

Efficacy of Stylet-Oil® in suppressing spread of TEV infections in a Scotch Bonnet pepper (*Capsicum chinense* Jacquin) field in Jamaica

Sharon A. McDonald¹

Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0319, U.S.A.

Sue A. Tolin

Department of Plant Pathology, Physiology and Weed Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330, U.S.A.

Brian A. Nault

Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, U.S.A.

The effect of Stylet-Oil® on the spread of tobacco etch virus (TEV) (Genus: *Potyvirus*, Family: *Potyviridae*) in a Scotch Bonnet pepper (*Capsicum chinense* Jacquin) field was investigated during 2 September 1998 through 17 March 1999. There were three treatments, each comprising 100 plants per experimental unit and replicated thrice. With the exception of the control treatment, seedlings were kept virus-free prior to transplanting by protecting them under aphid exclusion cages. Of these protected seedlings, one set was treated with JMS Stylet-Oil® after they were transplanted to the field. A backpack mist blower (low volume) was used to apply Stylet-Oil® at pressures of about 1000 kPa to pepper plants weekly, from transplant until the last harvest. Stylet-Oil® was effective in reducing incidence of tobacco etch virus (TEV) in Scotch Bonnet pepper by about 24% and delaying its spread by seven days. The TEV incidence was based on the appearance of symptoms in naturally infected pepper plants, and was confirmed serologically. The first symptoms of TEV appeared 56 days after transplanting and were correlated with aphid flight activities. The TEV symptom appearance was most closely associated with the presence of *Aphis gossypii* (Glover), a known vector of TEV, and two other aphids of unknown TEV vector status, *A. amaranthi* (Holman) and *Uroleucon ambrosiae* (Thomas) complex.

Keywords: Pepper; Stylet-Oil®; Disease incidence; Pest management; *Potyvirus*; TEV-vector

Occurrence of a mosaic of viruses in Jamaican hot pepper (*Capsicum chinense* Jacquin), fields has been associated with increased acreage of the crop due to commercial production (Ministry of Agriculture, unpubl. report) over the last three decades. McGlashan *et al.* (1993), Myers (1996), and Martin *et al.* (1998) reported that tobacco etch virus (TEV) (Genus: *Potyvirus*, Family: *Potyviridae*) and potato virus Y (PVY) (Genus: *Potyvirus*, Family: *Potyviridae*) are the main mosaic-causing viruses found on hot pepper farms surveyed in Jamaica. These two

viruses were found in samples taken from 80–100% of hot pepper farms surveyed (McGlashan *et al.*, 1993; Martin *et al.*, 1998). By six months after transplanting, most hot pepper crops are normally entirely infected with viruses (McGlashan *et al.*, 1993) and production ceases to be profitable. In the absence of these viruses, the crop can be harvested over several years. The TEV has been encountered more frequently than PVY in hot pepper crops in Jamaica and produces more severe symptoms in hot pepper than does PVY (McGlashan, 1993; Myers, 1996).

The TEV is carried on the maxillary stylets and the foregut of their aphid vectors (Pirone and Blanc, 1996; Bos, 1999) and is

¹Corresponding author: Research and Development Division, Ministry of Agriculture, Old Harbour P.O., St Catherine, Jamaica, West Indies

transmitted in a nonpersistent manner (Sylvester, 1969; Nault, 1997) as vectors probe prospective host plants. Five known TEV vectors, viz., *Aphis gossypii* (Glover), *A. spiraecola* (Patch), *Myzus persicae* (Sulzer) (Laird and Dickson, 1963), *A. craccivora* (Koch) (Herold, 1970), and *Lipaphis erysimi* (Davis) (Eckel, 1990; Eckel and Lampert, 1993), have been trapped on hot pepper farms in Jamaica (McDonald *et al.*, 2003a). Natural infections of TEV in hot pepper have been associated with flight activity of *A. gossypii*, and to a lesser extent, *A. spiraecola*, and *A. craccivora*, on a hot pepper farm in Jamaica (McDonald, 2001).

