Research Note

TEMPORAL AND SPATIAL DISSEMINATION OF BEAN GOLDEN YELLOW MOSAIC VIRUS (BEGOMOVIRUS) IN PHASEOLUS VULGARIS 'JAMAICA RED'12

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Bean golden yellow mosaic virus (BGYMV) is transmitted by whiteflies (*Bemisia* spp.) and causes significant losses in common bean (*Phaseolus vulgaris* L.) in Caribbean and Latin American regions (Beaver and Morales, 2000; Bird and Maramorosch, 1978). Symptoms observed in infected plants are yellowing, mosaic, stunting and distortion of leaves and pods (Bird et al., 1973; Morales, 2000; Morales and Niessen, 1988). This virus is difficult to transmit mechanically and has a limited host range.

A BGYMV inoculation method developed by Adames Mora et al. (1996) using viruliferous whiteflies was adapted to the study of dissemination of BGYMV under field conditions. This methodology ensured that bean plants were inoculated at the same stage of development with a uniform amount of inoculum. Symptom expression and enzymelinked immunosorbent assay (ELISA) were used to assess the spread of virus infection in the field. A monoclonal antibody, 3F7, developed by Cancino et al. (1995) has been used in Puerto Rico to detect BGYMV by using ELISA tests in *Phaseolus vulgaris* L. (Rivera et al., 2001). Monoclonal antibody 3F7 is a broad-spectrum antibody able to detect BGYMV isolates from the Dominican Republic, Guatemala and Puerto Rico.

Few epidemiological studies modeling begomovirus dissemination in the field have been reported. Fargette and Vié (1994) studied the temporal and primary spread of the African cassava mosaic virus (ACMV) in cassava plantings on the Ivory Coast, West Africa. They reported that ACMV epidemics over time resulted from primary spread and were dependent on crop age and planting date. They chose a monomolecular model as the best fit for the experimental data obtained in field experiments. In Israel, infections of tomato yellow leaf curl virus (TYLCV) occurred from primary spread at the beginning of the season and secondary spread as the season progressed. Polston et al. (1996) studied the spatial and temporal dynamics of tomato mottle geminivirus and its relationship with *B. tabaci* populations in Florida tomato fields. Immigrating viruliferous whiteflies rather than secondary spread within tomato fields appeared to be the driving force behind epidemics of tomato mottle in production systems characterized by frequent insecticide applications.

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⁵Researcher, Department of Agronomy and Soils, University of Puerto Rico, Mayagüez. In Puerto Rico, with a limited bean production, there are no studies on BGYMV spread under field conditions. Therefore, the objective of this research was to assess the temporal and spatial dissemination of BGYMV in common bean under field conditions during the summer, using MAb-based immunoassays and observations of symptom expression.

Field experiments to study natural BGYMV dissemination were established during the summer of 1999 at the Alzamora Farm, University of Puerto Rico-Mayagüez Campus. 'Indeterminate Jamaica Red', a BGYMV-susceptible common bean cultivar, was used in three adjacent plots to collect data on the dissemination of the virus. Artificial foci were created by using plants of the susceptible common bean cultivar Top Crop at the center of each plot. All foci contained three infected plants, each with 36 viruliferous whiteflies. Each ring consisted of eight bean plants around the foci. There were 20 rings per plot for a total of 160 plants per plot. In each plot, plant spacing within rows was 10 cm. Data of BGYMV incidence was collected every week for 37 days. All plants were visually inspected for symptom expression; 12% of the plants were selected at random for ELISA tests. This method was based on previous studies in which a sample size of 10% of a plant population had been sufficient to provide an estimate of viral assessments of tomato mottle geminivirus (Polston et al., 1996). Three leaves from young meristematic tissue were used for ELISA tests and the protocol was followed as previously described (Cancino et al., 1995; Rivera et al., 2001). Location and disease development for each plant was recorded.

Measurements of distance between virus-infected individuals were used to describe spatial pattern of virus dissemination. Average distance of BGYMV infected plants per number of plants in a row (rows were from A to H) were calculated and spatial maps were plotted. For temporal analysis of epidemics, three different models were evaluated, Gompertz, Monomolecular and Logistic, to select the appropriate model describing disease progress data. Only plots with disease incidence of 25% or greater were analyzed for temporal dissemination. The close fit between various models of disease progress curves was obtained by transforming the data with the following equations:

Model	$Equation^1$		
Gompertz	-ln [-ln (y)]		
Monomolecular	$-\ln[1/\ln(1/y)]$		
Logistic	ln (y/1-y)		

¹From Campbell and Madden (1990).

