

CHAPTER THREE - SPATIAL AND TEMPORAL MOVEMENT OF TOBACCO ETCH VIRUS WITHIN A JAMAICAN HOT PEPPER FIELD

Abstract

Spatial and temporal spread of *tobacco etch potyvirus* (TEV) (Genus: Potyvirus, Family: Potyviridae) was investigated within a 'Scotch Bonnet' pepper (*Capsicum chinense* Jacq.) field in Jamaica. The field contained 910 plants, each spaced 0.9 m apart. Plants were examined weekly for symptoms of TEV infection. Geostatistical analysis of the spread of TEV showed that it was spatially correlated throughout most of the study, with correlogram ranges of up to 25.5 m between pairs of infected plants. Maps produced by kriging showed that the spread of TEV was clustered about primary infections, which were scattered throughout the field. Therefore, secondary spread within the field was significant in the epidemiology of this virus. The incidence of TEV in the field increased with an increase in weekly aphid flight activity. A strong correlation also existed with a one-week lag between aphid flight activity and appearance of TEV symptoms. At least three aphid vectors of TEV, *Aphis gossypii* Glover, *A. spiraecola* Patch, and *A. craccivora* Koch were collected from within the field during the study.

KEYWORDS: aphid vector, epidemiology, epiphytology, geostatistics, Potyvirus

Introduction

Tobacco etch virus (TEV) (Genus: *Potyvirus*, Family Potyviridae) is the most important virus affecting hot peppers (*Capsicum chinense* Jacq.) in Jamaica (McGlashan 1993, Myers 1996, Martin *et al.* 1998¹). TEV is transmitted in a nonpersistent manner by aphids to over 150 plant species from more than 20 families (Edwardson and Christie 1997). Twelve species of aphids have been identified as vectors of TEV by various authors (Eckel and Lampert 1993). Five of these species, *A. gossypii* (Laird and Dickson 1963), *A. craccivora* Koch (Herold 1970), *A. spiraecola* Patch (Laird and Dickson 1963), *Lipaphis erysimi* Hille Ris Lambers (Eckel and Lampert 1993) and *M. persicae* (Laird and Dickson 1963), have been found in the pepper agroecosystem in Jamaica (Chapter Two).

The rate and pattern of spread of TEV within a pepper field is determined by the presence and behavior of its vectors, and the availability of a suitable source of TEV inoculum (Irwin and Ruesink 1986). Vectors must first probe on plants hosting TEV and then probe on uninfected pepper plants for the virus to spread within the crop. Even an inefficient vector can successfully spread TEV if it is common throughout the pepper-growing season (Raccah 1983). Non-colonizing vectors might be more important in spreading TEV if they occur in greater abundance than colonizing vectors that are more efficient (Raccah 1983). A vector that colonizes pepper immediately on arrival will not spread TEV as quickly as one that moves from plant to plant before settling and establishing a colony (Broadbent 1969, Zitter and Simons 1980) or as a non-colonizing species that moves from plant to plant before leaving the field in search of a host plant.

Researchers have used various methods to characterize the spatial and temporal movement of TEV within field crops. For example, Madden *et al.* (1997, 1998) examined spatial and temporal distribution of TEV within and among contiguous quadrats of 40 and 60 plants in individual

¹Martin, R., L. Myers, S. McDonald, and F. Ravlin. 1998. Incidence of pests of hot pepper in various agroecological zones in the parishes of St. Mary, St. Catherine and St. Elizabeth, IPM CRSP Annual report.

tobacco fields, while Eckel and Lampert (1993) investigated spatial and temporal spread of TEV among individual tobacco plants. The spread of TEV was spatially correlated among infected plants in all cases. Indices of aggregation (Madden *et al.* 1987), correlograms (Madden *et al.* 1987, 1988) and nearest neighbor technique with time lags (Eckel and Lampert 1993) have all been used to describe TEV spread in tobacco. Geostatistical methods such as correlograms, variogram estimation and kriging have overcome the barriers of traditional sampling methods when dealing with spatially correlated data (A. Sharov²).

