Annals of the Sri Lanka Department of Agriculture. 2003. 5:43-55.

SPREAD OF SRI LANKAN PASSIONFRUIT MOTTLE VIRUS IN YELLOW PASSIONFRUIT (*Passiflora edulis f. flavicarpa*) IN THE LOW COUNTRY WET ZONE OF SRI LANKA

E. M. DASSANAYAKE and W. G. S.PERERA
Plant Virus Indexing Centre, Gabadawatte, Homagama

ABSTRACT

Factors influencing the spread of passionfruit mottle potyvirus in the low country wet zone region of Sri Lanka were investigated. Observations were made within a newly planted passion fruit crop. Aphids of the species *Aphis spiraecola* were found colonizing the weed species *Mikania scandens* and *Eupatorium adoratum* and was the most common potential aphid vector. They were detected in yellow water traps placed at regular intervals within the crop. Ability of virus transmission by *Aphis spiraecola* was further confirmed by a series of artificial inoculations. A positive correlation between the number of *A. spiraecola* and the number of passion fruit plants infected with SLPFMV at weekly intervals was established. The numbers of trapped *A. spiraecola* were positively correlated with rainfall upto four weeks preceding the aphid counts.

KEYWORDS: Passionfruit mottle virus, *Aphis spiraecola*, Artificial inoculation.

INTRODUCTION

Several aphid borne viruses of *Passiflora spp* have been reported from various parts of the world. A potyvirus, named Sri Lankan passion fruit mottle virus, (SLPFMV) was isolated from yellow fruited passion fruit (*Passiflora edulis f. flavicarpa*) which shows mottle symptoms. This virus was reported to be transmitted, under laboratory conditions, by several species of aphid in a non-persistent manner (Dassanayake and Hicks, 1992). Although this was the first published report of the occurrence of an aphid-borne virus from *Passiflora* in Sri Lanka, there is little information on the ecology of SLPFMV or on its spread under natural or semi-natural conditions. To provide such information, a field trial was set up at Pahana Estate, Kalutara, Sri Lanka. The objective of the trial was to monitor disease progress within a new passion fruit planting and to assess the relative abundance of aphid vectors. This paper presents the results of our observations.

MATERIALS AND METHODS

Passion Fruit Seedlings

Passion fruit seeds (which do not carry any virus infections) of the yellow-fruited cultivar (*P.edulis f. flavicarpa*) were collected from apparently virus free mother plants and sown in a nursery at the Regional Agricultural Research Centre, Bombuwela, Sri Lanka. After six weeks in the nursery when 10-12 mature leaves were present, seedlings were transplanted to a field at the experimental site at Pahana Etate close to Bombuwela Agriculture Research Centre.

Experimental Field

The experimental plot (figure 1) consisted of six contours of 20 plants with 360 cm between the plants and approximately 210 cm between the rows. A fully grown passion fruit crop, approx. 90% of which was naturally infected with SLPFMV (as evidenced by symptom expression and infectivity tests) continuously surrounded the healthy plot on the northern, western and southern borders, at a distance ranging from 10 – 50 metres. *Pueraria phaseoloides* and *Centrosema pubescens* grown as cover crops, and various weeds, were removed at monthly intervals, within a radius of 60 cm from the base of the vines. *Crotalaria* species*, Cassia tora, Mikania scandens, Eupatorium odoratum* were the predominant weeds at the experimental site. *Vigna unguiculata, Citrus* sp. and a few banana (*Musa paradisica*) plants were also present in the vicinity of the plot. *Gliricidia maculata* was used as a 'live post' to support the mature passion fruit crop.

Assessment of winged aphids

After initial experiments to find the optimum trap size and location (Dassanayake, 1989), six plastic circular yellow water pan traps, approx. 30 cm in diameter and 15 cm deep, were placed in the field to monitor the movement of winged aphids (alates). The traps were placed 900 cm apart, on wooden platforms, 75 cm above ground, running diagonally across the field. Trap contents were removed for analysis at weekly intervals and after separating other insects in the laboratory; the aphids were placed in 98% alcohol for preservation before identification. The preparation of aphids prior to microscopic examination and identification was done by the method of Blackman and Eastop (1984). The effect of various climatic factors (temperature, sunshine, rainfall) on total aphid catch was tested by regression analysis.

