected from the field. Potyvirus particles were consistently isolated from guar cvs. ZDPS, Lewis, HSB-130, and TX-79-2471 leaves showing green-sterile symptoms over three consecutive growing seasons. No other viruslike particles were noted in any leaf-dip or purified preparations. From the extraction method of Jafarpour et al (14), the average concentration of viral protein was estimated to be 0.5 mg/100 g of fresh guar leaves. These figures were obtained spectrophotometrically using the absorption coefficient of 2.5 but were only rough estimations, because all contaminating proteins could not be eliminated during purification of the virus. The virus purification procedure for potyviruses developed by Hammond and Lawson (9) gave only 0.06 mg/100 g of fresh leaves. Long, flexuous virus particles, averaging 750 × 15 nm, were observed in low numbers in leaf-dip preparations (2) from infected guar cvs. ZDPS and TX-79-2741 and from purified virus prepara-

tions from all cultivars tested. No viruslike particles were observed from mechanically inoculated or from aphid-fed indicator plants, although symptoms were observed. This could be due to low virus concentrations or hypersensitive restriction of virus spread. Small numbers of potyvirus particles were observed from purifications done with infected pods.

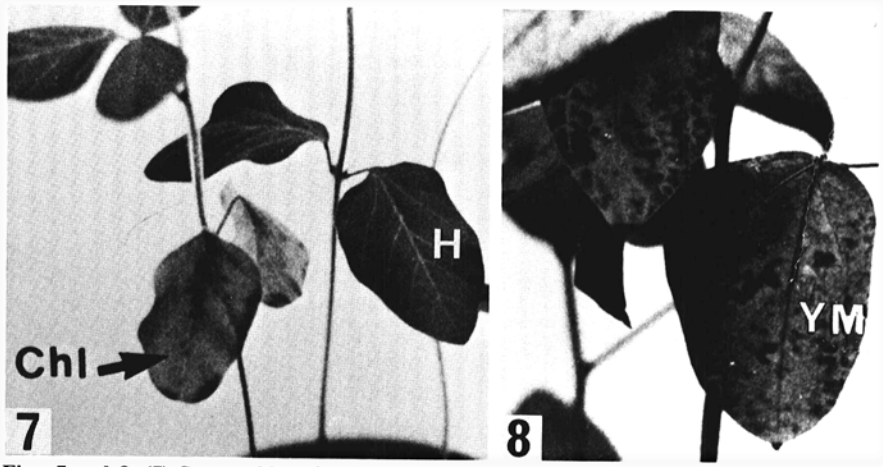
Serology. ELISA tests were taken as positive if the infected sample readings were higher than the healthy readings plus twice the standard deviation. The A_{405} readings for the $F(ab')_2$ ELISAs (Table 2) are the averages of eight tests. *Pisum sativum* and *P. vulgaris* cvs. Prince 3, Top Crop, and Double White Settler, which were mechanically inoculated with infected guar sap, were strongly positive for BCMV and weakly positive for BYMV. Guar cv. TX-79-2741 was strongly positive for both BCMV and BYMV. All samples were negative with SBMV antiserum.

Indirect plate ELISAs. All leaf samples of infected guar cvs. Lewis, ZDPS, TX-79-2741, and HSB-130 were strongly positive for BCMV, PVY, and SMV but negative for TRSV and SBMV (Table 3). Weak positive reactions were obtained with BYMV antiserum. Purified virus preparations (four methods) from guar cv. ZDPS plants showing green-sterile symptoms and pods from infected ZDPS plants were also strongly positive for BCMV, PVY, BYMV, and SMV but negative for TRSV and SBMV. Results of plate-trapped antigen ELISAs with TRSV, BCMV, and BYMV antisera were similar to those of indirect ELISAs. The same results were obtained in two additional seasonal studies.

Dot-blot immunoassays. Results of the dot-blot immunoassays were identical to those of the plate ELISAs (Table 4). Guar with green-sterile symptoms showed a strong binding to the homologous antiserum raised against the purified potyvirus preparation. In addition, the two guar plants that developed lesions after inoculation with a purified virus preparation also showed positive cross-reactivity with the homologous antiserum. Furthermore, leaf extracts (dilutions up to 1:1,000) of indicator plants showing symptoms from secondary and tertiary serial mechanical transmissions were dot-blotted onto nitrocellulose and reacted with PVY (dilution 1:500) and BCMV (dilution 1:500) antisera. *P. vulgaris* cvs. Top Crop and Contender as well as *V. unguiculata* and *G. max* gave positive results with both antisera. Guar plants showing necrotic symptoms (Fig. 4) after serial transmissions were positive for BCMV and PVY as well as for homologous antiserum (dilution 1:500) raised to a virus preparation from plants showing green-sterile symptoms (Fig. 9). Guar symptomless virus antiserum was recently obtained after the serological studies were completed. Positive binding to the GSV antiserum was obtained with field-infected guar cvs. ZDPS and TX-79-2741 leaf material as well as with mechanically inoculated *P. vulgaris* cvs. Wintergreen, Top Crop, and Contender.