The correlogram ($\rho(\mathbf{h})$) is the correlation coefficient between pairs of data values separated by distance \mathbf{h} (Isaaks and Srivastava 1989). The correlation coefficient may also be plotted against distance as $1 - \rho(\mathbf{h})$, producing an inverted correlogram. Correlogram parameters measured are the range and the nugget. The range is defined as a distance within which data points (in this case, infected plants) are spatially correlated (A. Sharov). The range is also the distance lag at which an inverted correlogram ($1 - \rho(\mathbf{h})$) reaches 1, its maximum value (Isaaks and Srivastava 1989). The distance lag, \mathbf{h} , may be a scalar if only distance is important, or it may be a vector if both distance and direction are important in the spatial relationship between two data points (Isaaks and Srivastava 1989, Journel 1989). If \mathbf{h} is a scalar the correlogram is said to be omnidirectional (Isaaks and Srivastava 1989). The nugget measures the extent to which the spatial distribution is random and is equal to the correlogram value ($1 - \rho(\mathbf{h})$) at $\mathbf{h} = 0$ (Isaaks and Srivastava 1989). The nugget and the range provide useful information on the spatial continuity of sampled data and are used in a process known as kriging to develop probabilistic models and generate maps of related areas of spread, including unsampled areas (Journel 1989, Lecoustre *et al.* 1989).

There is no information available on the pattern of spread of TEV in Scotch Bonnet peppers.

² Sharov, A. 1997. Quantitative Population Ecology: Online lectures. Department of Entomology, Virginia Tech, Blacksburg, VA.

The first objective of this research was to describe the spatio-temporal distribution of TEV-infected hot pepper plants (*C. chinense*, variety Scotch Bonnet) in a typical field in Jamaica. Specifically, we wanted to determine if the distribution of TEV infection in a Jamaican hot pepper field occurred by primary or secondary spread. Our second objective was to determine if the incidence of TEV was correlated with flight activity of aphids within a pepper field.

Materials and Methods

The experiment was conducted at the Ministry of Agriculture Experimental Station at Bodles, St. Catherine, Jamaica, W.I., from 26 June through 1 October 1998. Pepper seeds, *C. chinense*, obtained from CARDI, Antigua, were sown in sterile potting mixture (Easi-Grow, Bulrush Peat Co. Ltd., Londonderry, UK) and the seedlings kept under a screen cage to exclude aphids. Screen cages were supported by wooden frames, 0.5 m high x 0.9 m wide x 4 m long, and made of sheer curtain cloth with mesh sizes of 0.5 to 1 mm². The cloth with larger-sized mesh was used to cover the top of the cages to prevent excess humidity. Seedlings were transplanted in the field six weeks after sowing on 26 June.

The study site was 23.4 x 31.5 m (0.07 ha) with 26 rows spaced 0.9 m apart, each with 35 plants spaced at 0.9 m intervals (910 plants in total). Seedling holes were filled with a mixture of soil, dried cow manure (227 cm³ per hole) and N:P:K fertilizer (15:5:35 at 14 cm³ per hole) at transplanting. The fertilizer was applied to the soil at the base of each plant at twice the rate one month later. Weeds in the field were removed by hand while those around the borders of the field were treated with one application of paraquat (Grammoxone[®], Agroinsumos Guatemala S.A., under license from Zeneca Ltd) at a rate of 2.0 liters/hectare.

Monitoring TEV incidence

Before transplanting, tissue blots were taken from a random sample of 100 seedlings to confirm the absence of TEV. After the seedlings were transplanted, each of the 910 'Scotch Bonnet' plants was observed weekly, for 14 weeks, for symptoms of TEV infection. The first symptom of TEV appeared on new leaves as a dark and pale green mottling. The mottling soon coalesced

to produce characteristic light and dark green blotches. Symptomatic pepper plants were tagged with a different colored flag each week. Tissue blots were taken from each symptomatic plant as well as from 20 randomly selected nonsymptomatic pepper plants each week. Virus infection was confirmed by serological assay of the tissue blots.

Tissue blot immunoassay

Tissue blots were prepared and processed as described in Lin *et al.* (1990) and modified by Srinivasan and Tolin (1992), using NBT-BCIP substrate (Zymed Laboratories, South San Francisco, CA). Nitrocellulose membranes (Micron Separations Inc., Westborough, Mass.) were overlain with paper templates having an array of punched holes (0.7 cm in diameter and 0.5 m apart). A symptomatic leaf was removed from a pepper plant, rolled and torn. The torn end was pressed onto the nitrocellulose membrane until a uniform green color was seen.