Artificial inoculation of SLPFMV under Glasshouse conditions

Aphid colonies found in different plant species were raised in the insectory.to study their potential as virus vectors. Aphids were collected from healthy plants of tobacco (White burly), *Glyricidia maculate, Vigna unguiculata, Solanum melongena, Mikania scandens* and were reared on their natural hosts. The first generation offspring were used for the transmission study.

Aphids starved for 2 hours were allowed to feed on a portion of passion fruit leaves infected with SLPFMV for 5 minutes. Fifteen to twenty aphids were immediately transferred and allowed to feed on each of passion fruit seedlings for another 5 – 30 minutes. Results are given in table 1. All the insects were destroyed after transmission. Test plants were assayed for infection by observing for symptom expression and by enzyme linked immunosorbent assay.

Table 1. **Effect of acquisition and inoculation period on the transmission of SLPFMV by different aphid species.**

Aphid spp.	*No. of Aphids Per plant*	*Acquisition feeding time*	*Inoculation feeding time*	*No. plants inoculated /No. of plants infected*	*% transmission*
M. peesicae	15	5	5	10/10	100
- do -	15	5	30	6/10	60
A. craccivora	20	5	30	5/10	50
- do -	15	5	30	4/10	40
- do -	15	5	5	6/10	60
A. gossypii	15	5	30	7/10	70
- do -	15	5	5	8/10	80
A. spiraecola	15	5	30	6/10	60
- do -	15	5	5	8/10	80

Assessment of SLPFMV transmission in the field

This was determined by exposing field-planted passion fruit seedlings to natural infection. Counts of diseased plants were recorded weekly on the basis of symptomatology supported by confirmatory infectivity tests on to *Passiflora foetida* and *Chenopodium amaranticolor* (Dassanayake and Hicks, 1992).

Weather Data

Weather data (May 1993 – May 1994) were obtained using the apparatus installed at the Regional Agricultural Research Centre, Bombuwela.

RESULTS AND DISCUSSION

Survey of host plants for aphid vectors

Several aphid species including *Myzus persicae, Aphis spiraecola* and *A. gossypii,* have been shown to transmit SLPFMV in the laboratory (Dassanayake and Hicks, 1992). Attempts were made to find vector colonies on plants in the vicinity of our experimental field. *Aphis spiraecola* was found infesting *Mikania scandens* Willd, and *Eupatorium odoratum,* perennial weeds (*Compositae*) common within and around the experimental site. *A. gossypii* was found on seven weeds (in five families) in the area including *Hibiscus esculentus, Solanum melongena* and *Capsicum* spp., while large populations of *A. craccivora* were found on *Gliricidia maculata* and *Vigna unguiculata.*Other species found included *Pentalonia nigronervosa* and *Toxoptera citricidus* on banana and citrus respectively . However *Myzus persicae* was not observed among these host plants. Aphids did not appear to colonise *Passiflora* although small groups of *A. spiraecola* were occasionally found on the young shoots.

Sources of virus

A variety of weed and wild plant species in the area had virus-like symptoms, with mottle and mosaic being the most common. Attempts to isolate SLPFMV from twelve weed or wild plant species (in five families) by sap inoculation of indicator hosts were negative, except one sample from *P. foetida*, which caused symptoms typical of SLPFMV on *P. edulis f. flavicarpa. Passiflora foetida* should be regarded as a potential source of infection along with *Cassia occidentalis,* which was also present in the locality and is an experimental hosts of SLPFMV (Dassanayake and Hicks, 1992).

Table 2. Winged aphids trapped during the period of investigation (May 1993 – May 1994).

Aphid species	***May***	***June***	***July***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***	***Jan***	***Feb***	***Mar***	***Apr***	***May***	***Total***
Aphis spiraecola	1356	1386	358	401	3428	934	244	411	307	63	5	17	18	8928
A. gossypii	15	18	12	9	2	7	49	39	37	5	4	2	0	199
A. craccivora	0	3	5	5	2	0	0	15	4	3	3	1	0	41
Toxoptera citricidus	0	1	2	1	0	4	6	8	0	0	0	0	0	22
T. auranti	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Pentalonia nigronervosa	0	1	1	0	0	1	1	0	3	0	3	2	0	12
Melanaphis sacchari	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Rhopalosiphum maidis	0	0	0	0	0	1	26	13	1	0	0	0	0	41
Tetraneura nigriabdominalis	0	0	0	0	0	0	6	2	2	0	10	0	0	20
Cerataphis variabilis	0	0	0	0	0	0	2	0	0	0	0	1	0	3
Unidentified spp.	0	0	0	0	0	0	0	0	0	0	1	9	0	10
Total	1371	1409	378	417	3432	948	334	488	354	71	26	32	18	9278