The TEV can reduce yield of *C. chinense* var. Scotch Bonnet by up to 50–80% (Myers *et al.*, 1998; McDonald *et al.*, 2003b). Scotch Bonnet is the hot pepper variety grown by about 80% of Jamaican farmers. Farmers have no effective management programme for viruses infecting peppers in the field. Moreover, pepper seedlings are sometimes infected before they are transplanted to the field (S.A. McDonald, pers. observ.).

Infections of nonpersistently transmitted aphid-borne viruses, like TEV, have been delayed and (or) reduced by protecting seedlings and transplants with aphid-excluding covers and regular application of oil sprays. Vos and Nurtika (1995) showed that in West Java, Indonesia, hot pepper seedlings (*Capsicum* spp) maintained under aphid exclusion screens for 1.5 months from sowing until transplanting were protected from the aphid-transmitted viruses. Such plants matured faster and produced more fruit than plants from unprotected nurseries (Vos and Nurtika, 1995).

Oils are known to suppress acquisition and inoculation of nonpersistently transmitted aphid-borne viruses (Loebenstein *et al.*, 1970; Zitter and Simons, 1980) by preventing attachment of the viruses to the stylets (Powell, 1992; Wang and Pirone, 1996). Use of many early formulations of oils for virus management was limited due to phytotoxicity, but a new less toxic formulation, JMS Stylet-Oil[®], was developed later (Simons and Zitter, 1980; Zitter and Simons, 1980; Simons, 1982). Weekly applications of 0.75% emulsion of JMS Stylet-Oil[®] have been shown to effectively control several aphid-transmitted viruses in pepper, tomato, and squash (Simons and Zitter, 1980; Zitter and Simons, 1980; Simons, 1982). For JMS Stylet-Oil[®] to be most effective, it should be applied with a high volume air blast sprayer at pressures of about 2800 kPa (Simons and Zitter, 1980; Zitter and Simons, 1980; Simons 1982). However, Thomas (1984) was successful in controlling aphid-transmitted viruses in cucurbits by applying JMS Stylet-Oil[®] with a hand pumped CP-3 knapsack sprayer, equipped with high volume cone jets which delivered the oil emulsion at a pressure of only 200 kPa. Loebenstein *et al.* (1970) used a motorized

knapsack sprayer to deliver a commercial oil [Blanco[®] (Pazchem Ltd, Tel Aviv, Israel)] emulsion at low volume with a single nozzle to pepper (*C. annuum*). Sprays were very effective in reducing the spread of PVY and cucumber mosaic virus (Genus: *Cucumovirus*, Family: Bromoviridae) to pepper at oil concentrations of 1–2% (v:v) in nurseries and 2.5% (v:v) in the field (Loebenstein *et al.*, 1970).

Successful application of Stylet-Oil[®] with a motorized knapsack mist blower, such as the Solo[®] 423 model, which produces a pressure of about 1000 kPa, would be ideal for most Jamaican pepper and vegetable farmers. The average size of a pepper field in Jamaica is less than one hectare (McGlashan, 1993; Martin *et al.*, 1998) and often located on hillsides (McGlashan, 1993), which would not accommodate tractor-mounted, boom sprayers. The motorized knapsack mist blower would also be convenient because many farmers already own such a sprayer or can afford to purchase one. Attempts to control viruses of pepper plants with JMS Stylet-Oil[®] in Jamaica in the 1980s failed because application techniques were poor (McGlashan, 1993). There is no report of phytotoxic effects of Stylet-Oil[®] on Scotch Bonnet pepper plants.

The objective of this study was to compare the incidence and timing of TEV infection in a hot pepper field in which seedlings were either protected with aphid exclusion cages and JMS Stylet-Oil[®] or not protected at all. Additionally, an association between timing of TEV infection in pepper plants and flight activity by known aphid vectors of TEV was investigated.