Serological assays were conducted using four virus antisera, TEV (ATCC 69-PVAS; Wisconsin isolate; depositor: D.E. Purcifull), PVY (ATCC 50A-PVAS; depositor: W.B. Raymer), cucumber mosaic (CMV) (S. Tolin's laboratory, Blacksburg, VA) and tobacco mosaic (TMV) (S. Tolin's laboratory, Blacksburg, VA). These antisera were selected based on previous knowledge of viruses likely to be found infecting peppers in Jamaica (McGlashan 1993, Myers 1996, Martin *et al.* 1998).

Tissue blots were decolorized in 5% Triton X-100 for about 30 minutes, then rinsed for 3 minutes in 1X potassium phosphate buffered (pH 7.4) saline (KPS) + 0.05% Tween-20 before being placed in blocking solution (5% non-fat dry milk + 0.5% bovine serum albumin in 1X KPS) for 10 minutes. Next, tissue blots were agitated in 1:10,000 dilutions of polyclonal antisera of the respective viruses, TEV, PVY, CMV and TMV for 30 minutes. After three buffered rinses (10 + 5 + 5 minutes, 1X KPS + 0.05% Tween) the tissue blots were transferred to 1:20,000 dilutions of alkaline-phosphatase labeled goat-antirabbit serum (Sigma-Aldrich, St. Louis, MO) (30 minutes), three additional buffer rinses as before, and then NBT-BCIP substrate for about 5-10 minutes for color development. Tissue blots were then rinsed in distilled water for 3-5

minutes. Blots from test plants were compared against positive and negative control blots for each virus.

Monitoring aphids

Aphid flight in the field was monitored using five pan traps. One trap was placed at each corner, about 2.5 m from each edge of the field, and one trap was placed in the center of the field (between the 13th and 14th rows [14.8 m in] and in line with plant number 18 of both rows [16.5 m down]). Each trap (Chapter Two) was filled with approximately 500 ml 1:1 ratio of monoethylene glycol:water (modified from DiFonzo *et al.* 1997b). The glycol:water mixture was collected and replaced weekly. In the laboratory, aphids were removed from the mixture and counted. Aphid species were identified by Dr. Susan Halbert³ and by Dr. David Voegtlin⁴.

Statistical analysis

The data on virus incidence were analyzed by correlogram estimation to detect the extent of spatial correlation in the spread of TEV. The range and nugget were obtained from the estimated correlogram for each week and used in kriging to produce probability maps of the progression of TEV in the field. The following non-linear regression model (least square method) was used to fit the correlogram [1- (h)] points:

$$\tilde{c}(h) = 1 - (1 - \text{nugget}) * \exp^{-\text{distance}/\text{range} * 3}$$

where *distance* is the distance between any two infected plants, *nugget* is the correlogram value at $h = 0$, and the range is the distance where the correlogram reaches a plateau. Rate of spread of TEV was fitted to the following logistic model:

$$\% \text{ TEV infection} = 100 / (1 + \exp(-(t_x - t_{50\%}) * \beta_1))$$

³ FDACS/DPI, Gainesville, Fl.

⁴ Center for Biodiversity, Illinois Natural History Survey, Champaign, Illinois.

where t_x = any given day after transplanting, $t_{50\%}$ = number of days after transplanting when 50% of the crop became infected with TEV, and β_1 = slope of the fitted line. Least square method was used to improve the fit between the observed and predicted values for the incidence of TEV. Time series cross-correlation analyses (ARIMA procedure, SAS Statistical Institute Inc. 1996) were conducted to determine the relationship between the total number of winged aphids captured and TEV incidence each week and at different lag weeks.

Results

The first symptomatic Scotch Bonnet pepper plant confirmed as having TEV was found 27 days after transplanting (DAT). No other virus was detected at any time. There was a positive correlation ($r = 0.96$, $N = 11$) between the number of plants that showed TEV symptoms and the proportion of blots that tested positive for TEV (Table 3.1). The number of plants with TEV symptoms was overestimated during the first three weeks of symptom appearance because we were learning to recognize TEV symptoms in field grown peppers. From the fourth week onward estimations were very accurate. The correlation was also high ($r = 0.97$, $N = 11$) between the number of nonsymptomatic plants and the proportion of blots from the random sample that tested negative for TEV (Table 3.1). The number of nonsymptomatic plants that tested positive for TEV was high on the last two sampling dates. In most cases, nonsymptomatic plants for which the random tissue blots tested positive became symptomatic for TEV by the following week indicating that Scotch Bonnet plants can become infected with TEV for about one week before developing symptoms.