Corn (*Zea mays*) was planted at the edge of each bean plot to reduce BGYMV movement through its vector. No insecticides were applied during the experiment. Meteorological data gathered during the experiment, from May to June 1999, were provided by the Department of Geology, University of Puerto Rico-Mayagüez Campus (Table 1).

The first BGYMV symptoms were observed 12 days after foci were established in plots 2 and 3. At the end of the experiment, BGYMV total incidence was 10, 74 and 19% in plots 1, 2, and 3, respectively (Table 2). Virus incidence measured by symptom expression and ELISA tests was higher in plot 2, located at the center of the experimental plots. ELISA tests detected BGYMV disease earlier than did the evaluations based on symptom expression (Figure 1). Twenty-six days after inoculation, ELISA was not sufficiently sensitive to detect the disease, even though plants showed clear viral symptoms. The low number of samples that were positive for ELISA on the 31th day might have been due to the low virus titer in plant tissues at the onset of secondary infections. Plants reached maximum virus titer around 19 and 37 days after inoculation, most likely because virus replication is at its highest during this time of the infection period in primary and sec-

Weeks	Temperature (°C)	Relative humidity (%)	Dew point (°C)	Wind speed (km/h)	Barometric pressure (cm Hg)	Precipitation (cm)
I	30.3	66.9	21.74	8.86	29.98	0.123
II	28.7	64.8	21.22	9.40	29.98	0.071
III	29.5	64.6	21.98	7.83	29.99	0.000
IV	29.7	65.3	22.30	7.70	30.00	0.033
V	29.4	67.8	22.68	8.50	29.99	0.145
Mean	29.0	66.0	22.00	13.19	73.99	0.150
Range	19 to 35	52 to 94	13 to 25	4.82-28.96	75.97-76.32	0-1.04

TABLE 1.—Meteorological data taken weekly during experimental period.¹

¹Average of meteorological data taken weekly is presented.

ondary infections, respectively (Rivera et al., 2001). Virus titer reduction in plant cells is related to the decline of physiological activity due to the diverse physiological changes that occur in infected cells such as vacuolization, chloroplast degradation and finally cell death. In plants infected with Cucumber Mosaic Virus a series of progressive metabolic changes occur and chlorosis is correlated spatially with increase of glycolysis and respiration, decrease in photosynthesis and photosynthetic enzymes, and decrease in total protein synthesis (Técsi et al., 1996).

In beans the critical period of virus acquisition is 30 days after planting because infection before flowering could severely affect yields. In these experiments, virus titer increased again after 37 days as new plants were infected as a result of secondary infections. In summary, we observed primary and secondary infections in the field, at 19 and 37 days after the beginning of the infection (Figure 1). These findings are in accordance with geminiviruses transmission by *B. tabaci*, in which whiteflies can transmit the virus for five to 20 days, with a gradual loss in transmission efficiency over time (Brown and Bird, 1992).

A detailed diagram of virus infection during the experiment was recorded for each plot to monitor BGYMV progress under field conditions. Details of the experimental design for plot 2 are shown in Figure 2. This information was used to develop spatial map patterns of BGYMV movement during the experiment (Figure 3). Average distance of infected plants per total number of plants in a row was greater in plot 2 than in plots 1 and

	BGYMV incidence (%) and number of infected plants ¹ by field plots			
Virus assessment	1	2	3	
Symptom expression ² ELISA Total infected plants ³	3.75(6) 6.25(10) 10.00(16)	60.00 (96) 18.75 (30) 73.75 (118)	7.50(12) 13.75(22) 19.37(31)	

 TABLE 2.—BGYMV incidence (%) and number of infected plants assessed by symptom

 expression and ELISA tests in bean plots after 37 days.

¹Number of BGYMV infected plants in parenthesis.

²Based on 160 plants examined per field plot.

³Detected by symptom observations and ELISA.



FIGURE 1. Bean golden yellow mosaic virus incidence as measured by symptom observations and ELISA tests in bean field plot 2 after 37 days.