Abundance of winged aphids

A total of 10 aphid species were caught and identified in the yellow water traps during the period of investigation (table 2). About 90% of all aphids trapped were *Aphis spiraecola,* the only species caught regularly throughout the year. The number of trapped *A.craccivora,* in contrast, were low compared to the number present on local weed hosts. Other species such as *Aphis gossypii, Toxoptera citricidus, Rhopalosiphum maidis* and *Pentalonia nigronervosa* were present, but in very low numbers. *Myzus persicae* was not detected.

Artificial inoculation of SLPFMV under Glasshouse conditions

Results shown in table 1 indicated that increasing the inoculation feeding time from 5 minutes to 30 minutes had little effect on the transmission. *Myzus persicae, Aphis gossypii, A. craccivora, A. spiraecola,* transmitted SLPFMV to passiflora seedlings. Results indicated that SLPFMV transmitted by aphids in a non-persistent manner and symptoms appeared 3 weeks after transmission. Furthermore results showed several aphid species were able to transmit SLPFMV by artificial inoculation.

Symptoms and disease development

Symptoms were first observed on plants in the field two to three weeks after planting. This was about the same time it took for symptom development when viruliferous aphids were used to inoculate *P.edulis* seedlings with SLPFMV under glasshouse conditions. Early symptoms on passion fruit seedlings were vein yellowing, yellow spotting and down curling of young leaves, in mature plants a severe foliar mottle was the most conspicuous symptom.

Infected plants initially clustered along the southern and western borders of the planting, downwind of the prevailing southwestern monsoon. Over half of the plant with SLPFMV two months after planting were found in the first six rows of the field, although by 20 weeks this aggregation had largely disappeared and infected plants were more uniformly distributed within the plot (figure 1). Disease levels were initially low as many plants lost leaves following transplantation and this had deterred vectors from alighting. From about the end of June, when plants began to produce new flushes of growth, diseased levels increased as the crop became more attractive to vectors until; as plants matured and flowered, the numbers of new infections started to decline. By the end of the experiment, a few other plants had developed ringspot symptoms caused by a potyvirus serologically related to SLPFMV (Dassanayake and Hicks, 1991).