The initial distribution of TEV as observed at 27 and 34 DAT was random. The correlograms for these weeks had pure nugget effects confirming that these infected plants were not spatially correlated ($p > 0.1$) (Figure 3.1. A). Distribution of TEV became spatially correlated ($p < 0.005$) from 42 DAT to 97 DAT (Figures 3.1. B-J). The range, h , was 4 m at 42 DAT and increased to 26 m by 97 DAT (Figures 3.1. B-J). The nugget effect ranged from 0.54 at 76 DAT to 1.0 at 27 DAT (Figures 3.1. A-J). This suggests increasing spatial correlation up to 76 DAT when 38% of

Table 3.1. Proportion of total *tobacco etch virus*-symptomatic and nonsymptomatic ‘Scotch Bonnet’ pepper plants that tested positive for TEV. Samples of tissue blots were taken during 23 July through 1 October 1998 at Bodles, St. Catherine, Jamaica, W.I.

Days after transplanting	Symptomatic	Nonsymptomatic
27	1/20 ^{ab}	0/20 ^{ab}
34	2/50	0/20
42	12/53	0/20
48	8/13	2/20
55	23/23	0/20
62	41/41	1/20
69	42/42	2/18
76	165/167	1/19
83	106/106	1/10
90	103/107	9/10
97	132/136	7/7

^a Numerator = number of plants that tested positive for TEV, denominator = number of plants sampled.

^b $r = 0.96$ and 0.97 for the proportion of confirmed symptomatic and nonsymptomatic plants, respectively.

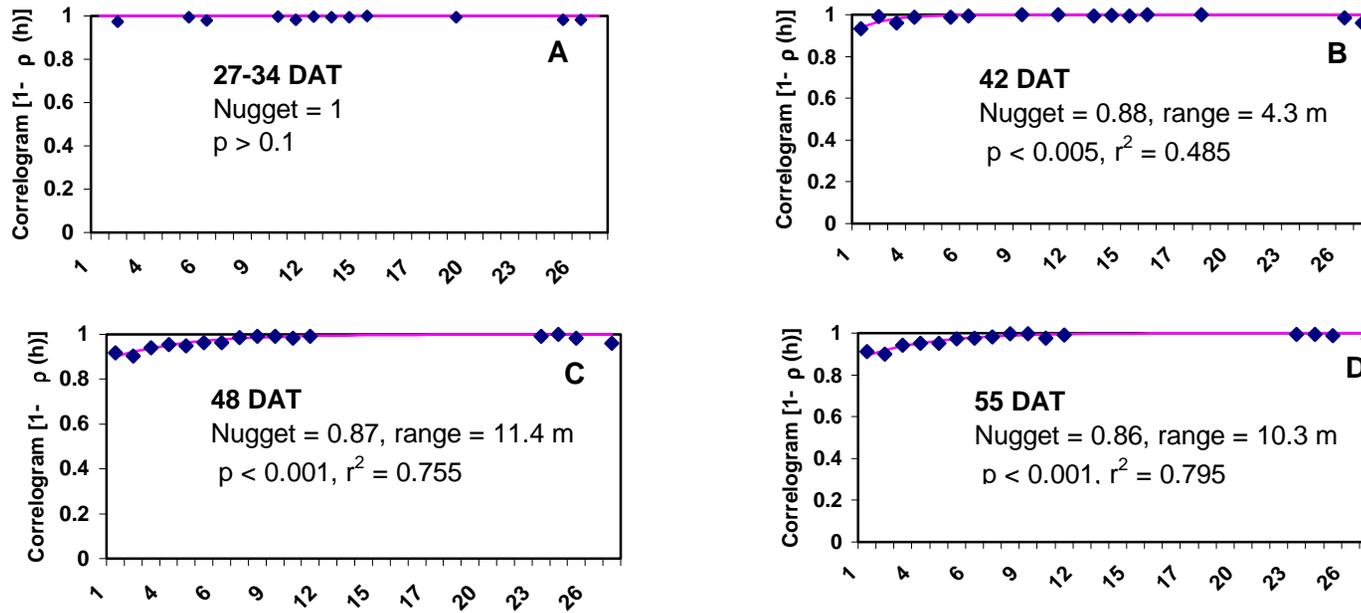


Fig. 3.1. Correlograms of TEV spread in a hot pepper (*C. chinense*) field. The study was conducted in Bodles, St. Catherine, Jamaica, W.I. during 26 June through 1 October 1998. Letters signify different times during the study: A = 27-34 days after transplanting (DAT), B = 42 DAT, C = 48 DAT, D = 55 DAT, E = 62 DAT, F = 69 DAT, G = 76 DAT, H = 83 DAT, I = 90 DAT and J = 97 DAT.

