

CHAPTER FIVE - EFFECTS OF TIMING OF TEV INFECTIONS ON GROWTH, YIELD AND QUALITY OF 'SCOTCH BONNET' PEPPER (*CAPSICUM CHINENSIS* JACQUIN) FRUIT IN JAMAICA

Abstract

The effect of *tobacco etch virus* (TEV) (Genus: Potyvirus, Family: Potyviridae) on growth and yield of *Capsicum chinense* Jacquin cv. 'Scotch Bonnet' and 'West Indian Red' peppers was investigated. Peppers were manually inoculated at 7, 28 and 56-57 days after transplanting (DAT), which corresponded with early vegetative stage, onset of flowering and completion of first stage of flowering, respectively. Plants in control plots were not inoculated with TEV. Reflective mulch and JMS Stylet-Oil[®] were used to protect the uninoculated and late-inoculated plants from natural infections. Scotch Bonnet plants inoculated with TEV 7 DAT were 33% shorter and produced 57% less canopy than plants in the control plots, which averaged 50 cm in height and 4,000 cm² in canopy. Height and canopy of Scotch Bonnet plants infected with TEV at 28 and 57 DAT were similar to heights and canopies of control plants. Total number of fruit produced per Scotch Bonnet plant inoculated with TEV 7 and 28 DAT were 69 and 36% less than the number (100 on average) of fruit produced per plant in the control plots, respectively. The marketable weight of fruit produced per Scotch Bonnet plant inoculated with TEV 7 and 28 DAT were 78 and 57% less than the marketable weight of fruit produced per plant in the control plots, which was 1 kg on average, respectively. Number and weight of fruit produced by Scotch Bonnet plants infected with TEV at 57 DAT were not significantly different from those by control plants. Results suggest that decreases in Scotch Bonnet pepper yield can be avoided by protecting plants from TEV infection from the seedling stage through the first stage of flowering (approximately 50-60 DAT). Height, canopy and yield of West Indian Red plants were not adversely affected by TEV.

KEYWORDS: timing of infection, Potyvirus, Stylet-Oil[®], reflective mulch, yield.

Introduction

Viruses often affect peppers, *Capsicum* spp. wherever they are grown. As early as 1963, Laird and Dickson reported that *tobacco etch virus* (TEV) and *potato virus Y* (PVY) (both, Genus: *Potyvirus*, Family: Potyviridae) occurred at rates of up to 100 % in peppers (*Capsicum* spp.) in southern California, USA. Benner and Kuhn (1985) also found up to 100% incidence of virus diseases in *C. annuum* in northeastern Georgia, USA that was due mostly to TEV. Similar reports of virus infestations of peppers have come from Mexico (Campodonico and Montelongo 1988) and Indonesia (Duriat 1989).

Two recent surveys (McGlashan *et al.* 1993, Martin *et al.* 1998) found viruses on over 80 % of Jamaican hot pepper, *C. chinense*, farms. Very often entire hot pepper crops were infected with viruses once they reached 6 months of age (McGlashan *et al.* 1993). TEV and PVY are the most prevalent viruses of pepper in Jamaica (McGlashan *et al.* 1993, Myers 1996, Martin *et al.* 1998). TEV is encountered more frequently than PVY and both viruses may occur in mixed infections (McGlashan *et al.* 1993, Myers 1996, Martin *et al.* 1998). Other viruses found in Jamaican hot pepper fields include *tobacco mosaic tobamovirus* (TMV) (McGlashan *et al.* 1993, Myers 1996, Martin *et al.* 1998), *cucumber mosaic cucumovirus* (CMV), *pepper mild mottle tobamovirus* (PMMV) and *tomato mosaic tobamovirus* (ToMV) (McGlashan *et al.* 1993).

Myers *et al.* (1998) studied the effect of natural TEV infection on the yield of two varieties of *C. chinense* Jacquin, 'Scotch Bonnet' and 'West Indian Red', in Jamaica. TEV caused 50% reduction in Scotch Bonnet but only 15 % in 'West Indian Red' peppers. The effects of TEV on different phenological stages of these pepper varieties are not known. The stage of development of a crop at the time of infection by a virus influences the severity of the disease, with the greatest reduction in plant growth and yield occurring when plants are infected during the earliest stages of their development (Matthews 1997). For example, CMV caused 76% yield loss in pepper inoculated 18 days after transplanting (DAT) compared with only 19% yield loss in pepper inoculated 65 DAT (Agrios and Walker 1985). Plants inoculated with CMV 18 and 65 DAT also were 50% and 7% shorter than the control, respectively (Agrios and Walker 1985).

