Occurrence of Bean Golden Mosaic Virus in Florida

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ABSTRACT

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An epidemic of bean golden mosaic virus (BGMV-H) was observed in the winter of 1993 in South Florida. The disease was found in common bean, *Phaseolus vulgaris*, and lima beans, *P. lunatus*, in southwest Dade County and southeast Palm Beach County. In a survey of 125 fields in Dade County, there was an average disease incidence of 26%, with higher disease incidences in fields of cranberry beans and pole beans than in snap beans. Approximately 30% of the estimated 11,000 ha planted to snap beans in South Florida was affected. In snap bean fields where BGMV-H was most severe, growers reported yields of 26–87 hL/ha compared to expected yields of 175 hL/ha. In some cases, fields were completely abandoned or destroyed. The disease was not detected in the other winter bean production areas in South Florida. An isolate of the virus from Homestead (BGMV-H) was mechanically transmissible to *P. vulgaris* cv. Topcrop, and the whitefly *Bemisia tabaci* (also known as *Bemisia argentifolii*) was an efficient vector of the virus in transmission tests. Plants with bright golden mosaic symptoms tested positive for geminivirus infection when extracts were probed (dot blots) with A component DNA from a geminivirus infecting the weed *Macroptilium lathyroides* or from the recently identified tomato mottle geminivirus, both from Florida. The bean samples did not react with probes prepared to the B components for either of these viruses. Hybridization probes prepared to A and B components of BGMV-H gave strong reactions with extracts from beans infected with BGMV isolates from Guatemala and from the Dominican Republic. This is the first report of an epidemic of BGMV occurring in the continental United States.

Bean golden mosaic virus (BGMV) is a New World geminivirus (8). BGMV-Puerto Rico (-PR) (16) is the type isolate of *Geminiviridae* Genus Subgroup III (17), and a number of closely related strains and isolates are found in the Caribbean and Central America (10). BGMV-Brazil (-BZ) and other isolates from South America differ significantly from the BGMV-PR in genome nucleotide sequence and in the inability to be mechanically transmitted (10,14). Some confusion exists in the literature regarding the designation of BGMV (10), since the name was derived on the basis of disease symptoms in beans before the availability of more definitive properties such as serology, biology, and genomic sequences.

BGMV is one of the most serious constraints to bean production in the lowland tropics of Latin America. BGMV was not previously reported as economically important in Florida, although it was prevalent on nearby Caribbean islands such as Cuba (7), Jamaica (19), Dominican Republic/Haiti (13), and Puerto Rico (5). The distribution of BGMV has continuously expanded since the disease was first described (9). The disease has spread into many new regions of Latin America, such as Honduras and northeastern Brazil, where it was previously unknown, and has become the principal bean disease within the course of one season (18). Once established, the disease has been hard to control or eliminate (12). Control measures include the use of systemic insecticides or regulation of the crop cycle and the production of alternative whitefly hosts (12).

Previously, a geminivirus causing symptoms similar to BGMV in mechanically inoculated bean, *Phaseolus vulgaris* L. cv. Topcrop, was isolated from the weed *Macroptilium lathyroides* (L.) Urb. found in South Florida. This geminivirus was described as a BGMV-Florida isolate (15) and was shown to have a high sequence similarity to BGMV-PR (1), but it has not been found naturally infecting field-planted beans (*P. vulgaris*). This geminivirus has now been renamed as a Macroptilium geminivirus (MaGVFL; 15). In January 1993, a BGMV epidemic in snap beans was first noticed in Dade County (South Florida) and developed into a serious disease throughout the spring. This was the first occurrence of an epidemic of BGMV in beans grown in Florida (6). In this paper, results that characterize and identify this BGMV isolate and study its new distribution in Florida are presented.