DISCUSSION

Investigation of the causal agent of green-sterile disease of guar in South Africa has revealed a flexuous potyvirus (750 × 15 nm). Strong evidence exists that this virus may be associated with the disease, but failure to induce green-sterile symptoms prevents the establishment of this virus as the unequivocal causal agent. However, several factors, such as consistent isolation of this potyvirus and no other virus from seed and infected guar leaves over 4 yr, cross-reactivity of antiserum raised to the potyvirus purified from green-sterile guar



Figs. 7 and 8. (7) Severe chlorosis (Chl) of leaf of *Glycine max* at 25 C; H = healthy leaf. **(8)** Leaves of *Vigna unguiculata* cv. Rhino showing yellow mottling (YM) after mechanical inoculation with *Phaseolus vulgaris* cv. Contender (showing red veins and chlorosis) at 25 C.

Table 2. Absorbance (A_{405}) values for $F(ab')_2$ ELISAs of guar (*Cyamopsis tetragonoloba* (L.) Taub.) and indicator plants using bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), and southern bean mosaic virus (SBMV) antisera (1:1,500)

	BCMV antiserum		BYMV antiserum		SBMV antiserum	
Samples[a]	Infected	Healthy	Infected	Healthy	Infected	Healthy
Pisum sativum L.	0.71[b]	0.20	0.29[b]	0.18	0.13	0.01
Phaseolus vulgaris L.						
cv. Prince 3	0.17[b]	0.05	0.33[b]	0.13	0.04	0.01
cv. Prince 2	0.17	0.12	0.27	0.19	0.04	0.03
cv. Top Crop	0.71[b]	0.10	0.27[b]	0.09	0.06	0.02
cv. Bountiful	0.11	0.09	0.33	0.29	0.02	0.03
cv. Double White Settler	0.80[b]	0.09	0.39[b]	0.24	0.08	0.01
Guar						
cv. TX-79-2741	1.20[b]	0.16	1.08[b]	0.22	0.32	0.29
Positive virus controls	1.99	···	1.17	···	0.41	···

[a] Extracts of homogenized leaves clarified and diluted 1:1,000.
[b] Positive.

Table 3. Absorbance (A_{405}) values for indirect ELISAs of guar plants healthy or with symptoms of green-sterile disease

	Antisera[b]									
	BCMV		SMV		SBMV		PVY[c]		TRSV[c]	
Guar cultivars[a]	I	H	I	H	I	H	I	H	I	H
Lewis	0.66	0.12	0.68	0.14	0.14	0.08	++	–	–	–
TX-79-2741	0.12	0.68	0.14	0.08	0.06	0.08	++	–	–	–
ZDPS	0.44	0.15	0.60	0.16	0.09	0.07	++	–	–	–
HSB-130	0.65	0.10	0.67	0.11	0.09	0.08	++	–	–	–
Positive virus controls	1.70	···	1.50	1.10	···	···	1.70	···	1.20	···

[a] Sap preparations from healthy or infected leaves diluted 1:1,000.
[b] IgG purified on protein-A sepharose affinity columns and diluted in phosphate-buffered saline. BCMV = bean common mosaic virus, SMV = soybean mosaic virus, SBMV = southern bean mosaic virus, PVY = potato virus Y, TRSV = tobacco ringspot virus; I = symptoms of green-sterile disease, H = healthy.
[c] ELISAs performed on dot blots of leaf preparations onto nitrocellulose; ++ = strongly positive, – = negative.

Table 4. Dot-blot immunobinding assays with antisera raised against the guar potyvirus (isolated from plants with symptoms of green-sterile disease), guar symptomless virus (GSV), bean common mosaic virus (BCMV), and potato virus Y (PVY)

Virus sources	Antisera[a]			
	GSV	BCMV	PVY	Guar potyvirus
Guar cv. ZDPS[b]	++	+	++	+
Guar cv. TX-79-2741[b]	++	+	++	+
Guar cv. TX-79-2741[c]	++	+	++	+
Phaseolus vulgaris cv. Top Crop[c]	++	NT	++	+
Vigna unguiculata[c]	++	NT	++	+
Glycine max[c]	++	NT	++	+
P. vulgaris cv. Top Crop[d]	++	+	++	+

[a] + = Positive, ++ = strongly positive, NT = not tested.
[b] Field plant showing symptoms of green-sterile disease.
[c] Plants showing symptoms after secondary and tertiary serial transmissions.
[d] Plants showing symptoms after primary inoculations.