Yields of pepper inoculated with CMV 1 and 7 weeks after transplanting, were 96 and 42% lower than yield of uninoculated plants, respectively (Avilla *et al.* 1997). Similarly, pepper yields were reduced by > 60% and 20 % of yields produced by uninfected plants when inoculated with PVY 1 and 7 weeks after transplanting, respectively (Avilla *et al.* 1997). Tomato plants naturally infected with tomato spotted wilt virus (TSWV) 24 DAT produced 91% less fruit yield than plants infected ≥ 67 DAT (Moriones *et al.* 1998). This reduced effect of viruses on plant growth and yield with increasing age of plants at the time of infection has been described as “mature plant resistance” (Matthews 1997). Mature plant resistance is a common phenomenon in plants and may also occur in response to infection by fungi (Dickson and Petzoldt 1993) and feeding by insects (Nicol and Wratten 1997).

Knowledge of the stage of plant development at which TEV has the least economic impact on *C. chinense* pepper yield is required to determine how long the plants should be protected from the aphids that vector TEV. The objective of this study was to examine the effect of timing of TEV infection on growth and yield in Scotch Bonnet and West Indian Red peppers.

Materials and Methods

Seedling preparation

Experiments were conducted at Mona, in the parish of Kingston, Jamaica, at the

Field Station of the Caribbean Agricultural Research and Development Institute (CARDI) from 4 May through 3 September 1999 (Scotch Bonnet), and from 24 September 1999 through 7 February 2000 (West Indian Red). Pepper seeds obtained from CARDI, Antigua, were sown in sterile potting mixture (Easi-Grow, Bulrush Peat Co. Ltd., Co Londonderry, UK) and the seedlings kept under screen cages (described in Chapter Three) to exclude aphids. Before transplanting, leaves were taken from a random sample of 50 seedlings to confirm the absence of TEV and PVY by tissue blot serology as described in Chapter Three. Seedlings were transplanted to the field about six weeks after sowing. A portion of the original batch of seedlings was transplanted into seedling bags (100 plants in 7.5 x 12.5x 15 cm bags and 50

plants in 12.5 x 23 x 25.5 cm bags) and maintained in the greenhouse to replace seedlings that failed to establish within 6 weeks after transplanting.

Experimental design

The field was prepared so that each seedling received 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. Each plant received a second application of fertilizer (28 cm³ of N:P:K, 15:5:35) 4 weeks after transplanting and a third application (28 cm³ of urea), 4 weeks later. Weeds within test plots were removed by hand while those on the perimeter of the plots were treated with Paraquat (Grammoxone[®], Agroinsumos, Guatemala, S.A., under license from Zeneca Ltd. Rate = 2.0 liters/hectare) and glyphosate (Roundup[®], Monsanto Co., St. Louis, MO. Rate = 0.05 to 1.0 litres/ha). Mites and aphid populations were managed with soil application of imidacloprid (Admire 2F[®], Bayer Corp., Kansas City, MO. Rate = 1.5 to 3.0 litres/hectare).

The Scotch Bonnet pepper experiment was established as a completely randomized design with four treatments and six replicates. Each plot contained 10 plants spaced at 0.9 m. There were two plots per row, separated by a 1.8 m distance. The surfaces of the 1.2 m wide rows were flat and covered with 0.9 m wide strips of aluminum coated plastic mulch (total width of plastic = 1.2 m), under which drip irrigation lines were placed. A space of 0.3 m separated adjacent rows.

The West Indian Red pepper experiment had a randomized complete block design with four treatments and six replicates. Plots contained five plants spaced 0.6 m apart. There were four plots per row, separated by 1.2 m distances. Rows were prepared similarly to those used in the Scotch Bonnet pepper experiment.