MATERIALS AND METHODS

Prevalence and distribution of bean golden mosaic virus. Snap beans are produced during the winter months in five production areas in southern Florida: Homestead (Dade County), Pompano/Delray (Broward/Palm Beach Counties), Belle Glade (Palm Beach County), Immokalee (Collier County), and Naples (Collier County) (11). All five production areas were surveyed during February, March, and April 1993. Disease incidence data were collected from a plot of 100 consecutive plants in a randomly chosen row-site found at least 25 m from the border of the field. Plants were rated visually as diseased or not diseased based on the presence of typical symptoms of BGMV, which consisted of bright golden mosaic or chlorosis in systemically infected leaves (Fig. 1). Samples of symptomatic bean leaf tissue were collected from a subset of the surveyed fields in Pompano/Delray and Homestead to confirm geminivirus infection and to characterize the new disease. Confirmation of geminivirus infection in the representative samples was done by dot blot hybridization, as described below.

In Dade County, an extensive survey was conducted in the last week of February 1993, involving 125 bean fields in the areas southwest, west, northwest, and north of Homestead (Fig. 2). Dade County accounts for 75% of the 11,000 ha of snap

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beans grown in South Florida. In terms of hectarage, beans are the major winter vegetable for that county (11). The locations of the bean fields in the survey were mapped onto 1:24,000 scale quadrant maps. Beans are grown interspersed with tropical fruit orchards, ornamental plant nurseries, and other agricultural crops such as tomatoes. Each bean field was classified according to the type of bean grown and the approximate growth stage. Three major types of common beans are grown in Dade County: snap beans, cranberry beans, and pole beans. They differ in their growth habit, growing season, and cultural management. Beans were examined at four general growth stages: preflowering, flowering, pod fill, and maturity. The Homestead region was divided into four regions (Fig. 2): north (Kendall area), northwest, west, and southwest. The boundaries for these regions were the east-west roads Hainlin Mill Rd., Waldin Dr., and Mowry Rd., respectively (Fig. 2).

Fig. 1. Symptoms of infection with a bean golden mosaic virus isolate from Homestead, Florida (BGMV-H) **(A)** in Topcrop bean (mechanical inoculation) and **(B)** in field snap bean (natural infection).

Extraction of viral DNA. Infected leaf tissue (100 mg) was ground with the aid of a Kontes pestle/eppendorf tube in 500 µl of extraction buffer (pH 7.0, 0.1 M NaH_2PO_4, 2 mM EDTA, 10 mM Na_2SO_3, and 1% polyvinylpyrrolidone) in a 1.5-ml microfuge tube. The tissue extract was vortexed with 325 µl of chloroform for 15 min and then centrifuged for 5 min at 10,000 *g*. The aqueous phase was transferred to another microfuge tube. An equal volume of isopropanol was added, and the DNA was collected by centrifugation for 5 min at 10,000 *g*. The pellet was washed with 70% ethanol. It was dried under vacuum, and the DNA was resuspended in 100 µl of dH_2O. The resuspended DNA was treated with RNase before amplification by polymerase chain reaction (PCR).

Amplification of geminivirus sequences. The primers selected for geminivirus sequence amplifications were according to Rojas et al (21). For the A component DNA, primers PAL1v1978 and PAR1c496 were used. For the B component DNA, primers PBL1v2039 and PCRc1 were used. Both sets of primers amplify the intergenic regions of the two genomic components. These intergenic regions are the least conserved among whitefly-transmitted geminiviruses (3), and the hybridization probes prepared from these regions are useful in distinguishing geminiviruses. The PCR reaction of 100 µl contained 5 µl of DNA extracted from infected tissues, 40 µM deoxynucleoside triphosphate, 0.2 mM each primer, 0.15 mM Mg^{2+}, and 2–5 units of Taq polymerase. The reaction mixture was covered with 60 µl of mineral oil. Viral DNA was amplified in a BIOS Thermal Cycler by 35 cycles as follows: 94 C, 2 min; 55 C, 2 min; and 72 C, 3 min. A final (1 cycle) DNA extension for 10 min at 72 C was done.

Cloning of the amplified DNA. The amplified viral DNA was purified by electrophoresis in low-melting agarose and recovered with a Bio-Rad Prep-a-Gene kit. The purified DNA was ligated into PT7-Blue T-Vector (Novagen, Madison, WI) according to the supplier's instructions.

