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TRANSMISSION AND PATTERN OF SPREAD OF CHILLI VEINAL MOTTLE VIRUS BY *APHIS GOSSYPII* GLOVER (HOMOPTERA: APHIDIDAE)

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By

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LIST OF ABBREVIATIONS

AzMV	Arizona Mosaic Virus
CBRSV	Cabbage Black Ring Spot Virus
CMV	Cucumber Mosaic Virus
CVMV	Chilli Veinal Mottle Virus
PeMV	Pepper Mottle Virus
PMMV	Pepper Mild Mottle Virus
PMoV	Pea Mosaic Virus
PLRV	Potato Leaf-roll Virus
PRPMV	Puerto Rico Pepper Mosaic Virus
PVMV	Pepper Veinal Mottle Virus
PVY	Potato Virus Y
SBM	Sugar Beet Mosaic
TEV	Tobacco Etch Virus
TsEV	Tobacco severe Etch Virus
YvSB	Yellow virus infected Sugar Beet
min	minutes
sec	seconds

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Chilli veinal mottle virus (CVMV) is a serious problem and has become a major limiting factor in growing and production of chilli in Malaysia. This virus is transmitted non-persistently by aphid, *Aphis gossypii*. CVMV control efforts have been unsuccessful due to lack of knowledge on the epidemiology of the virus.

Studies on transmission and spread of CVMV to chilli using nymphs and adults of *A. gossypii* were conducted in the laboratory and glasshouse. Aphids and test plants (*Capsicum annuum* cv. MC 4) were cultured and maintained in insect proof glasshouse.

Fifteen seconds and thirty seconds acquisition feeding time were found to be the optimum time for *A. gossypii* to transmit CVMV. All aphid forms were equally efficient in CVMV transmission.

A single *A. gossypii* was able to transmit and retain CVMV regardless whether it was fed or starved. *A. gossypii* lost CVMV infectivity more rapidly in serial transfer (when feeding) than in post-acquisition starvation (when starving). Transmission level decreased as the period of serial transfer (0 - 60 min) and post-acquisition starvation time (1-120 min) increased, however, the level of transmission increased with aphid density.

Colony establishment of *A. gossypii* was faster when aphids were initially released on healthy plants than on CVMV infected plants. Apterous vectors spread CVMV more efficiently than alate vectors. However, CVMV spread faster when *A. gossypii* was placed on the inoculum source. Apterous adults released near the inoculum source achieved the highest level of transmission compared with the alates. In the beginning, CVMV spread occurred mostly near the inoculum source and moved outward thereafter.

Weeds species infected by CVMV included members of family Amaranthaceae, Boraginaceae, Compositae and Solanaceae. CVMV failed to infect plant species from the family Acanthaceae, Capparidaceae, Chenopodiaceae, Convulvulaceae, Labiatae, Leguminosae, Malvaceae, Onagraceae, Portulaceae, Rubiaceae, Scrophulariaceae and Sterculiaceae.

Amongst the non-colonising aphids, *Aphis craccivora*, was found to be the most efficient in transmitting CVMV followed by *Hysteneura setariae, Rhopalosiphum maidis, Lipaphis erysimi* and *Toxoptera aurantii*.

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TRANSMISI DAN CORAK PENYEBARAN CHILLI VEINAL MOTTLE VIRUS OLEH *APHIS GOSSYPII* GLOVER (HOMOPTERA: APHIDIDAE)

oleh

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Chilli veinal mottle virus (CVMV) merupakan satu masalah yang serius dan menjadi satu faktor penghad dalam penanaman dan pengeluaran cili di Malaysia. Virus itu ditransmit secara tidak kekal oleh kutu daun, *A. gossypii*. Usaha-usaha untuk mengawal CVMV belum lagi berjaya kerana kekurangan pengetahuan mengenai epidemiologi virus tersebut.

Kajian penjangkitan dan penyebaran CVMV kepada cili telah dijalankan di dalam makmal dan rumah kaca dengan menggunakan peringkat nimfa dan dewasa *A. gossypii*. Kutu daun dan tanaman kajian (*Capsicum annuum* cv. MC4) telah dikultur dan dipelihara di dalam rumah kaca.