TEV inoculation procedure

Hot pepper plants were manually inoculated with TEV at 7, 28 and 57/56 DAT (57 - Phase 1, 56 - Phase 2) when the plants were in the early vegetative, late vegetative (\leq 15% flowering) and fruiting ($>$ 90 % flowering) stages, respectively. Fully expanded leaves (two leaves - pepper

plants \leq 1 month old; 4 leaves (1 leaf from each quarter of the canopy) - pepper plants 2 months old) were rubbed firmly with a pestle that was dipped repeatedly in the TEV inoculum, using fresh inoculum after every 10 to 20 plants. The control consisted of plants that were not inoculated by this process.

The TEV used for inoculation was obtained from infected Scotch Bonnet pepper leaves at the Bodles Field Station in St. Catherine, Jamaica, W.I. Potential source plants exhibited TEV symptoms; serological tests confirmed the presence of TEV and absence of PVY. After the first set of field inoculations, leaves from these newly infected plants were used as a TEV source. Some TEV infected plants in the nursery were maintained as initial inoculum for the West Indian Red experiment. Inoculum was prepared by crushing 2-3 young leaves in about 10 ml of 0.01M cold potassium phosphate buffer (pH 7.0) within a chilled mortar and carborundum was added at the rate of 0.1 g per 500 ml. Both the inoculum and extra buffer were maintained on ice at all times during the inoculation process.

For each set of inoculations, an additional 33 seedlings were inoculated and maintained under screen cages in the greenhouse. Inoculated plants were observed for TEV symptoms, and tissue blots were prepared from about 50 % of the plants to confirm the presence of TEV at least two weeks after inoculation. Single leaves were taken from each of 50 uninoculated plants in the field at 52 DAT to confirm the absence of TEV (and PVY) by tissue blot serology before the final inoculation was done. No symptomatic or TEV positive plants were inoculated. To avoid natural infection of TEV, aluminum-coated plastic was used to repel aphids. In addition, all uninoculated plots were sprayed weekly with Stylet-Oil[®] using the rates and techniques described in Chapter Four.

Measuring effects of timing of TEV inoculations on growth and yield

Alternate Scotch Bonnet plants (5 plants) within each plot in each of six replicates were tagged for measuring and harvesting. At 80 DAT, the height (from the base to the highest point) and

canopy (the product of widths perpendicular to and parallel to the beds) for each tagged plant were measured. The growth stages of tagged plants were also recorded at 84 DAT about one week before harvesting began. The growth stages were based on a study of Scotch Bonnet peppers in 1996 that defined¹ three vegetative stages (V_1 , V_2 and V_3) and several reproductive stages (R_1 , R_2 ,... R_n) (S. McDonald unpublished report). All 5 West Indian Red plants were used to obtain measurements of height and canopy. Mature fruit were harvested weekly from the five selected Scotch Bonnet plants within each plot (of six replicates), beginning 93 DAT, and 85 DAT for three randomly selected West Indian Red plants per plot. Number and weight of fruit produced by each plant were recorded over time. Fruit were separated into marketable and unmarketable groups (as described in Chapter Four), counted and weighed.

Statistical analyses

For the purpose of data analysis, growth stages were converted to growth indices, 1 to 3 for the three vegetative stages and 4 onward, for the first and successive reproductive stages. Data on plant growth stage, height, canopy, number and weight of fruit, and mean weight per marketable fruit were analyzed for the effects of treatments using Generalized Linear Models Procedures (GLM procedure) and means among treatments were compared using Tukey's HSD test at $P < 0.05$ (SAS Institute. 1996). Repeated measures analysis (MIX procedure, SAS Institute. 1996) was conducted to determine the effects of harvest dates and the interactions of harvest dates and treatments on yield.