Preparation of tissue extracts for geminivirus dot blot hybridizations. Crude sap extracts from leaf tissues were prepared by crushing 4 leaf disks (picked from separate parts of a test plant and cut with the microfuge tube cap as a punch) in 75 µl of 0.4 M NaOH (freshly prepared) with the aid of a Kontes pestle/eppendorf tube. The extracts were centrifuged (microcentrifuge maximum speed 5 min) before spotting 3- to 6-µl samples onto nylon membranes (Amersham Hybond N+). The DNA on the membranes was fixed by UV cross-linking. The membranes were prehybridized (15 min) and then hybridized with a ^{32}P-labeled probe

(15 h) at 55–65 C in 1% sodium dodecyl sulfate (SDS), 1 M NaCl, and 10% polyethylene glycol (PEG, MW 8000). Cloned A and B components from MaGVFL (15) and from tomato mottle geminivirus (TMoV; 3,20) were used initially in the dot blot hybridization studies. Later, cloned fragments from BGMV-H were used in the hybridization tests. Geminivirus probes were prepared by random-primed labeling or by PCR random-primed labeling, using commercial kits. The membranes were washed briefly two times in 2× SSC (1× SSC = 150 mM sodium chloride, 15 mM sodium citrate, pH 7.0) at room temperature and then washed twice in either 2× SSC with 1% SDS at 65 C (moderate stringency conditions) or in 0.2× SSC with 1% SDS (high stringency) for 30 min each. The final washes were at room temperature in 0.1× SSC. The membranes were exposed to X-ray film for autoradiography at –70 C.

RESULTS

Prevalence and distribution of BGMV. Bean plants with bright, golden mosaic symptoms, characteristic of BGMV in Latin America, were first observed in the winter crop (1993) around Homestead (Fig. 1). Disease symptoms affecting production and crop value included fewer pods per plant, reduced length and width of pods, pod deformation, and poor seed set. In the surveys of the bean crop in Florida, BGMV-H infections were only found in Homestead (Dade County) and Pompano/Delray (Broward and Palm Beach Counties). The disease was not detected in the other winter bean production areas in southern Florida during the 1992–93 season (Table 1).

The average BGMV incidence in Dade county was 26% for all the fields surveyed. Disease incidence for individual fields varied from none to 100%. At least 30% of the estimated 8,100 ha of snap beans in Dade County were affected to some degree. In Pompano/Delray Beach, the farm visited had an average of 20% BGMV incidence in snap beans at the flowering stage.

The predominant type of bean grown in Dade County was snap bean, with 108 of the fields surveyed. Smaller amounts of cranberry beans and pole beans were grown, with 6 and 10 fields visited, respectively. Cranberry beans were only found west of Homestead, while snap beans were grown throughout the region. Pole beans were grown west of Homestead and in the northern region around Kendall Avenue. Disease incidences in fields of cranberry beans (72.3%) and pole beans (37.5%) were higher than in fields of snap beans (21.4%). For the west region with the highest disease levels, disease incidences in cranberry beans (85.0%) and pole beans (86.9%) were higher than in snap beans (35.0%). Individual fields of all three types of beans had up to 100% infection. BGMV was also identified in one field of lima beans (*Phaseolus lunatus* L.).

Beans of all developmental stages, from recently germinated to mature pods, were surveyed. The majority of the snap bean fields were at flowering (40 fields) or preflowering (36 fields). A substantial number of snap bean fields were at pod fill (23 fields) and close to harvest (Table 2). In all areas of the survey, beans of different ages were mixed, so that the distribution of beans at any one growth stage was even throughout Dade County. Early seedling stage bean fields were adjacent to bean fields that were being harvested.

Plantings in later growth stages had the highest incidences of BGMV. Both pole beans and snap beans are normally harvested before they mature, during the pod fill growth stage. However, seven fields of snap beans and two fields of pole beans were abandoned because of the BGMV epidemic and left to mature (Tables 2 and 3). Cranberry beans, which are normally harvested at physiological maturity, had high average incidence at the pod fill stage (Table 2).