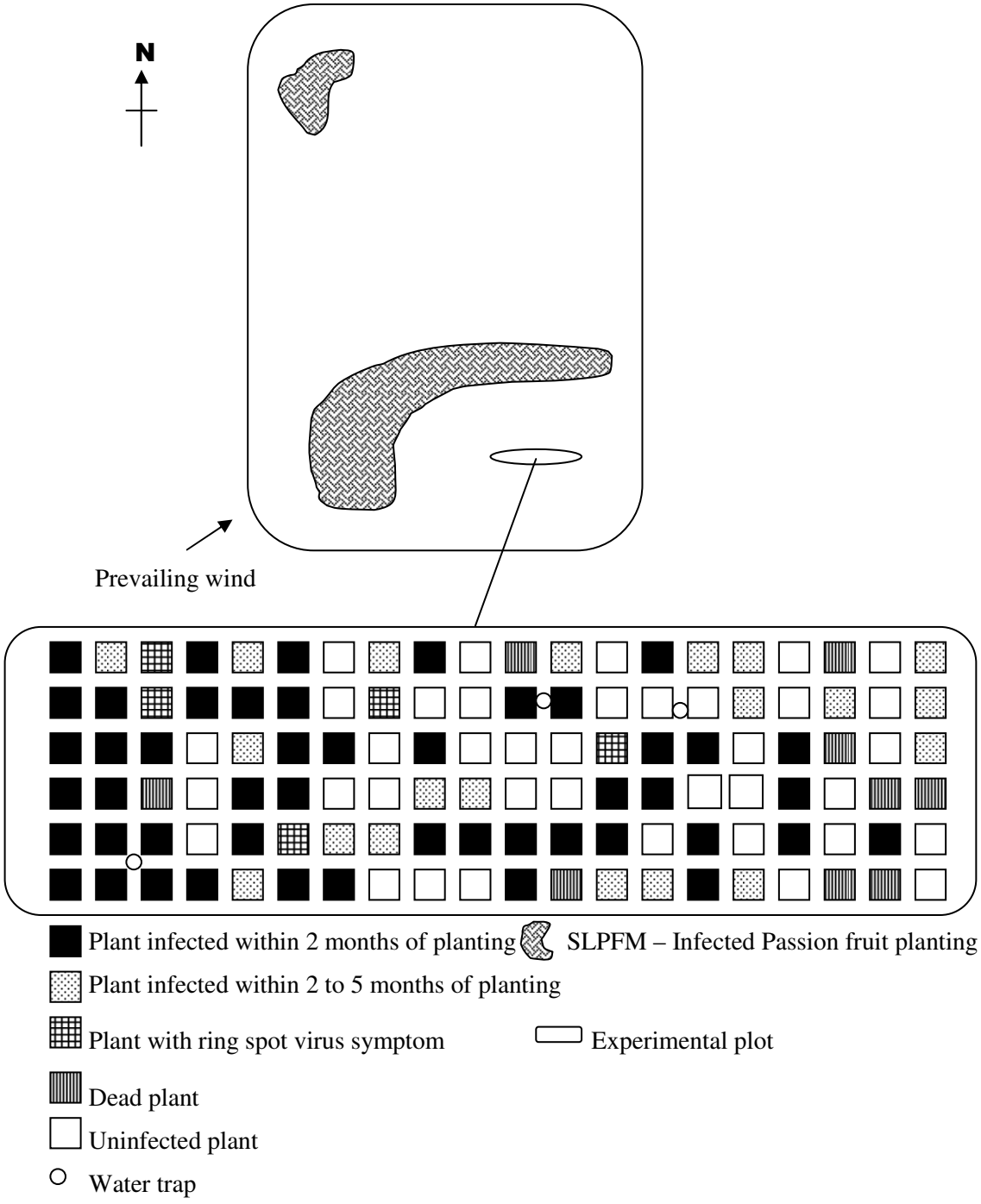

Figure 1. Experimental Area showing the location and distribution of infected passion fruit plants, two and five months after planting.

As with other non-persistently transmitted aphid borne viruses the disease progress curve was sigmoidal and could be linearised by regression analysis of logit transformed data (figure 2). The rate of the epidemic spread was given by the slope of the line (regression coefficient).

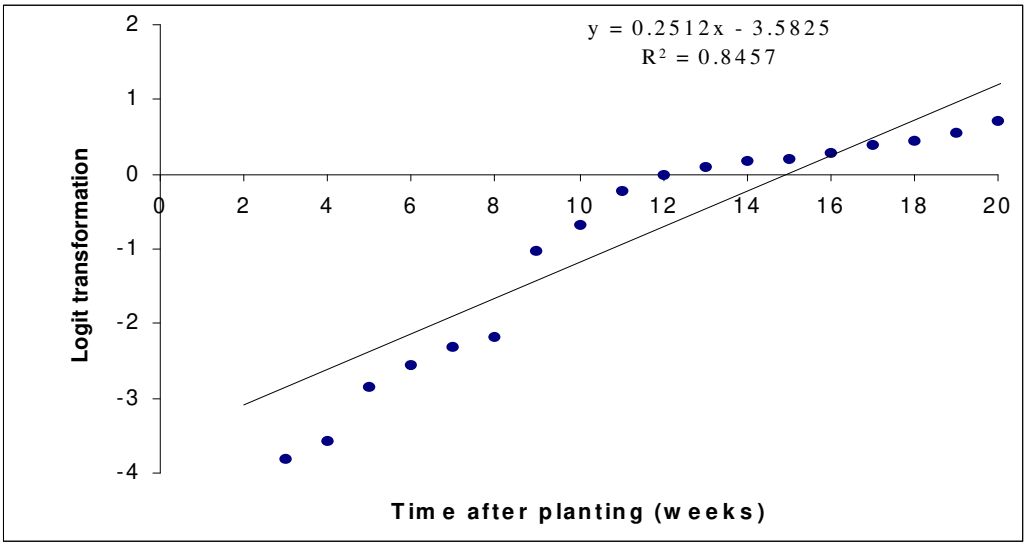

Figure 2. Disease progress curve for Sri Lankan passion fruit mottle virus over eighteen weeks. Percentage incidence of infected plants (y) was transformed to log y/(y-1) and the virus infection rate was determined by the slope of the regression equation.