Results

Effects of timing of TEV inoculations on growth

Scotch Bonnet: Inoculated plants were uniformly infected with TEV and symptoms appeared 7 days after inoculation. Except for five plants, which became symptomatic with TEV by natural means during the 9th week after transplanting (week 9 = 63 DAT), most of the control plants did

¹ Plants produce two alternate (secondary) branches during vegetative growth. During the reproductive stage, each plant produces dichotomous branches and flowers develop at the internodes. Reproductive stages can be determined by following this dichotomy from the main stem.

not become symptomatic until weeks 12 – 14. The average time of natural infection of control plants was at about week 13 (93 DAT). Timing of TEV infection significantly affected plant height ($F = 6.4$; $df = 3, 20$; $p = 0.003$) and size of plant canopy ($F = 5.2$; $df = 3, 20$; $p = 0.008$). Plants inoculated with TEV 7 DAT were 33% shorter (Figure 5.1. A) and produced 57% less canopy (Figure 5.1. B) than plants in control plants. However, heights and canopy of plants inoculated at 28 and 57 DAT were not significantly ($p > 0.05$) different from the control plants. Timing of TEV inoculations had no significant impact ($F = 2.0$; $df = 3, 20$; $p > 0.1$) on developmental stages. At 84 DAT plants inoculated 7 DAT had a growth index of 12 (growth stage = R_9) while all other plants had a growth index of 13 (growth stage = R_{10}).

West Indian Red: West Indian Red pepper inoculated at different ages showed no significant difference in height ($F = 1.6$; $df = 3, 15$; $p > 0.1$) or canopy ($F = 0.3$; $df = 3, 15$; $p > 0.1$) when compared with the control. The overall mean height and canopy per West Indian Red plant were 51 cm and 5080 cm², respectively.

Effects of timing of TEV inoculations on yield

Scotch Bonnet: The total number of fruit per treatment was not significantly ($F = 1.8$; $df = 12, 80$; $p = 0.055$) affected by a harvest date x treatment interaction. Significant increases were observed in the total number ($F = 7.1$; $df = 3, 20$; $p < 0.002$) and marketable number ($F = 7.3$; $df = 3, 20$; $p < 0.002$) of fruit per plant with delayed TEV inoculation (Figures 5.2. A and 5.2. B). Total number of fruit produced per Scotch Bonnet pepper plant inoculated with TEV 7 and 28 DAT were 69 and 36% less than the total number of fruit produced per control plant, respectively (Figure 5.2. A). Plants inoculated 57 DAT the same total number of fruit per plant as in the control plots ($p > 0.05$). The proportion of marketable yield (number and weight of fruit) was not different ($p > 0.1$) among treatments, with less than 4 % of all fruit being unmarketable in each treatment.