The distribution of BGMV in Dade County was uneven. The west and north regions had higher than average incidence (Table 3). A focal point of high disease

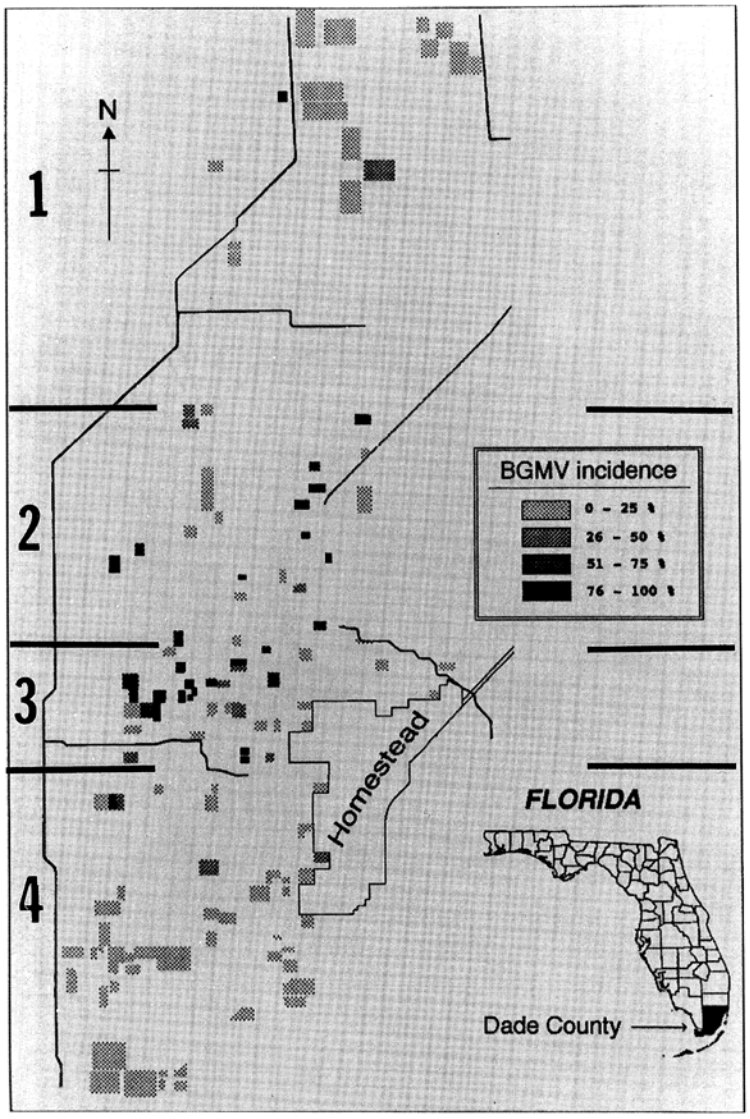

Fig. 2. Location and level of bean golden mosaic virus incidence for 125 common bean fields surveyed in Dade County, Florida, in February 1993. Regions were designated as Kendall (1), Northwest Homestead (2), West Homestead (3), and South Homestead (4). The boundaries (identified as lines on the sides of the figure) for these regions were the east–west roads: Hainlin Mill Rd. (region 1 and 2), Waldin Dr. (region 2 and 3), and Mowry Rd. (region 3 and 4).

Table 1. Survey of bean production areas in South Florida for average bean golden mosaic virus incidence[a]

Production area	Farms surveyed	Incidence (%)
Naples	1	0
Immokalee	1	0
Belle Glade	4	0
Pompano/Delray	1	20
Homestead	125	26
Ruskin	1	0

[a] Survey conducted during February–March 1993.

incidence was observed directly northwest of the city of Homestead (Fig. 2). Lower disease incidence was observed in the more peripheral areas further north and south of Homestead. A declining gradient was evident in a northerly direction from the focal point west of Homestead. In the west and north regions with high incidence of BGMV, some snap bean fields were left to mature. Meanwhile, none of the fields of snap beans in the south and Kendall regions, where BGMV was less severe, were left to mature (Table 3).