Relationship between infection, the abundance of winged aphids, and weather

The number of infected plants in the early part of the season was greatest around the first three traps, which collected more aphids than other traps, showing higher vector activity in this part of the field. Trap data suggested that viruliferous alates were entering the crop and initiating infection mainly from southwestern corner. There was no correlation between the weekly counts of alate *A.spiraecola* and the numbers of new infections of passion fruit with SLPFMV over a three month period ($r = 0.25$; $n = 13$, $P < 0.05$). A positive correlation could be found, however, between the percentage weekly new infections and the number of alate *A.spiraecola* trapped in the preceding one or two weeks respectively, ($r = 0.58$; $n = 13$; $P < 0.05$; $r = 0.74$; $n = 13$, $P < 0.01$). The occurrence of rows containing two, three or more infected plants in a sequence, suggested that a major component in the spread of SLPFMV was the acquisition and transmission of virus from sources within the crop.

The numbers of *A.spiraecola* fluctuated seasonally and trap data showed two main peaks – one in June/July and one in September. Based on the data in figure 3, most spread appeared to be occurring during the first peak of activity. Of the environmental factors investigated only rainfall proved to be correlated to the numbers of *A.spiraecola* with higher populations of aphids following periods of high rainfall . A positive correlation ($P< 0.01$) was found between number of aphids trapped and the rainfall for the second ($r = 0.62$), third ($r = 0.54$), or fourth ($r = 0.39$) preceding weeks (figure 4).

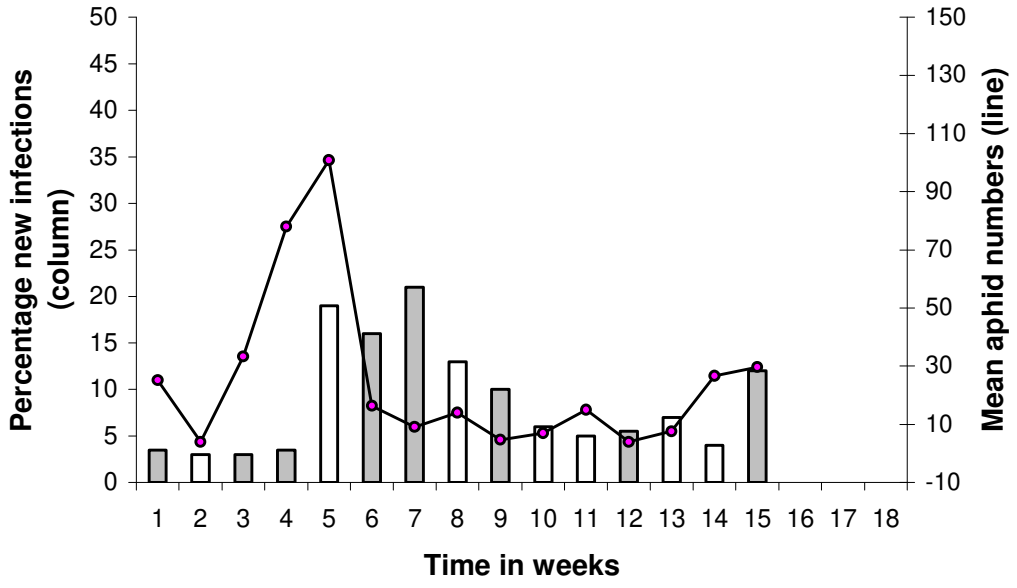

Figure 3. The change in percentage SLPFMV infection over time in relation to the number of trapped alate *A. spiraecola.*

Rainfall usually prevents aphids flying and fewer aphids were caught during maximum periods of precipitation. Wet weather, however, stimulated the growth of *Mikania scandens* and *Eupatorium odoratum,* which were heavily colonized by *A.spiraecola.* Rapid multiplication of aphids on these hosts would occur during conditions favourable for plant growth and this would result in over-crowding and the production of alates. Vector activity and trap counts, therefore, were higher following wet weather. Wet weather also encouraged the expansion of yellowish new leaves on the passion fruit plants, which would attract the alates.

There is no evidence to suggest SLPFMV is transmitted through *Passiflora* seed (Dassanayake 1989) and very little evidence, was from the present study, that it is present in local weeds or wild plants. The most likely source of infection for our experimental field was the adjacent, mature SLPFMV-infected passion fruit plants (figure 1). Trap and survey data implicated the aphid *A. spiraecola* and, by association with its perennial wild hosts *Mikania scandens* and *Eupatorium odoratum*, in the epidemiology of SLPFMV. The virus is known to be transmitted by several species of aphids (Dassanayake and Hicks, 1992) after short acquisition and inoculation feeds.