Materials and Methods

Seedling preparation

The experiment was conducted on a private farm in Bushy Park, St Catherine parish, Jamaica, West Indies, from 2 September 1998 through 17 March 1999. Scotch Bonnet pepper seeds were sown in sterile potting mixture (Easi-Grow, Bulrush Peat Co. Ltd, Co. Londonderry, U.K.). Two-thirds of the seedlings were grown under aphid-proof screen cages until transplanting, whereas the remainder of the seedlings was grown in the open. Seedlings were transplanted in the field six weeks after sowing on 2 September 1998. Before transplanting, tissue blots were taken from a sample of seedlings to confirm the absence of TEV. Tissue blots were prepared and processed as described in Lin *et al.* (1990) and modified according to Srinivasan and Tolin (1992), using NBT-BCIP substrate (Zymed Laboratories, South San Francisco, CA, U.S.A.). Polyclonal antisera of TEV (ATCC 69-PVAS; Wisconsin isolate; depositor: D.E. Purcifull) and PVY (ATCC 50A-PVAS; depositor: W.B. Raymer) were used.

The field site

The field site was 0.1 ha (16 m × 61 m) and comprised 13 rows, about 1.2 m apart with pepper plants spaced within rows at 0.8 m. Seedling holes were hand dug, filled with a mixture of soil, farmyard manure (227 cm³ hole⁻¹), and N:P:K fertilizer (11:22:22 at 14 cm³ hole⁻¹). Additional applications of fertilizer (28 cm³ of N:P:K, 11:22:22 plant⁻¹) were made 7 and 25 weeks after transplanting. All plots were treated as per the recommendations of Johnson (1993) and Chung (1995) with dimethoate (Dimethoate[®], Agriculture Chemical Plants, Jamaica W.I., Rate = 1–1.5 L ha⁻¹) and diafenthiuron (Pegasus[®] 500, Novartis, Cartagena, Colombia, S.A., Rate = 0.125–0.300 L ha⁻¹) to suppress broad mite and aphid populations. Dimethoate and diafenthiuron are recommended for use with Stylet-Oil[®] (Simons *et al.*, 1995).

Experimental design

Three treatments, each comprising 100 plants experimental unit⁻¹, replicated three times, were arranged systematically due to restricted land space. All plots treated with Stylet-Oil[®] were located north-west from plots not treated with Stylet-Oil[®] in order to minimize drift to these unsprayed plots. Treatment 1 comprised plants that were covered with screen cages as seedlings and sprayed weekly with JMS Stylet-Oil[®] (JMS Flower Farms, Inc., Vero Beach, FL, U.S.A.) after transplanting. In Treatment 2, plants were covered with screen cages as seedlings, but were not treated with Stylet-Oil[®] after transplanting. In Treatment 3, plants were neither covered as seedlings nor treated with Stylet-Oil[®] after transplanting. Stylet-Oil[®] was mixed in water at a concentration of 0.75% and applied at the manufacturer's recommended rate of 234–1401 L ha⁻¹ (rates varied with the size of the plants) using a 12 L Solo[®] model 423 motorized knapsack mist blower (single nozzle) at a pressure of about 1000 kPa.

Monitoring virus symptoms

The progress of TEV spread was monitored based on symptoms. The first symptom of TEV appeared on new leaves as a dark and pale green mottling (vein clearing). The mottling soon coalesced to produce characteristic light and dark green blotches (etching). The presence of TEV and absence of PVY were confirmed serologically. Tissue blots were taken from one leaf on each of 10 randomly chosen non-symptomatic plants to confirm the absence of TEV within each experimental unit. Samples were taken weekly until 28 days after transplanting (DAT) and then monthly until symptoms were observed on some plants. Once symptoms of TEV appeared within the plots, symptomatic plants were tagged with a different colour for each week (beginning at 133 through 196 DAT).