Yield losses due to BGMV incidence. In those plantings where BGMV was most severe, growers reported snap bean yields of 26–87 hL/ha compared to expected yields of 175 hL/ha. In some cases, fields were completely abandoned or destroyed. Growers also reduced bean plantings when losses due to BGMV became evident as the season progressed. The volume of snap beans marketed at two packing houses in Dade County indicate a significant loss in the bean production in 1992–93 and 1993–94 compared to 1991–92, when BGMV incidence was not observed.

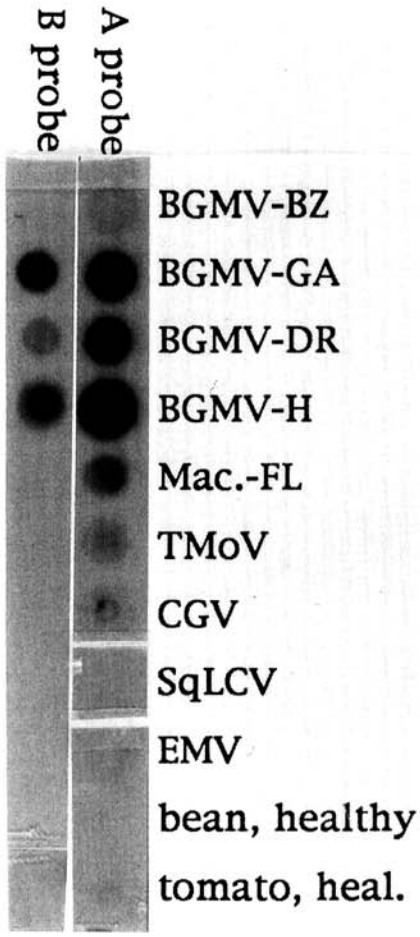

Fig. 3. Dot blot hybridization test with extracts from bean golden mosaic virus isolates from Brazil (BGMV-BZ), Guatemala (BGMV-GA), Dominican Republic (BGMV-DR), and Homestead, Florida (BGMV-H), from a geminivirus infecting the weed *Macroptilium lathyroides* in Homestead (MaGVFL), tomato mottle virus (TMoV), cabbage geminivirus (CGV), squash leaf curl virus (SqLCV), euphorbia mosaic virus (EMV), and from noninfected bean and tomato. A probe represents a 1.1-kb cloned DNA from BGMV-H A component, and B probe represents a 0.6-kb cloned DNA from BGMV-H B component. Hybridization was under high-stringency conditions.

Identification of the geminivirus. Dot blot hybridization of tissue extract samples from beans showing characteristic BGMV symptoms were positive when tested with the A component DNA from TMoV and MaGVFL, but not with the B component DNA from these geminiviruses. Probes prepared to cloned fragments of the BGMV-H A and B components reacted very efficiently in hybridization tests with extracts from field samples showing BGMV symptoms. The BGMV-H specific probes were tested under high stringency conditions against known geminiviruses including Latin American BGMV isolates, geminiviruses identified recently in Florida (TMoV, MaGVFL, cabbage geminivirus), squash leaf curl virus (SqLCV), and euphorbia mosaic virus (EMV). Dot hybridization results (Fig. 3) show strong reactions with BGMV-PR related isolates (13) from Guatemala and Dominican Republic, and not with other geminiviruses.

The virus was readily transmitted mechanically with 0.1 M potassium phosphate buffer, pH 7.8, 0.2% 2-mercaptoethanol, and Carborundum abrasive from field samples to *P. vulgaris* cv. Topcrop and maintained in this host (Fig. 1A). The whitefly *Bemisia tabaci* (Genn.) (also known as *Bemisia argentifolii* Bellow & Perring) was an efficient vector of the virus in transmission tests.

DISCUSSION

During the 1992–93 bean production season, the distribution of BGMV in southern Florida was limited to the southeastern coastal region. BGMV was found in two discontinuous production areas, Homestead and Pompano/Delray Beach, which are separated by 130 km. The disparate distribution of BGMV in South Florida could be due to separate introductions or to the same pathogen moving northward.