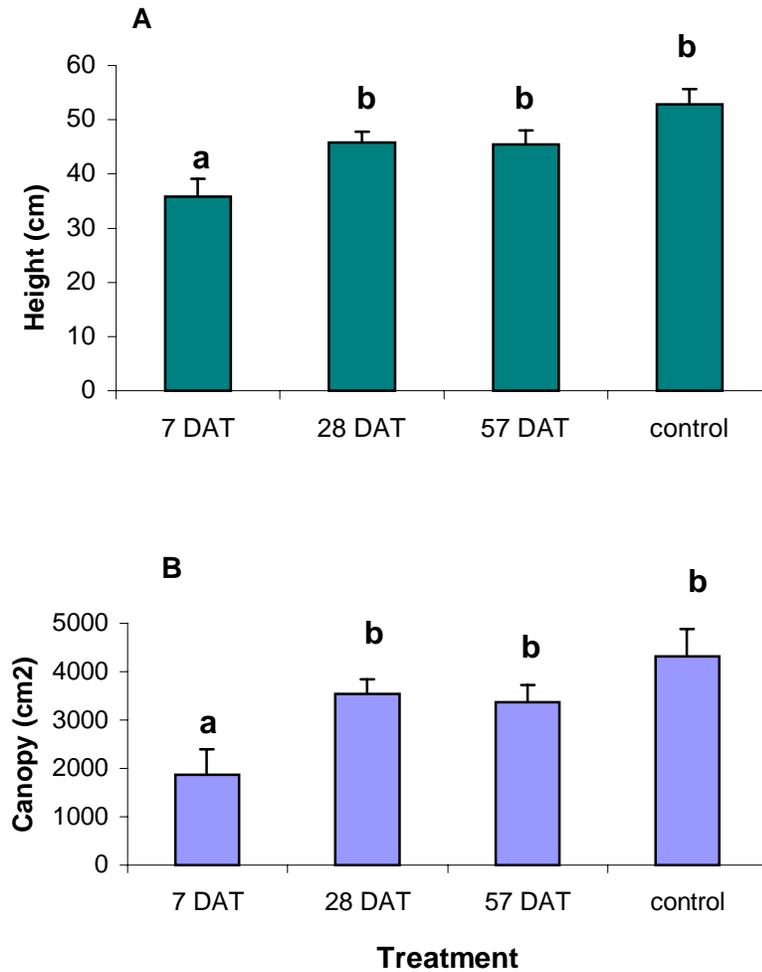


Figure 5.1. Mean (\pm SE) height (A) and mean (\pm SE) canopy (B) of 'Scotch Bonnet' pepper plants inoculated with TEV at different times, days after transplanting (DAT). Plants in the control treatment were not inoculated. Height and canopy were measured 80 DAT, during July 1999 in Kingston, Jamaica, W.I. Treatment bars with similar letters are not significantly different from each other (Tukey's HSD, $p > 0.05$).

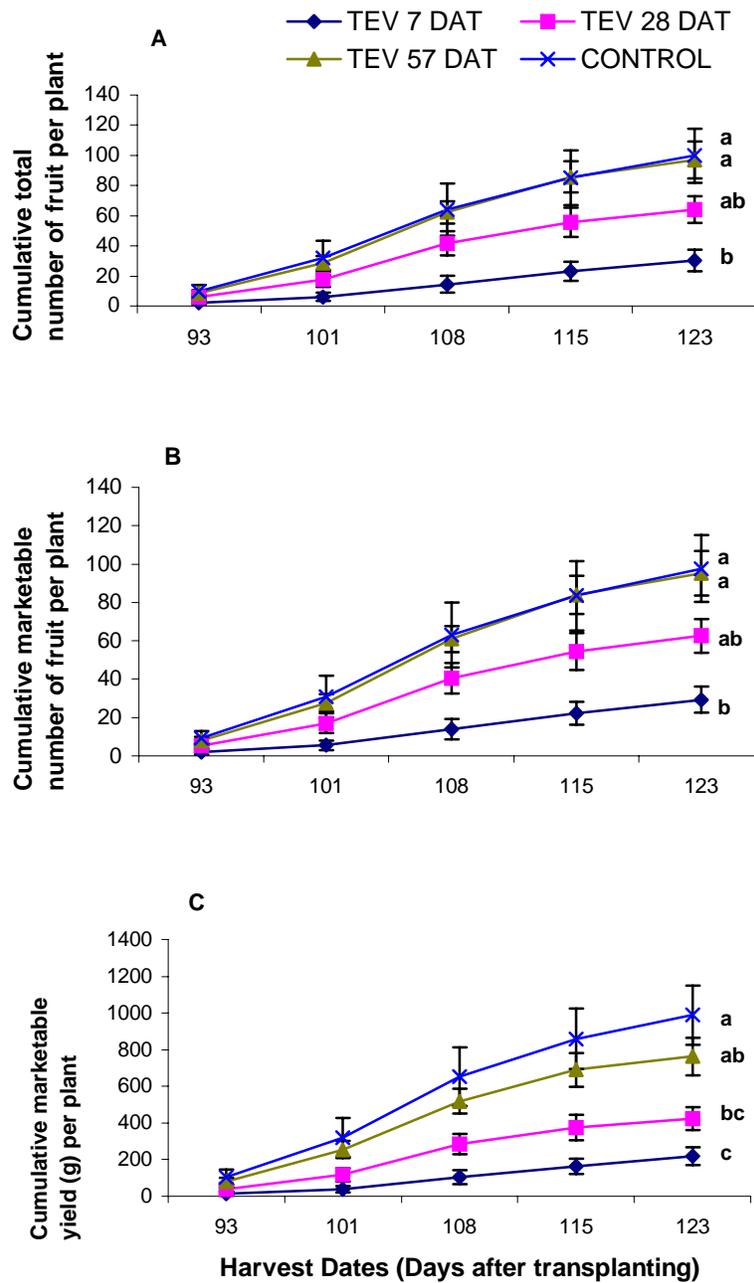


Figure 5.2. Cumulative yield expressed as mean (\pm SE) total number (A), mean (\pm SE) marketable number (B), and mean (\pm SE) marketable weight (C) of fruit per 'Scotch Bonnet' pepper plant in plots inoculated with TEV at various times, days after transplanting (DAT). Plants in the control treatment were not inoculated. Plants were harvested for five weeks, from 5 August through 3 September 1999 in Kingston, Jamaica, W.I. Treatment lines followed by the same numbers are not significantly different after five cumulative harvests (Tukey's HSD, $P < 0.05$).