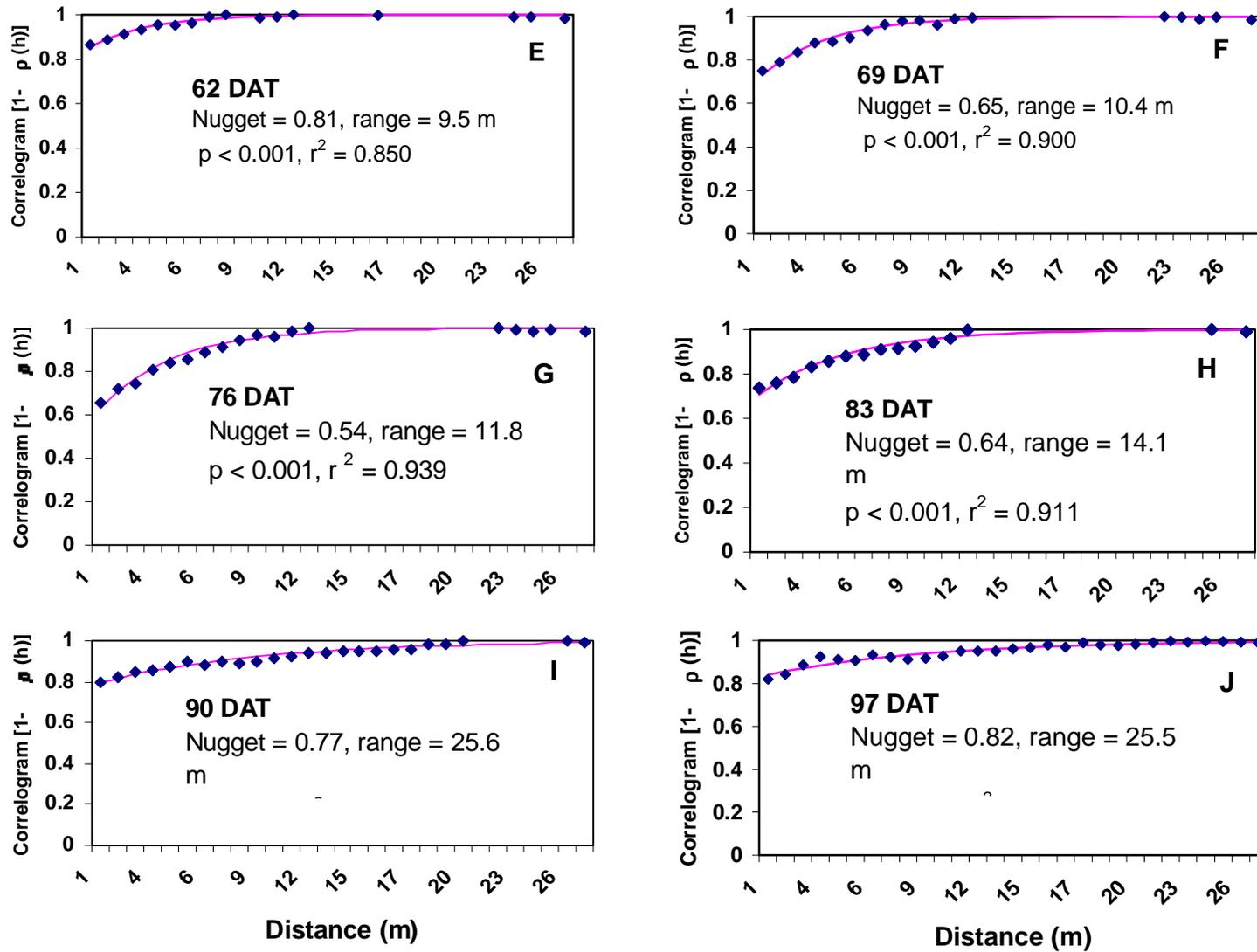


Figure 3.1. (Cont'd) Correlograms of TEV spread in a hot pepper (*C. chinense*) field

the plants were infected with TEV and then decreasing spatial correlation from 83 DAT when the infection rate was 49%.