Monitoring of aphids and other field observations

Aphid flight in the field was monitored using three pan traps, placed 10 m apart down the middle of the field. Each trap (McDonald, 2001) was hand painted in two shades of green and filled with approximately 500 mL 1:1 ratio of monoethylene glycol:water (modified from DiFonzo *et al.*, 1997). The glycol:water mixture was collected and replaced weekly. In the laboratory, alate aphids were removed from the mixture, counted, and stored in 70% ethanol until identified. Aphid species were identified by the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, U.S.A., and by the principal author. Each week colonizing aphid populations were assessed by observing one lower leaf on each of four sections of 10 randomly selected pepper plants within each subunit. Casual observations were also made to detect aphids on plants within or near pepper plots. Frequently observed weeds were noted.

Statistical analyses

Time series cross-correlation analyses (ARIMA procedure, SAS Institute, 1996) were conducted to determine an association between number of aphids trapped per week and the percentage of pepper plants with TEV symptoms within the three treatments. Mean rates of TEV disease progress in the three treatments were fitted to the following logistic model:

$$\% \text{ TEV infection} = t_{\max} / \{1 + \exp [-(t_x - t_{\text{median}})^* \beta_1]\}$$

where, t_{\max} = maximum TEV infection at 196 DAT, t_x = any given day after transplanting, t_{median} = number of days between transplanting and median percentage TEV infection, 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. Ninety-five per cent confidence intervals were obtained for the parameters of interest by calculating a range within which the residual sum of squares (RSS_{\min}) for the model was less than the critical level (CL) (Sharov *et al.*, 1995):

$$CL = RSS_{\min}(1 + F_{v_1/v_2})$$

where, F is the critical value of the F statistic at $P = 0.05$, v_1 and v_2 are 1 and $n-1$ degrees of freedom, and n is the number of observations (weeks) over which TEV incidence was calculated.

Results

The TEV was the only virus confirmed from random samples of leaves for tissue blots taken from non-symptomatic and symptomatic Scotch

Bonnet plants. All the randomly sampled leaves taken from non-symptomatic pepper plants during the first 28 DAT tested negative for TEV. Some randomly sampled leaves from non-symptomatic pepper plants sampled on 49 DAT tested positive for TEV for all treatments, but complete data were not available because some of the blot membranes were damaged.

The first symptoms of TEV were observed 56 DAT during routine monitoring (Figure 1A). In addition, serological testing of all plants exhibiting symptoms of TEV during a census of the field 56 DAT, confirmed the presence of TEV on 0.3% of all 300 covered seedlings and Stylet-Oil® (CS) treated plants, 4.0% of all 300 covered seedlings and non-Stylet-Oil® (CN) treated plants, and on 3.7% of all 300 uncovered seedlings and non-Stylet-Oil® (UN) treated plants. These plants exhibited very early TEV symptoms, in which the youngest leaves had initial vein clearing but symptoms did not develop, and there appeared to be a recovery from the disease in these plants by 63 DAT.

With the exception of three additional plants that exhibited symptoms of TEV 63 DAT (Figure 1A), no new symptomatic plants were observed during routine monitoring of plots until 111 DAT. However, from 84 DAT through 133 DAT, >60% of random samples from all three treatments tested positive for TEV. Leaves sampled from plants showing symptoms of TEV

disease during 147 DAT through 182 DAT also tested positive for TEV.

Incidence of TEV symptoms (Figure 1A) was correlated to flight activity of aphids within the field (Figure 1B). A two-week lag was found between aphid flight activity and the appearance of TEV symptoms in CN ($r = 0.49$) and UN plots ($r = 0.45$) but a three-week lag between aphid flight activity and the appearance of TEV symptoms in CS plots ($r = 0.67$). There was an increase in the mean aphid flight between 35 and 42 DAT (Figure 1B), two weeks before the first TEV symptoms were observed (Figure 1A). Then between 112 and 140 DAT, there was a second increase in aphid activity two weeks preceding an increase in virus symptoms (Figure 1A, B).