3. Plot 2 was located at the center of the experiment. Spatial patterns of virus-infected plants were nonrandom, on the basis of observations of a higher frequency of viral infections in plants close to the infection foci (Figure 3). Blocks of infected plants were also observed as new foci of infections developed from infected plants located at plot edges (Figure 3B).

The majority of geminiviruses are not mechanically transmitted and must be inoculated exclusively through whitefly vectors. These vectors feed on the phloem by a stylet, thus effectively acquiring and transmitting the virus (Brown and Bird, 1992). In our experiments whitefly populations in plots were not assessed, but we observed numerous flies, especially in the central plot. In general, whiteflies preferred to feed from young tissues at the apical meristem of the plants in which they reproduced. Whiteflies migrated from infected bean plants in the foci to colonize new food sources. No virus reservoirs of infected weeds such as *Macroptilium lathyroides* or other crops were close to experimental plots to provide for an external source of infected whiteflies or viral inoculum (Bracero et al., 2003).

The majority of viral infection was located in the southwest quadrants of experimental plots 1 and 2 (Figures 3A and B). Wind direction for the duration of the experiment showed that 46% of the time the wind was blowing from the east and northeast. This might cause the movement of whiteflies to the southwestern quadrants of plots 1 and 2 (Figures 3A and B). These findings are in agreement with those of Fargette and Vié (1994) in their studies with African cassava mosaic virus in Africa, in which virus inci-



FIGURE 2. Detail of the experimental design for plot 2 used to monitor BGYMV progress under field conditions. This figure illustrates bean plant arrangement and data of BGYMV infection by monitoring symptom expression and positive (+) ELISA tests (S = South; W = West; N = North; and E = East).

dence was determined by wind direction and time of planting. In their studies, primary spread accounted for the largest portion of infected cassava plants. Our conclusions agreed with theirs and those of other epidemiological studies in which primary infections accounted for the largest portion of infected plants (Fargette and Vié, 1994; Polston et al., 1996). In addition, we observed secondary infections of BGYMV in the field consistent with BGYMV capability to disseminate rapidly in the field.

On the basis of the evidence presented, we concluded that the Logistic and Gompertz models are the most appropriate to describe BGYMV progress data for the cultivar Indeterminate Jamaica Red under field conditions. Coefficient of determination (\mathbb{R}^2) values for the Logistic and Gompertz models were 97.3 and 94.3, respectively, compared to 75.8 for the Monomolecular model (Table 3). However, we concluded that the Gompertz model is superior to Logistic in describing BGYMV progress for the cultivar Indeterminate Jamaica Red, on the basis of a lower mean square error (MSE) value and smaller standard error for the slope. The Gompertz model provided the best fit to the field data (Table 3).

Even though our data reflect erratic and unpredictable BGYMV dissemination patterns in experimental fields, we managed to establish whitefly colonies in the field, and we observed a nonrandom pattern of primary and secondary infections. In addition, we provided a model for temporal distribution. The Gompertz model was the best fit to what we observed in plot 2, which had the highest BGYMV incidence. These findings on BGYMV temporal and spatial patterns emphasize the importance of secondary infections in incrementing disease incidence to epidemic levels. Studies in the epidemiology



FIGURE 3. Spatial map patterns of BGYMV infection in three different fields plots: A) Plot 1; B) Plot 2; and C) Plot 3. Letters from A to H represent plant rows; 0 to 160 represent the number of plants. Corn plants were planted at the eastern and western edges of each bean plot.

Model	${ m R}^2(\%)^1$	MSE^2	Intercept	Slope	St. error of intercept ³
Logistic	97.3	0.175	-8.01	0.222	0.0213
Gompertz	94.3	0.058	-2.89	0.086	0.0012
Monomolecular	75.8	0.034	-0.48	0.029	0.0095

TABLE 3.—Summary of linear regression statistics used in the evaluation of three models for appropriateness for describing disease incidence of BGYMV in field plot 2.

 ${}^{1}\mathrm{R}^{2}$ = coefficient of determination.

 $^{2}MSE = mean square error.$

³St. error of intercept = Standard error of the intercept.

of BGYMV are crucial for understanding this disease and for implementing management strategies. Further studies would be needed to validate this model and to explain the complexity of the interactions among BGYMV, its vector, host plant and the environmental factors.

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