The progress of TEV throughout the field was centered about initial infections (Figures 3.2. A-J). The maps produced by kriging at 27 and 34 DAT indicated there was a 100% chance that the field was not infected with TEV (Figure 3.2. A). By 42 DAT there were three initial points of TEV infection located inside the lower and mid left-hand side, and inside the upper right-hand corner of the field (Figure 3.2. B). TEV infection increased around these three points as well as from few other new infection points until all the infected areas coalesced (Figures 3.2. C-J). At 97 DAT almost the entire field was infected with TEV (Figure 3.2. J). The main movement of TEV appeared to have been from the left to the right of the field. An older infected pepper field located about 80 m to the left of this field was likely to have been the original source of the virus.

The number of plants that became infected with TEV increased logistically over time (Figure 3.3), which is characteristic of secondary infection (Thresh 1974). The predicted incidence of TEV over time could be described by the following equation:

$$\% \text{ TEV infection} = 100 / (1 + \exp(-(t_x - 83.756)^2 / 100))$$

where t_x = days after transplanting ($F = 3081.4$, $df = 1, 13$, $p < 0.0001$, $R^2 = 0.996$).

Aphids were caught in each trap located within the study area from 13 DAT (two weeks before the first symptoms of TEV were observed) until 97 DAT when the experiment was completed (Figure 3.3). *A. gossypii*, was the most commonly encountered aphid species and is a known vector of TEV. Other aphid species collected included *A. craccivora* Koch, *A. nerii* Boyer de Fonscolombe, *A. spiraecola* Patch, and *Teteraneura nigriabdominalis* (Sasaki). *A. craccivora* and *A. spiraecola* are known vectors of TEV.