Aphis gossypii accounted for 90% of the total aphid species captured during 35–42 DAT (Figure 2A), but only represented 32% of the total aphids during 85–119 DAT and 5% of the total aphids during 120–147 DAT (Figure 2A). *Aphis gossypii* was found to colonize pepper but no correlation ($r = 0.07$) was found between the *A. gossypii* population on the plants and alates in the traps. *Uroleucon ambrosiae* (Thomas) complex accounted for 53% of the total number collected during 85–119 DAT and 85% of the total aphids during 120–147 DAT. During 148–175 DAT, *U. ambrosiae* only accounted for 8% of the aphid

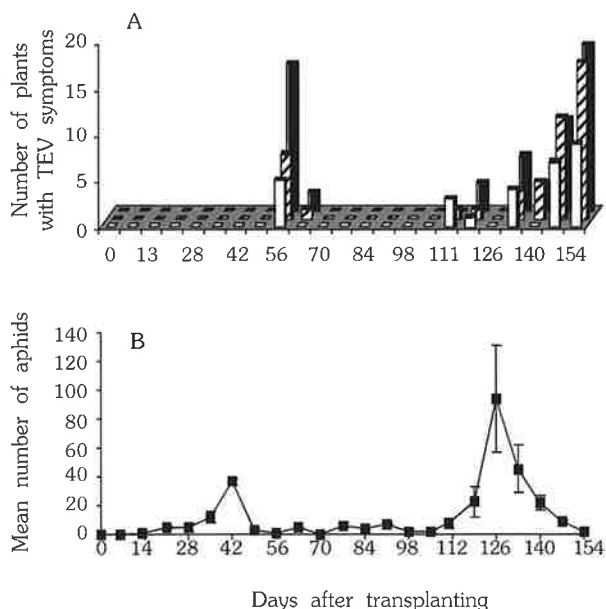


Figure 1 Number of Scotch Bonnet pepper plants with TEV symptoms per week from a total of 30 plants monitored in plots treated weekly with Stylet-Oil® and from untreated plots (A) Mean (\pm SE) number of alate aphids captured with pan traps (\diamond), \square , Covered seedling/Stylet Oil®; \boxtimes , Covered seedling/no Stylet Oil®; and \blacksquare , Uncovered seedling/no Stylet Oil® (B) during 2 September 1998 through 3 February 1999 (0–154 days after transplanting). The experiment was conducted at Bushy Park, St Catherine, Jamaica, W.I.

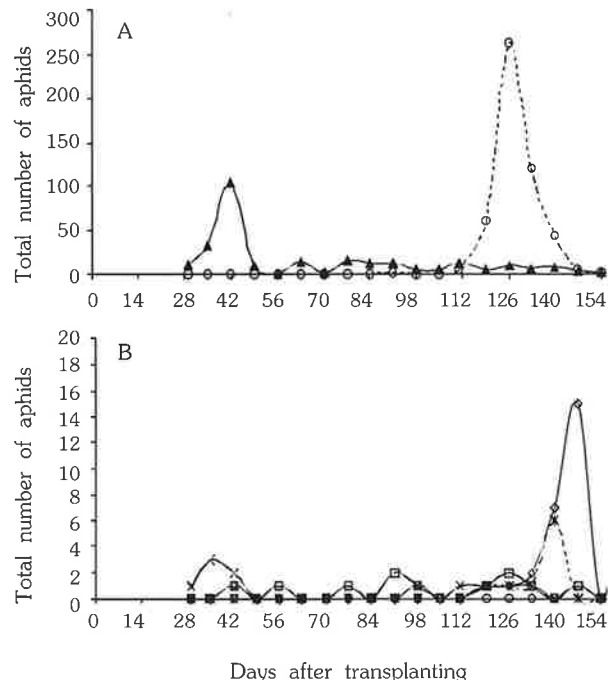


Figure 2 Total numbers of selected alate aphids captured with pan traps (A and B) from 2 September 1998 to 3 February 1999 (0–154 days after transplanting) The experiment was conducted at Bushy Park, St Catherine, Jamaica, W.I. (A) \blacktriangle , *Aphis gossypii* and \circ , *Uroleucon ambrosiae*. (B) \diamond , *Aphis amaranthi*; \times , *Aphis spiraeicola*; \circ , *Myzus persicae*; \square , *Aphis craccivora*; and $*$, *Lipaphis ermsyimi*