The focal point of the BGMV epidemic was in the area west of Homestead and may indicate that the source of inoculum was there. Hurricane Andrew swept through the Homestead area in late summer 1992. The sequence similarity of the BGMV-H isolate to the Caribbean BGMV isolates (10), based on hybridization (Fig. 3) and on preliminary sequence comparisons (A. M. Abouzid and E. Hiebert, *unpublished*), have led to speculations that the bean geminivirus may have been introduced into Florida by Hurricane Andrew. However since both Homestead and Delray Beach are southwest of major ports of entry, Miami and Palm Beach, respectively, the virus may have been introduced accidentally during traffic at these entry ports from the Caribbean.

BGMV-H has the potential to become endemic to the state. In southern Florida, snap beans have a long, continuous growing season, from August to April, in which whitefly populations and virus inoculum can build up. The crop is planted successively, so that the young plantings whiteflies prefer are always available. In this survey, newly planted fields were found adjacent to old and abandoned fields. Thus, BGMV-H inoculum was easily transmitted by whiteflies from the mature bean field to the developing field. BGMV also delays flowering in infected beans and the onset of senescence. These infected beans in a prolonged vegetative state serve as inoculum sources for the whitefly vector. The occurrence of frost in

Table 2. Average incidence of bean golden mosaic virus in snap, cranberry, and pole beans at four growth stages in Dade County, Florida, March 1993

	Growth stage			
Bean variety	**Preflowering**	**Flowering**	**Pod fill**	**Maturity**
Snap	6.3 (36)[a]	22.5 (40)	33.4 (23)	52.3 (7)
Cranberry	...	...	66.8 (4)	83.5 (2)
Pole	10.0 (2)	57.0 (3)	35.3 (3)	38.8 (2)

[a] Number of fields sampled for each growth stage and variety shown in parentheses. Incidence given in percent.

Table 3. Average bean golden mosaic virus incidence for snap beans at four growth stages in four regions of Dade County, Florida, March 1993

	Growth stage			
Region	**Preflowering**	**Flowering**	**Pod fill**	**Maturity**
North[a]	0.8 (12)[b]	0.7 (13)	10.9 (9)	...
Northwest	8.5 (11)	42.1 (18)	60.0 (5)	100.0 (3)
West	12.6 (8)	27.8 (4)	46.2 (5)	52.4 (4)
Southwest	4.8 (5)	4.4 (5)	35.8 (4)	...

[a] Regions of Dade County are shown in Figure 2.
[b] Number of fields sampled for each growth stage and region shown in parentheses. Incidence given in percent.

midwinter interrupts the growing season for beans in central and northern areas of Florida and may prevent the northward spread of BGMV-H. However, the virus could overseason in alternative hosts in these areas. Other whitefly-transmitted geminiviruses have become well-established throughout Florida on tomatoes (3,20), cabbage (2), and several weed species (1).

All three types of bean grown in Dade County were very susceptible to BGMV-H. Snap beans were susceptible to pod distortion caused by BGMV-H. Deformed pods are of little commercial value and are removed at the packing house. Thus, BGMV-H caused a reduction in marketable yield through reductions in quality as well as in total yield. Significant losses were reported by the growers, and records at two packing houses verify the impact of the BGMV epidemic. Abandoned bean fields show that BGMV-H can have a devastating effect on bean production. Unfortunately, resistant varieties of snap, cranberry, and pole beans are not available. In Latin America, BGMV-resistance has been used effectively to control the disease for dry beans (4).

The higher disease incidence in pole beans and cranberry beans compared to snap beans is probably due to the combination of indeterminant growth habit and longer growing period. Both pole beans and cranberry beans have indeterminate growth habit and begin flowering about 2 wk later than snap beans, which are determinate. Pole beans remain in the field a longer time because of the multiple harvests of the pods; whereas cranberry beans are harvested when the plants are approaching physiological maturity. Pole beans are considered a 90-day crop; whereas snap beans take less than 60 days before harvest.

An integrated pest management plan is urgently needed to control BGMV-H in southern Florida, where growers are not accustomed to dealing with this disease. Cultural practices, such as crop sanitation, avoidance of sequential plantings in the same or adjacent fields, avoidance of planting downwind from older bean fields, field rotation, and regionalization of bean production could help reduce the incidence of BGMV-H.

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