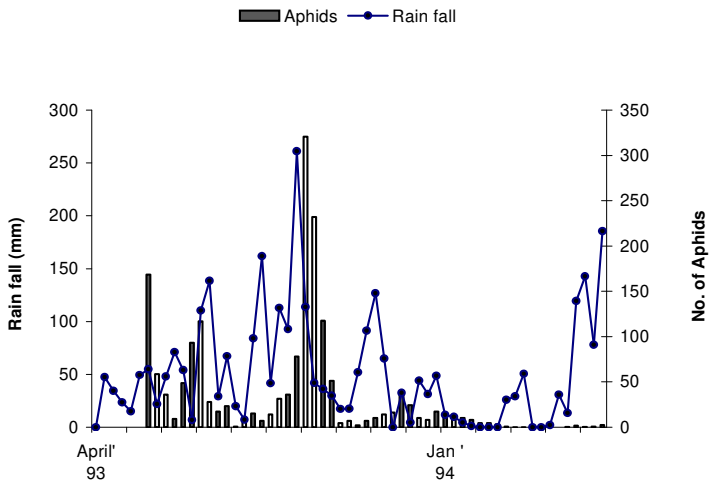

Figure 4. Number of *Aphis spiraecola* trapped in yellow water pans and weekly total rain falls (mm) data during May '93 to May '94.

Primary infection of newly planted seedlings was probably initiated by vectors moving from weed hosts onto the new planting having first acquired virus *en route* from the adjacent infected vines. Once the virus has reached sufficient concentration in the epidermal cells to be acquired by visiting alates, these newly infected plants would then act as sources of infection for secondary virus spread. The evident clustering of infected plants along rows (figure 1), clearly suggested within-crop spread. The majority (80 %) of additional infections at the final assessment (after five months) occurred next to plants, which were infected within two months.

The role of *apterae* in the spread of SLPFMV between passion fruit, a non-breeding host, is a probable minor. The transitory and non-colonizing alates, however, are known to spread non-persistent viruses efficiently in a range of crops (Jayasena and Randles 1984; Raccah *et al.,* 1985) and are most likely responsible for both primary

and secondary infections in passion fruit. They may, however, acquire and inoculate viruses less frequently than species, which colonize the crop. The climatic conditions experienced in the low country wet zone of Sri Lanka, would probably ensure there were breeding colonies throughout the year and virus spread could presumably occur over a similar period. Disease and vector levels, however, should be monitored over several seasons to provide a more accurate epidemiological picture. It was likely, for example, that the final infection rates observed in our studies would have increased from about 60 %, five months after planting, to the 90% or more invariably found in mature crops in the region.

CONCLUSIONS

Although the most important vector of SLPFMV was probably *A. spiraecola, which* represented the bulk of the total catch, and which correlated with the percentage of mottle infected passion fruit plants, peaks of aphid numbers do not necessarily correlate with peaks of transmission. SLPFMV is transmitted efficiently by several other aphid species (Dassanayake and Hicks, 1992), which, in our study, were trapped in very low numbers. The role of vectors other than *A.spiraecola* in field transmission, therefore, needs to be evaluated. It is possible that some other important vectors of SLPFMV were not being caught efficiently in our traps. Aphid species are differently attracted to insect traps (Eastop, 1955) and some species, for example, are more attracted to green than to yellow.

It was of interest that the overall weekly collection of aphids correlated with rainfall although many other abiotic and biotic factors, including the presence of other viruses, are likely to be important in the epidemiology and ecology of SLPFMV through effects of vector behavior, and the physiology and susceptibility of host plants. The pattern of infection suggested that virus spread into, and within the crop was occurring downwind, presumably through direct effects on vector movements. Our results are similar to those reported for other aphid-borne, non-persistently transmitted viruses in row crops (Swanson, 1968; Thresh, 1974; 1980; Conti *et al.,* 1979; Gonzales and Rawlins 1969; Knoke *et al.,* 1974; Watson and Healy 1953). De Wijs, (1974) noted the importance of *A. spiraecola* in the spread of a passion fruit ringspot virus to passion fruit plantings in West Africa.