species caught, while *A. gossypii* accounted for 24%. It was observed that *U. ambrosiae* complex formed dense colonies on *P. hysterothorus* and this weed was removed from the plots on 126 DAT. *Aphis amaranthi* Holman accounted for 1, 5, and 36% of the aphids collected during 85–119, 120–147, and 148–175 DAT, respectively. Four other aphid species, all of which are reported to be TEV vectors, *A. craccivora*, *A. spiraecola*, *L. erysimi*, and *M. persicae*, together accounted for 9, 3, and 24% of the total aphid species during 85–119, 120–147, and 148–175 DAT, respectively (Figure 2B).

Weekly census of the field from 133 through 196 DAT showed that the appearance of TEV symptoms closely followed the logistic model within CS plots ($F = 1809.5$; $df = 1, 8$; $P < 0.0001$, $R^2 = 0.99$), CN plots ($F = 1700.9$; $df = 1, 8$; $P < 0.0001$, $R^2 = 0.99$), and UN plots ($F = 1120.1$; $df = 1, 8$; $P < 0.0001$, $R^2 = 0.99$), (Figure 3). At 196 DAT, the predicted TEV incidence (t_{max}) was 74% within CS plots, but 100% within the two non-Stylet-Oil® treated plots. The 95% confidence interval (CI) for t_{max} within the CS treatments ranged from 71 to 76% and was therefore significantly ($P < 0.05$) different from 100. The slope of the logistic model for the spread of TEV within CS plots was 0.19 (95% CI = 0.16–0.24) whereas the slopes of the logistic models for the spread of TEV in CN and UN plots were 0.15 (95% CI = 0.13–0.19) and 0.11 (95% CI = 0.09–0.14). The median infection time ($t_{37\%}$) of TEV in CS plots occurred 155.6 DAT (95% CI = 154.4–155.9 DAT), while the median infection times ($t_{50\%}$) of TEV were 152.7 DAT (95% CI = 151.4–154.0 DAT) in CN and 152.9 DAT (95% CI = 151.2–154.6 DAT) in UN plots (Figure 3). The predicted $t_{50\%}$ for

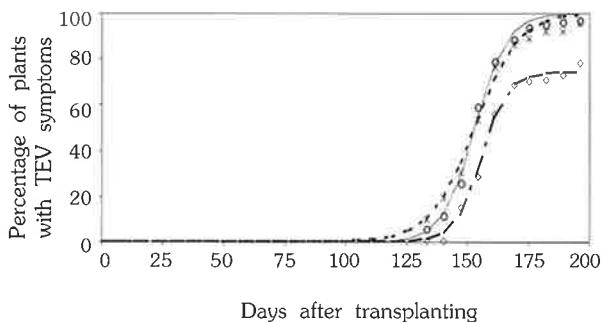


Figure 3 Logistic model: predicted (lines) and observed (symbols) incidence of TEV symptoms (mean % of plants with symptoms) over time within (A) covered seedling/Stylet-Oil® treated (\diamond), (B) covered seedling/non-Stylet-Oil® treated (\circ), and (C) uncovered seedling/non-Stylet-Oil® treated ($*$) Scotch Bonnet pepper plots during 2 September 1998 through 17 March 1999 (0–196 days after transplanting). The experiment was conducted at Bushy Park, St Catherine, Jamaica, W.I. The logistic models were, (A) $Y = 74/[1 - \exp\{-(x - 155.6)^{0.19}\}]$; (B) $Y = 100/[1 - \exp\{-(x - 152.7)^{0.15}\}]$; and (C) $Y = 100/[1 - \exp\{-(x - 152.9)^{0.11}\}]$

TEV infection within the Stylet-Oil® treated plots occurred at 159.4 DAT, about 6.6 days later than that predicted for non-Stylet-Oil®-treated plots.