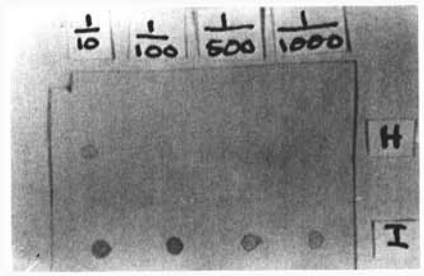


Fig. 9. Dot-blot immunoassay of healthy (H) and infected (I) guar preparations on nitrocellulose and reacted with homologous antiserum (dilution 1:500).

plants showing lesions after mechanical inoculations, and detection of a similar potyvirus in guar seeds from the United States, India, and Zaire (10), suggest that a potyvirus may be associated with green-sterile disease. The inability to obtain green-sterile symptoms in guar after mechanical inoculations may be explained by the slow spread of the virus and by symptoms manifesting at the flowering stage under field conditions only. If guar plants inoculated with purified virus preparations were allowed to grow to full maturity and size in the field, symptoms of sterility might develop. This has been impossible to achieve, however, for two reasons. First, infectious virus preparations can be obtained only from fresh material collected toward the end of summer when guar shows sterility; the virus occurs in low concentrations and remains infectious in mechanical inoculations to bean cultivars for a short time. Second, to grow to full height and set seed, guar requires high temperatures (28–32 C) and deep soil for its long taproot; consequently, guar does not grow well when confined to pots in a greenhouse. Thus, it has not yet been possible to inoculate guar seedlings with purified infectious virus at the end of summer and then grow the plants to full maturity over winter in order to observe symptom development. However, in a few cases in which local lesions were observed on inoculated guar leaves, the plants had positive reactions to homologous antiserum raised against the potyvirus isolated from plants with green-sterile disease. Furthermore, mechanical transmissions to several *P. vulgaris* cultivars with purified virus preparations resulted in positive cross-reactivity with homologous antiserum, suggesting that the green-sterile symptoms may be linked to the localized symptoms observed in mechanically inoculated indicator hosts and guar itself.

The symptoms of infected guar (Fig. 1) in South Africa are unlike the symptoms reported for top necrosis (6) and an aphid-transmitted mosaic disease reported in the United States (17). The potyvirus isolated in this study appeared to be transmitted by *M. persicae* to several *P. vulgaris* cultivars, but again no symptoms were observed in guar. Symptoms observed at 30 C differed from the hypersensitive red vein necrosis observed at 25 C, and the different symptoms obtained with aphids do not exclude the possible existence of more than one strain. It is also possible that the virus is able to spread systemically when introduced directly into the phloem by aphids, as opposed to mechanical inoculations. Host ranges, serological tests, and biological and physical properties place the virus isolated in this study in the potyvirus group. Failure to observe potential aphid vectors in the field, the random appearance of isolated infected plants in nature, and detection of virus in seed suggest that the causal agent may have been imported into South Africa via seed obtained from the United States. A similar potyvirus, GSV, has been isolated from seed of several guar isolates in the United States (10).

We compared the potyvirus in our study with GSV and BCMV because some evidence pointed to legume-seed transmission. Serological tests with green-sterile guar were positive with the GSV antiserum provided by J. Vetten, but earlier studies with this virus were inconclusive because their antiserum did not display any serological activity. The potyvirus isolated from green-sterile plants and GSV have some common features, including morphology, seed transmission, low virus concentration in all hosts, and lack of systemic hosts. However, investigators of GSV failed to obtain symptoms in naturally infected and inoculated guar, so it is not possible at this stage to establish any relationship between the two viruses. One may speculate that the failure of research workers to obtain symptoms with GSV and the inability to induce sterility in this study may be related. Host range studies of the two viruses differ, however. Our potyvirus induced hypersensitive red vein necrosis and chlorosis, typical of some BCMV-inoculated beans (4), in several *P. vulgaris* cultivars, whereas GSV was not transmissible to *P. vulgaris* cultivars. In addition, GSV induces red local lesions on *C. amaranticolor*, whereas the potyvirus isolated in South Africa was not able to infect this indicator host. BCMV also is unable to induce symptoms in *C. amaranticolor* (4).

Host range studies and symptom expression suggested that the potyvirus may be related to a strain of BCMV. The local strain of BCMV produces necrotic lesions or veins and chlorosis in *P. vulgaris* cvs. Sanilac and Peru 0251 and is hypersensitive above 26 C (8). In our study, however, Sanilac and Peru 0251 showed no symptoms. *P. vulgaris* cv. Monroe is known to be resistant to most common BCMV strains (groups I–VI) (7). In our study, no systemic spread (only local vein necrosis and chlorosis) occurred in inoculated Monroe. Again, however, the possibility of more than one virus strain cannot be ignored, since serial transmissions to Top Crop and Contender and aphid transmissions occasionally resulted in yellow mottling and blistering, not observed in primary inoculations at 25 C.

This study describes a new disease symptom of guar. Evidence indicates the possible involvement of a seed-transmitted legume potyvirus, but the potyvirus has not yet been unequivocally established as the causal agent. Further attempts are being made to induce green-sterile symptoms, and the relationship of the potyvirus to GSV and, with use of monoclonal antibodies, to BCMV is being elucidated.

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