Keputusan kajian menunjukkan masa pemerolehan optimum untuk *A. gossypii* mentransmit CVMV adalah 15 saat dan 30 saat. Semua peringkat kutu daun adalah sama cekap di dalam transmisi CVMV.

A. gossypii tunggal berkeupayaan menyebar dan mengekalkan CVMV samada ia diberi makan atau dilaparkan. *A. gossypii* hilang kejangkitan CVMV lebih cepat dalam pemindahan bersiri (semasa makan) berbanding dengan kelaparan selepas pemerolehan (semasa dilaparkan). Tahap transmisi berkurangan dengan penambahan tempoh pemindahan bersiri (0-60 minit) dan masa kelaparan selepas memperolehan (0-120 minit), begitupun, paras kejangkitan bertambah dengan kepadatan kutu daun.

Perkembangan koloni *A. gossypii* adalah lebih cepat apabila kutu daun dilepaskan ke atas pokok yang sihat berbanding dengan pokok yang dijangkiti CVMV. Penyebaran CVMV oleh vektor tidak berkepak adalah lebih berkesan berbanding dengan vektor yang berkepak. Walau bagaimanapun, penyebaran CVMV adalah lebih cepat apabila *A. gossypii* diletakkan di atas sumber inokulum. Tahap transmisi tertinggi diperolehi dari kutu daun dewasa tidak berkepak yang dilepaskan berhampiran dengan sumber inokulum berbanding dengan kutu daun berkepak. Pada mulanya, CVMV disebarkan berdekatan dengan sumber inokulum dan kemudiannya merebak ke arah luar.

Spesies rumpai yang dijangkiti CVMV termasuk ahli-ahli dari famili Amaranthaceae, Boraginaceae, Compositae dan Solanaceae. CVMV didapati gagal menjangkiti spesies dari famili Acanthaceae, Capparidaceae, Chenopodiaceae, Convulvulaceae, Labiatae, Leguminosae, Malvaceae, Onagraceae, Portulaceae, Rubiaceae, Scrophulariaceae dan Sterculiaceae.

Kutu-kutu daun tak berkoloni, *Aphis craccivora* didapati paling cekap dalam penyebaran CVMV, diikuti oleh *Hysteneura setariae*, *Rhopalosiphum maidis*, *Lipaphis erysimi* dan *Toxoptera aurantii*.

CHAPTER 1
INTRODUCTION

Chilli or Cayenne pepper (*Capsicum annuum L.*), is a lowland crop grown throughout Peninsular Malaysia. Johore, Perak and Kelantan have frequently been reported to have higher acreage compared to other states (Anon, 1986). The acreage of *C. annuum* in 1979 was 913 hectares and in 1986 was approximately 1200 hectares. The obvious increase in acreage indicates a high demand for chilli from year to year. The total acreage of this crop is still small, although Malaysia's climate is favourable for the growing of chilli and despite the high ex-farm price of the commodity. As such, Malaysia's import of chilli has exceeded 25 million ringgit annually (Anon, 1987).

One of the major problems limiting the production of chilli is the high incidence of virus infection which could reached 100% when the crop reached maturity (Abdul Samad, 1984). A survey conducted by Fujisawa *et al.* (1986) reported that 80% of the cultivated chilli crops manifested infection by mosaic virus diseases in the field in Peninsular Malaysia.

The virus disease showing mosaic symptom in *C. annuum* was first described by Ong (1975) and named it as CVMV. CVMV belongs to potyvirus group with particles ranging from 750 to 900 nm and produces the characteristic pinwheel inclusions in thin sections of leaf tissues (Abdul Samad, 1986). CVMV is transmitted by aphids in a non-persistent manner. Chemical control for vectors of non-persistent viruses had been ineffective because these viruses could be transmitted during brief acquisition and inoculation feeding, while insecticidal action comparatively takes

a longer period to kill the aphid vectors (Broadbent, 1956; Ribbands, 1964; Gonzalex and Rawlins, 1969; Smith *et al.*, 1969; Zitter and Simon, 1980).

Informations on the epidemiology, in particular transmission, virus-vector relationship, epidemiology, host range and symptomatology of CVMV and its vectors are still incomplete and badly needed to develop new strategies for CVMV management.