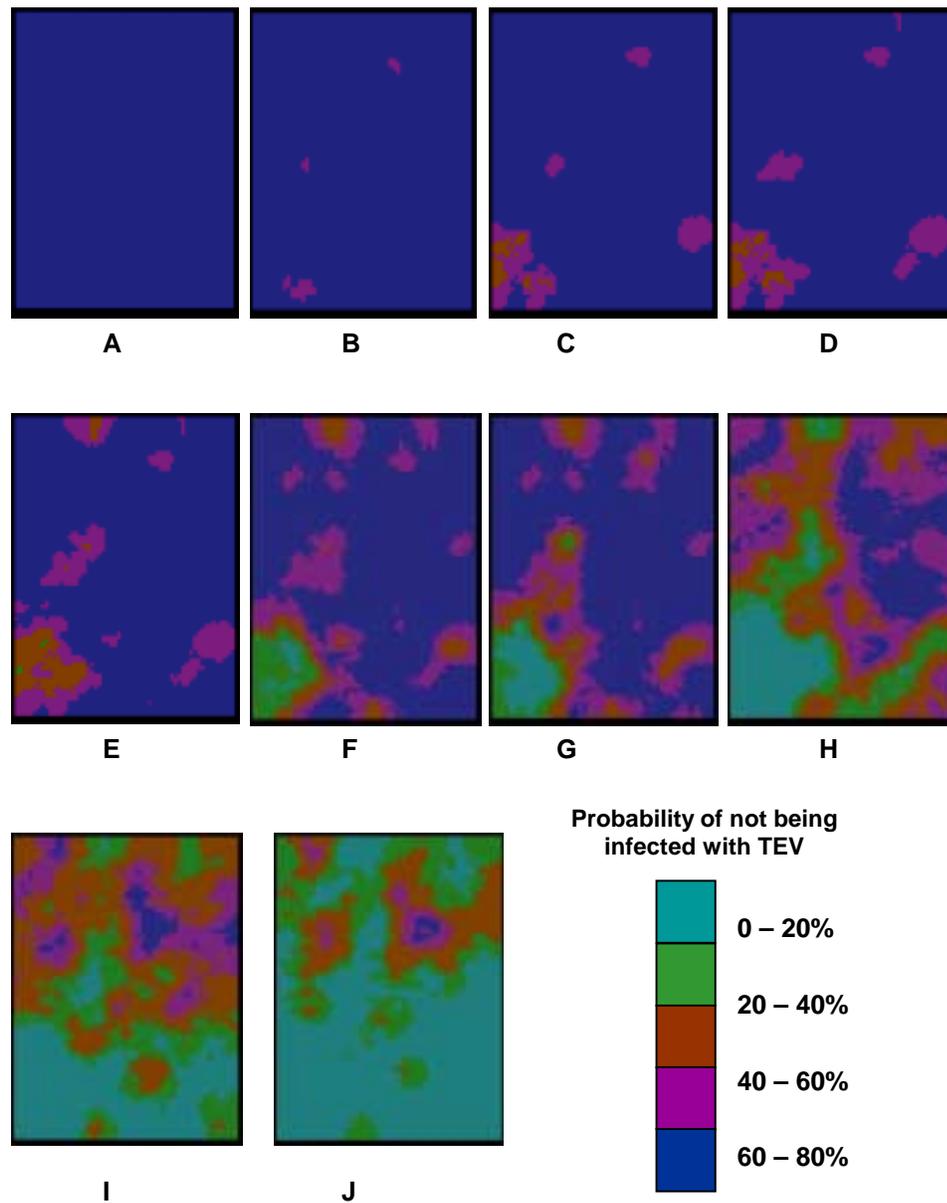


Fig. 3.2. Probability maps of TEV occurrence within a hot pepper (*C. chinense*) field. The probability is highest when actual incidence of TEV is low. The study was conducted in Bodles, St. Catherine, Jamaica, W.I. during 26 June through 1 October 1998. Letters signify different times during the study: A = 27-34 days after transplanting (DAT), B = 42 DAT, C = 48 DAT, D = 55 DAT, E = 62 DAT, F = 69 DAT, G = 76 DAT, H = 83 DAT, I = 90 DAT and J = 97 DAT.