Discussion

Protection of seedlings with aphid exclusion netting before transplanting did not significantly reduce the overall incidence of TEV during the season compared with seedlings that were not initially protected. Perhaps activity of viruliferous aphids at the location where the seedlings were grown during the study was very low or absent and was responsible for the lack of TEV infection in unprotected seedlings early in the season. In most situations, however, viruliferous aphids transmit TEV to pepper seedlings before they are transplanted in the field. Hence, protecting pepper seedlings with aphid exclusion netting prior to transplanting is highly recommended.

Disappearance of virus symptoms and apparent recovery of plants from virus diseases is not a new phenomenon (Benda and Bennett, 1964) and is often the result of environmental conditions, such as water stress (Matthews, 1991). Water stress causes the plants to become hardened and symptoms to become less obvious (Matthews, 1991). The pepper plants had undergone some degree of water stress during the period when the first symptoms of TEV disappeared.

Despite high aphid pressure and prevalence of TEV, applications of Stylet-Oil® reduced the incidence of this virus in plantings of hot pepper plants by 28% and delayed the progression of the disease by seven days compared with plants that were not treated. A greater difference in per cent virus reduction and delay in virus progression between Stylet-Oil®-treated plots and non-Stylet-Oil®-treated plots might have been observed if larger plots were used. Aphids were likely to move from virus-infected plants in untreated plots into adjacent Stylet-Oil®-treated ones to transmit the virus, whereas this type of movement could have been reduced if plots were larger and further separated. More frequent applications of Stylet-Oil®, especially during high aphid flight activity, might have further reduced the virus incidence as well.

Varied results have been reported regarding the efficiency of mineral oils in reducing the spread of viruses transmitted by aphids. Benner and Kuhn (1985) found no significant difference in the incidence and severity of TEV, PVY, and CMV in pepper (*C. annuum* L.) with and without JMS Stylet-Oil® treatment in Georgia. In their experiments, field sizes ranged from 0.007–2 ha. Umesh *et al.* (1995) reported that Stylet-Oil® failed to suppress field spread of watermelon mosaic 2 potyvirus and CMV under heavy inoculum pressure, but that it was able

to delay the onset of these diseases. Simons and Zitter (1980) and Simons (1982) confirmed that oil sprays lose their effectiveness when the percentage of virus-infected plants exceeded 10–20%.

Thomas (1984) reported successful control of aphid-transmitted viruses in cucurbits with applications of Stylet-Oil®. Loebenstein *et al.* (1970) were able to effectively delay and reduce the spread of CMV and PVY in pepper with a mineral oil, Blancol®. Both groups of researchers used knapsack sprayers to apply the mineral oils as was done in this experiment. Further studies need to be conducted to determine the efficacy of motorized knapsack sprayers as tools for applying mineral oils because their success could offer a practical means of managing aphid-transmitted viruses in hot pepper and other vegetable cropping systems in Jamaica.

The TEV symptoms appeared and increased shortly after peak aphid flights. The *U. ambrosiae* complex and *A. amaranthi* were implicated as possible vectors of TEV, although they have been tested neither for their transmission capability nor efficiency. These two species of aphids were common on *Parthenium hysterophorus* and weed species of amaranths found within the pepper field. The *U. ambrosiae* complex is known to transmit other potyviruses (Abney *et al.*, 1976; Koike, 1977; Orozco *et al.*, 1994). Studies need to be conducted to ascertain the abilities of these two species to transmit TEV and to determine their transmission efficiencies.

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