The objectives of this research were:

1. To study the efficiency of different forms of *A. gossypii* with different acquisition period in transmitting CVMV.

2. To determine the influence of acquisition time and density of *A. gossypii* on the retention and persistency of CVMV while feeding (serial transfer) and when starving (post-acquisition starvation).

3. To determine the pattern of spread of CVMV as related to *A. gossypii* colonisation.

4. To investigate the possibility of CVMV transmission from common weed species found in chilli growing areas.

5. To determine the ability of other non-colonising aphids in transmitting CVMV.

CHAPTER 2
LITERATURE REVIEW

Non-persistent Chilli (*Capsicum annuum*) Viruses

Non-persistent viruses, also known as stylet-borne or non-circulative viruses, are the most common type of viruses transmitted by aphids and causing great losses to crops (Bradley, 1964). These viruses can be acquired and transmitted during probing of less than 60 sec (Swenson, 1969; Harris, 1977; Markham *et al.*, 1987).

The non-persistent viruses of chilli reported in Malaysia are chilli veinal mottle virus (CVMV) and cucumber mosaic virus (CMV) (Fujisawa *et al.*, 1986). Other non-persistent viruses of chilli which belong to the potyvirus group have also been reported in Florida (Simon,1956; Zitter, 1971, 1972, 1973; Pieczarka and Zitter, 1981), India (Raychaudhuri, 1969), Israel (Cohen and Marco, 1973), Arizona (Nelson and Wheeler, 1972), Nigeria (Lana *et al.*, 1975), North Carolina (Lapp and Gooding, 1976) and Texas (Mclean, 1962). Seth and Dhanraj (1972) also reported other groups of viruses on chilli in India. The tobamovirus in Sicily, Italy (Wetter *et al.*, 1984) and pepper yellow vein virus are some of the other more recent viruses reported on chilli (Fletcher *et al.*, 1987).

Chilli Viruses in Malaysia

In Malaysia a mosaic disease on chilli was first described by Ong (1975) and named it chilli veinal mottle virus (CVMV). The virus belongs to the potyvirus group with particles ranging from 750 - 900 nm and produces the characteristic pinwheel inclusions (Abdul Samad, 1986). The

CVMV could be transmitted in a non-persistent manner by several aphid species, namely *A. craccivora (Koch), A. gossypii (Glover), A. spiraceola (Patch), Myzus persicae (Sulz), Toxoptera citricidus (Kirk), Hysteroneura setariae (Thos)* and *Rhopalosiphum maidis (Fitch)* (Ong, 1975). The disease is endemic, affecting *C. annuum* and *C. frutescens* grown in Peninsular Malaysia (Abdul Samad, 1984). CVMV produces a symptomatic mosaic on the leaves and causes stunting of the plants. Yield could be markedly reduced if the plants were infected at an early stage of growth. Other viruses namely tomato spotted wilt virus (TSWV) (Abdul Samad, 1986), cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV)(*Fujisawa et al.*, 1986) had also been reported to infect chilli.

Transmission of Plant Viruses by Aphids

Worldwide, it has been estimated that more than half of the plant viruses are transmitted by aphids (Eastop, 1977), where both the viruses and aphid vectors are found on crops and weeds (Bos, 1981). In Malaysia and other parts of the world where chilli is grown, chilli viruses are frequently transmitted by aphids. *M. persicae* and *A. gossypii* are the two aphid species which have been reported to be the vectors frequently transmitting non-persistent viruses (Eastop, 1977).

The process of virus transmission covers uptake, carry over and inoculation of the virus (Storey, 1939; Bradley, 1964). Success of transmission depends on several factors (Eastop, 1977) which directly or indirectly influence the process of transmission. Amongst these, the abiotic factors (eg. environmental condition) may affect vector's behaviour which in turn may affect transmission efficiency (Pirone and Harris, 1977;

Harris, 1983). For example, abiotic factors such as light intensity, exposure time and temperature had been found to influence aphid's behaviour (Cook and Sylvester, 1964; Sylvester and Richardson, 1966;Wyatt and Brown, 1977; Kemp and Troup, 1978). On the other hand, the biotic factors such as vector distribution and vector-plant interaction played an important role in virus transmission (Gill, 1970; Zitter, 1977; Zitter and Simons, 1980; Raccah, 1986; Markham *et al.*, 1987), while virus titre affected virus transmission. Watson and Plumb (1972) and Altieri (1981) reported that very low virus titre would decrease uptake and carry over resulting in an unsuccessful transmission of the virus.