Passion fruit cultivation is of considerable economic importance to Sri Lanka (Dassanayake, 1989) and control measures are needed to reduce losses caused by SLPFMV and other viruses. By identifying potential vectors and sources of infection our study has provided the first step for rationalizing such measures. Measures recommended for the control of some non-persistent viruses, such as isolation of new

plantings from sources of infection (Broadbent, 1957), the use of sticky yellow sheets to trap (Cohen and Marco, 1973), and use of synthetic pyrethyroid insecticides (Jutsum *et al.,* 1984) may prolong the economic lifetime of the crop. In the long term, however, the introduction of virus and/or vector resistance through conventional breeding, or genetic engineering, may probably be the only practical solution.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. R. G. T. Hicks of School of Biological Sciences, University of Bath U.K. for advice and guidance in preparation of the script and the British Museum of Natural History, U.K. for aphid identification.

REFERENCES

Blackman, R. L. and V. F. Eastop. 1984. Aphids on the World's Crop. An identification guide. John Wiley and Sons.

Broadbent, L. 1957. Investigations of virus diseases of Brassica crops. Agr.Res.Coun.Rep.Ser.14. Cambridge University Press, Cambridge.

Cohen, S. and S. Marco. 1973. Reducing the spread of aphid transmitted viruses by trapping the aphids on sticky yellow polythene sheets. Phytopathology 63:1207-9.

Conti, M., P. Caciagki, and A. Casetta. 1979. Infection sources and aphid vectors in relation to the spread of cucumber mosaic virus in pepper crops. Phytopathol. Mediterr. 18: 123-128.

Dassanayake, E. M. 1989. Studies on virus diseases of *Passiflora.* Ph.D.thesis, University of Bath, England.

Dassanayake, E. M. and R. G. T. Hicks. 1991. The serological relationships among *Passiflora* virus isolates determined by enzyme-linked immunosorbent assay. Sri Lanka Journal of Agric. 28: 43-52.

Dassanayake, E. M. and R. G. T. Hicks. 1992. Sri Lankan Passion Fruit Mottle Virus, a potyvirus infecting golden passion fruit in Sri Lanka. Ann.appl.Biol. 120: 459-469.

De Wijs, J. J. 1974. The correlation between the transmission of passion fruit ringspot virus and population of flying aphids. Neth.J. Pl. Path. 80: 130-144.

Eastop, V. F. 1955. Selection of aphid species by different kinds of insect traps. Nature, London 176, 396

Gonzales, D. and W. A. Rawlings. 1969. Vector behavior, environmental stimuli, and the dynamics of plant virus epidemics. In Spatial components of plant disease epidemics Eds. M. J. Jeger Prentice Hall, New Jersey.

Jayasena, K. W. and J. W. Randles. 1984. Patterns of spread of the non-persistently transmitted bean yellow mosaic virus and the persistently transmitted sunterranean clover red leaf virus in Vicia Faba. Ann.appl.Biol. 104: 249-260.

Jutsum, A. R., M. D. Collins, R. M. Perrin, D. D. Evans, R. A. H. Davies and C. N. E. Ruscoe. 1984 . A novel pyrethroid insecticide. Proc. Br. Crop Prot. Conf. Pests Dis. 1: 421-428.

Knoke, J. K., L. Ramond, R. J. Anderson and D. T. Gorden. 1974. Distribution of maize dwarf mosaic and aphid vectors in Ohio. Phytopathology. 64: 639-645.

Raccah, B., A. Gal-On and V. F. Eastop. 1985. The role of flying aphid vectors in the transmission of cucumber mosaic virus and potato virus Y to pepper in Israel. Ann.appl.Biol. 106: 451-460.

Swanson, K.G. 1968. Role of aphids in the ecology of plant viruses. Annual Rev.of Phytopathol. 6: 351-374.

Thresh, J. M. 1974. Temporal patterns of virus spread. Annual Rev. Phytopathology 12: 111-120.

Thresh, J. M. 1980. An ecological approach to the epidemiology of plant virus diseases. In Comparative Epidemiology Eds. J. Paiti and J. Kranz, Pp 57-70, Pudoc, Wageningen, The Netherlands.

Watson, M. A. and M. J. R. Healy. 1953. The spread of beet yellow and beet mosaic viruses in the sugarbeet root crop. Ann. Appl. Biol. 40: 189-193.