Marketable fruit weight per treatment was significantly ($F = 2.4$; $df = 12, 80$; $p < 0.01$) affected by a harvest date x treatment interaction. Weight of fruit from plants inoculated 7 DAT was significantly less ($p < 0.002$) than that from uninoculated plants on the second and third harvest dates but not on the first, fourth and fifth harvest dates ($p > 0.05$). Plants inoculated 7 DAT only produced lower ($p < 0.05$) fruit weight than plants inoculated 57 DAT on the second and third harvest dates. There was no significant difference ($p > 0.05$) between the weights of fruit produced by plants inoculated 7 and 28 DAT at any of the harvest dates. Except for the third harvest date, when fruit weight from plants inoculated 28 DAT was significantly less ($p < 0.02$) than that from uninoculated plants, there was no significant difference ($p > 0.05$) among plants inoculated 28 and 57 DAT and uninoculated plants in the weight of fruit harvested on any given date.

Generally, significant increases were observed in total marketable weight over five harvests ($F = 11.0$; $df = 3, 20$; $p < 0.002$) of fruit per plant with delayed TEV inoculation (Figure 5.2. C). The total marketable weights of fruit produced by plants inoculated with TEV 7 and 28 DAT were 78 and 57% less than that produced by plants of the control, respectively. Plants inoculated 57 DAT produced similar weights of fruit to plants in the control plots ($p > 0.05$) (Figure 5.2. C).

Mean weight per (marketable) fruit from all inoculated plants was significantly ($F = 12.3$; $df = 3, 20$; $p < 0.0001$) less than the control (Table 5.1). There was no significant difference among inoculated plants in the weight per fruit produced, although those fruit from plants inoculated 7 DAT tended to weigh more than those from plants inoculated 28 DAT (Table 5.1).

West Indian Red: There was no significant difference among treatments of West Indian Red pepper in total number (over all average = 122; $F = 0.3$; $df = 3, 15$; $p > 0.1$) or marketable weight (over all average = 1.35 kg.; $F = 0.5$; $df = 3, 15$; $p > 0.1$) of fruit produced by each plant. Over 93% of total numbers of fruit within all treatments were marketable ($F = 0.4$; $df = 3, 15$ $p > 0.1$). Weight per marketable fruit was also similar among treatments (over all average = 11.3 g.; $F = 0.5$; $df = 3, 15$; $p > 0.1$). Although not significant, yield and fruit size were always lowest in

plants inoculated 28 DAT, which coincided with the observation that flowering began at about this time.

Table 5.1. Mean (\pm SE) weight per marketable fruit produced by ‘Scotch Bonnet’ plants inoculated with TEV at various times, days after transplanting (DAT). Plants were harvested five times from 5 August through 3 September 1999 in Kingston, Jamaica, W.I.

Age at time of manual inoculation (DAT)	Number of replicates	Weight per fruit (g)
7	6	7.7 \pm 0.42 a
28	6	6.6 \pm 0.44 a
57	6	8.0 \pm 0.54 a
Control (not inoculated)	6	10.3 \pm 0.37 b

F = 12.3; df = 3, 20; p < 0.0001

Means followed by the same letter are not significantly different (Tukey’s HSD, $p > 0.05$)

Discussion

The appearance of symptoms 7 days after plants were inoculated with TEV concurs with the 1-week lag found between aphid flight activity and the appearance of TEV symptoms in naturally infected pepper plants described in Chapter 3. The virus pressure within the plots increased as more plants were infected with TEV, yet Stylet-Oil[®] and reflective mulch together were effective in delaying the symptoms of TEV in control plants for up to 13 weeks after transplanting. Mansour (1997), who studied the effectiveness of JMS Stylet-Oil[®] alone, Stylet-Oil[®] with formothion, and Stylet-Oil[®] with aluminum mulch in protecting squash from aphid transmitted mosaic viruses, found satisfactory protection only when Stylet-Oil[®] was combined with aluminum mulch (Mansour 1997).