Spread of Non-Persistent Viruses by Aphids

Three conditions, namely the presence of a vector, a sufficient level of inoculum and mobility of vector must be fulfilled before a virus could be spread (Broadbent, 1956; Irwin and Ruesinks, 1986). The interrelationship of these three conditions will then determine the level of the spread (Broadbent, 1956).

The source of inoculum is of prime importance in virus spread. For instance, the spread may be faster if the source of inoculum is near the target crops as compared to a more distant source of inoculum (Garrett and Mclean, 1983). The time of onset of the initial inoculum is also important in spreading the virus (Thresh, 1986). The vector has a greater chance of acquiring the virus if the onset of initial inoculum occurs in the early part of the crop growth period. The age of the infected plants in the entire crop may also determine the severity and spread of the disease; younger plants not only serve as a source of infection but they are more

susceptible and may become stunted while infection on older plants may cause less damage to the crop (Conroy, 1965; Sievert, 1971; Eastop, 1977; Rose, 1978; Ong *et al.*, 1980; Francki, 1984).

The presence of vectors, especially migrant alate vectors coming from outside a crop provides the initial spread of the virus, whereas the nymphs and apterous vectors are later responsible for the secondary spread within the crop (Taylor, 1986). Muniyappa (1983) and Sigvald (1986) showed that the time of migrant aphid entering the crop correlated stronger with virus spread than with the aphid numbers.

Host Range of Viruses and Vectors

Infected weeds and other wild plants which thrive near the crops are important sources of inoculum during the primary spread of the virus (Thresh, 1978). Crop plants other than chilli also become the host of the vectors. These plants are important for survival, spread, establishment of the virus as well as being the refuge for the vectors (Bos, 1981). Therefore, it is important to know the host range of vectors and the virus for the development of effective control measures.

Plants of the families such as Solanaceae, Compositae, Chenopodiaceae and Convolvulaceae have been reported to be hosts of chilli viruses in Malaysia and elsewhere. Natural infection on weeds (eg. *Datura ferox, Physalis mendocina*) by CMV, PVY and TMV had also been reported in Mandoza, Argentina (Feldman and Gracia, 1972). This is anticipated since most aphid pests spend part of the time colonising wild plants or weeds which in some instances became alternative hosts of the virus (Eastop, 1981).

Most aphids have a restricted range of host plants. However, eleven species of aphids have been reported to be polyphagous; namely *Aphis citricola, A. craccivora, A. fabia, A. gossypii, Aulacorthum circumflexum, A. solani, Macrrosiphum euphorbia, Myzus persicae* and *Toxoptera aurantii* (Eastop, 1981). Amongst these *M. persicae, A. gossypii* and *A. craccivora* have been reported to be the main vectors of CVMV in Malaysia (Ong, 1975).

CHAPTER 3
GENERAL MATERIALS AND METHODS

Virus Isolate

An isolate of CVMV was collected from infected *C. annuum* in the field at Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia. The virus was propagated in chilli plants by mechanical inoculation at two-leaf stage and maintained in an insect-proof glasshouse. The leaves that developed mosaic symptoms were used as virus sources.

Aphid Culture

Colonies of *A. gossypii* were started from a single apterous adult aphid collected in the field. The aphids were continuously reared on *C. annuum* cv. MC4 in an insect-proof cage made of nylon mesh (1444 mesh/cm^2) in a glasshouse. The colonies were transferred fortnightly to fresh host plants to prevent accidental infection by viruliferous aphids from outside and to ensure healthy condition of the aphids. Alate aphids used in certain experiments were obtained from overcrowded colonies. To ensure that the aphid colonies were virus free, transmission tests were carried out from time to time.

All six stages of *A. gossypii* were used in all the experiments, namely 1st, 2nd, 3rd and 4th nymphal instars and apterous and alate adult forms, unless otherwise stated.