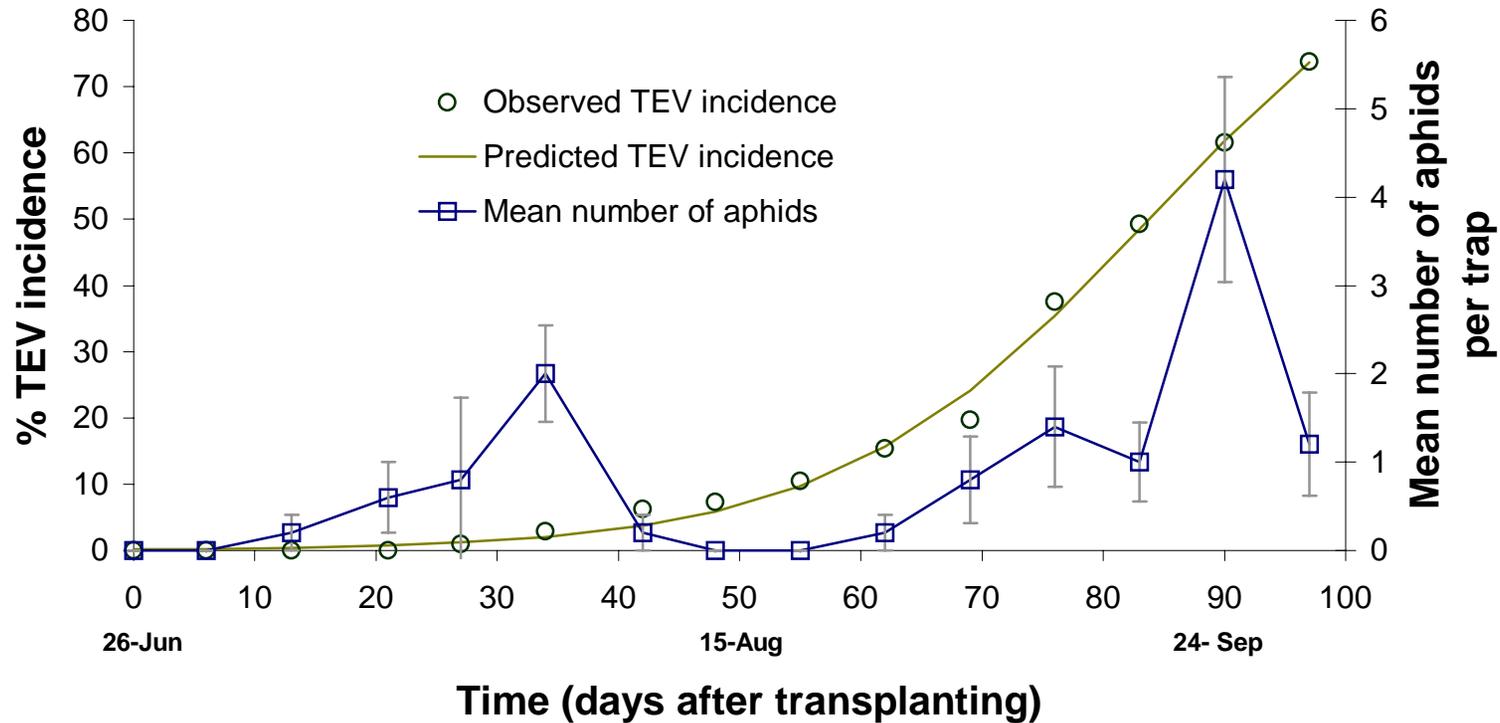


Figure 3.3. Observed and predicted incidence (%) of TEV within a 'Scotch Bonnet' pepper field and mean \pm SE number of aphids collected from 5 pan traps within the field at Bodles, St. Catherine, Jamaica, W.I. during 26 June through 1 October, 1998. The logistic model was used to predict the incidence of TEV over time.

The total number of aphids captured each week was positively correlated ($r = 0.60$, $N = 14$) with the percentage of plants that became infected with TEV. There was a slightly higher correlation ($r = 0.66$, $N = 14$) with a one-week lag between the total number of aphids caught and the incidence of TEV. The mean number of aphids caught in the five traps ranged from almost 0.2 to 2 during the first 34 DAT. During this time only 3% of the crop showed symptoms of TEV. Over the next 35 days (during the month of August) aphid flight was very low (mean catch ranging from 0-0.2 each week) but TEV continued to spread slowly. During September, mean aphid catch ranged from 0.8 to 4.2 aphids each week and the proportion of TEV infected plants ranged from 20 to 60% (Figure 3.3).

Discussion

The earliest TEV infections were random. This is indicative of primary spread, that is, the source of TEV for the infection was from outside the field (Broadbent 1957, Simons *et al.* 1977). Spatial correlation among TEV infected plants was detected by the third week of TEV symptoms. The distance over which the distribution of TEV was correlated increased between neighboring plants with time. This shows that during this period, secondary spread of TEV occurred, indicating that the main source of new infections was from within the field (Broadbent 1957, Simons *et al.* 1977). Broadbent (1969), Thresh (1974), and Simons and Zitter (1980) found that in most cases, primary spread of viruses to plants in the field is followed by secondary spread from these initially infected plants.

The range between neighboring infected pepper plants increased with time and was accompanied by a decrease in randomness of the infection pattern as indicated by the nugget until about half of the field was infected with TEV. After about 50 % of the field was infected with TEV the nugget started to increase from 0.54 to 0.82 although the range still was increasing. Madden *et al.* (1987 and 1988) reported that the distribution of TEV and tobacco vein mottling potyvirus in tobacco fields is random at first, then becomes increasingly spatially correlated until some point near the end of the season when it begins to decline because most of the crop is infected and new infection sites become scarce. Eckel and Lampert (1993) also found the distribution of two

strains of TEV in tobacco fields to be aggregated initially but later became random as new sites of infections became limited.

Initial infections were near the edge as well as toward the middle of the Scotch Bonnet pepper field. Immigrant aphids tend to land near edges of fields (Thresh 1976), but when fields are small, aphids tend to land randomly throughout the field. In our experiment, the test site was small (0.07 ha) as are most Jamaican hot pepper fields (i.e., 0.04 to 4 ha.; median 0.12 ha. (Martin *et al.* 1998)). Edge effect, therefore, is an important factor in the epidemiology of TEV in most Jamaican hot pepper fields. Increased field size to reduce the proportion of plants that become infected (van der Plank 1948, Thresh 1976) should be considered when developing any management program for TEV or any other aphid-borne virus in Jamaica. For many Jamaican farmers, however, land is limited and the option to increase field size is not practical. Alternatively, increased planting densities reduce the proportion as well as the actual number of plants infected in the field because aphids are deterred from flying through the crop (Broadbent 1969). The total yield per unit area will also increase with an increase in planting density. Difonzo *et al.* (1997a) also found that having a border crop with an area of bare soil immediately outside is effective in reducing virus spread, as aphids are attracted to the sharp contrast between the crop and soil. Farmers in Jamaica should not have problems increasing the cropping density of hot peppers. They will need a more vigorous weed management program to maintain a strip of bare soil around the field.

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