Plants inoculated 7 DAT were 17 cm shorter and had less than half the canopy of control plants. Plants inoculated at 28 and 57 DAT tended to be shorter with smaller canopies than control

plants, but these differences were not significant. Agrios and Walker (1985) also reported that size of bell peppers increased with the age at which they became infected with CMV, but height and top weight of plants (without fruit) were used as parameters for determining size.

TEV reduced yield of pepper fruit when plants were inoculated during the early vegetative stage (7 DAT) and at the onset of flowering (28 DAT). Significant increases in pepper yield were obtained by protecting pepper from TEV infection during seedling stage through first stage of flowering (approximately 57 DAT) with reflective mulch and oil sprays. Plants inoculated at 7 DAT appeared to have compensated for lower fruit set by producing larger fruit, equivalent to the weight of fruit produced by plants inoculated 57 DAT, but this was not enough to increase the total weight of fruit. TEV might have affected fruit set in our study, as some plants inoculated at 7 DAT produced no fruit during the five harvests while others were more than two weeks late in producing mature fruit. The vegetative and reproductive stages of Scotch Bonnet pepper plants were not affected by TEV.

Agrios and Walker (1985) found that growth and yield of *C. annuum* plants were greater as timing of CMV inoculations was delayed. Treatments consisted of weekly inoculations beginning 18 DAT and total number and total weight of fruit per plant increased significantly with delayed infection. They also found that while the earliest inoculated plants produced fewer fruit, the fruit were comparable in weight to fruit of the latest inoculated plants. Avilla *et al.* (1997) found similar effects of CMV and PVY on total weight of fruit, marketable weight of fruit, and number of marketable fruit produced by *C. annuum* plants inoculated at different ages. However, they reported decreases in the total number of fruit with delayed infection with PVY but not with CMV. Reduced fruit number in the early PVY infected plants was attributed to bud necrosis associated with the disease.

Unlike results reported by Agrios and Walker (1985), our study found no difference in the proportion of marketable fruit between plants inoculated at different ages. Our study showed that although the size of fruit was decreased by the early inoculations, the fruit were not reduced

to an unmarketable size (< 3 cm diameter/length) and the shape of Scotch Bonnet pepper fruit does not affect marketability. Unlike Scotch Bonnet pepper, both size and shape of bell peppers are critical factors in marketability (Agrios and Walker 1985).

The West Indian Red variety was tested because we anticipated it would be tolerant to TEV (Myers *et al* 1998). Myers *et al* (1998) had observed that natural TEV infection caused 15% reduction in weight of fruit of West Indian Red compared to 50% reduction in Scotch Bonnet plots. Our results suggest that West Indian Red is tolerant to TEV and that the effect of TEV in West Indian Red plants is greatest when inoculation occurs near 28 DAT, which is near the time of flower initiation. More detailed studies need to be conducted in this regard.

JMS Stylet-Oil[®] is normally applied with air blast sprayers, which deliver high volumes at pressures of around 2800 kPa (Simons 1982). We were able to delay TEV symptoms in peppers due to natural infections by about 13 weeks with aluminum coated plastic mulch in addition to weekly applications of Stylet-Oil[®] with a Solo[®] 423 mistblower. The Solo[®] 423 mistblower is a motorized backpack sprayer that delivers low volumes at pressures of around 1000 kPa. Loebenstein *et al.* (1970) used a motorized knapsack sprayer to deliver a commercial oil (Blancol) emulsion to pepper (*C. annuum*) and was able to reduce infections of CMV and PVY. Thomas (1984) was also successful in using a high volume hand pumped CP-3 knapsack sprayer equipped with cone jets, to delivered JMS Stylet-Oil[®] emulsion at a pressure of 200 kPa. Application of Stylet-Oil[®] with a knapsack mist blower, like the Solo[®] 423 model used in this study is appropriate for most Jamaican pepper/vegetable farmers because the average field size is less than 1 ha and most farmers use it.

In summary, this study showed that even under high inoculum pressure, when used with reflective mulch, Stylet-Oil[®] applied with a backpack mist blower could delay symptoms of TEV for up to 13 weeks after transplanting. This delay in virus infection would greatly reduce yield loss by 20-80% as seen in the results of this study.

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