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VIRUSES INFECTING CUCURBITS IN QUEENSLAND

By R. S. GREBER, M.Sc.

SUMMARY

Three viruses infecting cucurbits were investigated. The most common was watermelon mosaic virus (WMV), which resembled WMV type 2. Complete infection of pumpkin (*Cucurbita maxima*) and squash (*C. pepo*) crops by this virus often occurred. Transmission by four common aphid species was demonstrated and the host range for systemic infection was found to extend into four plant families. The long, flexuous rod particles measured approximately 760 mμ.

Cucumber mosaic virus (CMV) was comparatively rare, but the isolates obtained resembled QCMV in host reactions and serological tests.

Squash mosaic virus (SMV) was present in about 6% of the specimens examined. An antiserum prepared from a Queensland isolate reacted similarly in gel-diffusion tests to one prepared at Davis, California. Most symptoms produced by SMV were rather mild. Persistent type transmission by an unreported vector, the 28-spotted ladybird (*Henosepilachna vigintioctopunctata* (F.), was demonstrated, and the particle size shown to be approximately 30 mμ in diameter.

I. INTRODUCTION

The scope of this work covers investigations into three viruses infecting cucurbits in Queensland. These are watermelon mosaic virus (WMV), cucumber mosaic virus (CMV), and squash mosaic virus (SMV). The infection pattern in Queensland is characterized by a high incidence of WMV.

It is often difficult to determine with certainty just which viruses of cucurbits were actually being described in the early literature, but with the increase in volume of work published during the period 1940-1955 the confusion in nomenclature becomes increasingly evident. In order to assess the information from these papers it is first necessary to recheck the virus classification from the data presented.

Because of their ability to infect *Nicotiana* species systemically, CMV and tobacco ringspot virus (TRSV) have not proved particularly difficult to separate from the other cucurbit viruses. They were adequately described and recognized at an early date (Price 1934; Ainsworth 1935; Wingard 1928).

The first Queensland record of CMV on squash (*Cucurbita pepo* L.) by Mandelson in 1932 (Simmonds 1966) probably refers to WMV. Although CMV was isolated from both marrow (*C. pepo*) and cucumber (*Cucumis sativus* L.) during the present investigations, it represented less than 1% of isolates tested. The only pumpkin (*Cucurbita maxima* Duchesne) variety grown extensively in Queensland is immune to systemic CMV infection and glasshouse tests show that our common watermelon (*Citrullus vulgaris* Schrad.) varieties are resistant to this virus.

Some strains of CMV have proved difficult to purify (Hollings, Stone, and Brunt 1964). Typical purification methods were described by Tomlinson, Shepherd, and Walker 1959, and Scott 1963). Difficulties have also been experienced with electron microscopy of the virus particles (Francki *et al.* 1966; Murant 1965).

Two viruses previously included under watermelon mosaic virus were shown to be distinct by Webb and Scott (1965) and their work clarified a number of anomalies which had led to difficulty in interpretation of results with Queensland isolates of this virus group. The work of Kamuro (1962) in Japan evidently refers to a virus similar to Webb and Scott's WMV type 2. The melon mosaic described in New Britain by van Velsen (1960) would appear to resemble Queensland isolates, as well as the virus described by Nitzany and Friedman (1963), in the production of lesions on *Chenopodium amaranticolor* Coste & Reyn. (Figure 2). However, the virus described by Toba (1962; 1963) from Hawaii did not infect any tested plant outside the family Cucurbitaceae and thus resembles WMV type 1. Use of the differential reaction of the muskmelon

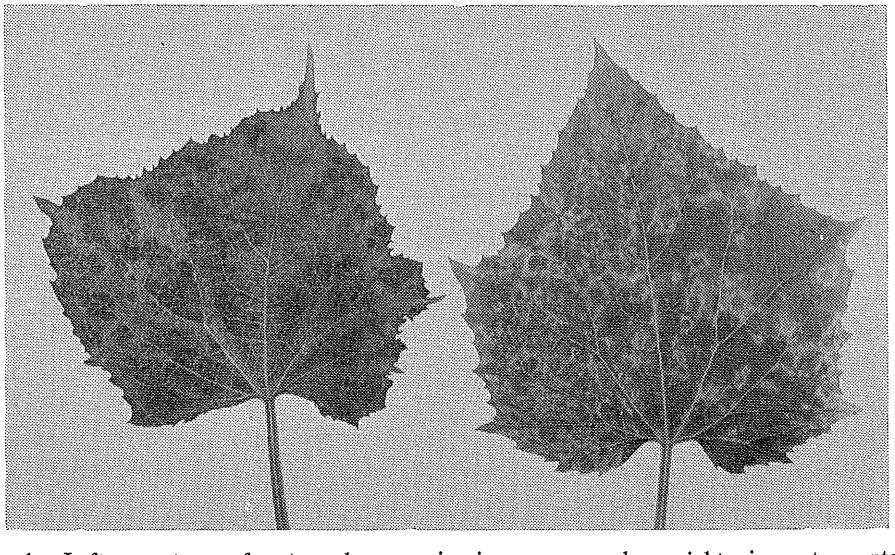

Fig. 1.—Left, symptoms of watermelon mosaic virus on cucumber; right, ringspot symptoms occasionally resulting from cucumber mosaic virus infection of cucumber.

633-3 (Webb and Scott 1965) is a convenient preliminary test for WMV type 1, while demonstration of a wider host range would exclude this type (Schmelzer and Miličić 1966).

Squash mosaic virus is distinguished by beetle transmission, higher thermal inactivation point and characteristic particle type. Many workers have used lack of systemic infection of watermelon to separate SMV from other cucurbit viruses. Although Queensland isolates and most others described elsewhere comply with this, a strain has now been described by Nelson, Matejka, and McDonald (1965) which will infect watermelon systemically. Serological methods appear to provide the most convenient and useful criteria for testing SMV isolates. However, because of the variability evident in described cucurbit viruses it would be unwise to rely on any one identification test.

The identification of SMV in Queensland is based on serological reaction with an antiserum prepared from a Californian isolate by Dr. R. Grogan and kindly provided by Mr. R. Taylor (Burnley, Victoria). The evidence of physical properties, beetle transmission and particle morphology presented here confirm the diagnosis.

II. MATERIALS AND METHODS

(i) *Glasshouse experiments.*—Plants were grown in pots of a size suitable for each species with a minimum of eight plants used in each test. An artificial peat-sand potting mixture was used, with additional nutrient solution added weekly if necessary.

The cucumber variety "Long Green" was used in most tests as an assay plant and sap source as well as a host for maintenance of virus isolates. White Scallop squash (*Cucurbita pepo*) was used as a sap source for purification of WMV.

Powdery mildew occasionally became a problem and was controlled by dinocap sprays when necessary. The short persistence aphicide, mevinphos, was used while vector work was in progress and a more persistent organic phosphorus insecticide at other times.

The earlier work was conducted in an insect-screened, naturally ventilated glasshouse where summer temperatures were sometimes high. Work from 1964 to 1966 was done in an evaporatively cooled and insect-proofed glasshouse. In this environment symptom expression was improved and host range work facilitated.

To obtain the WMV infectivity curve shown in Figure 2, half-expanded leaves of mechanically inoculated cucumber plants were assayed at 3-day intervals to four half-leaves of *Chenopodium*. The arrangement permitted the overlapping of two corresponding halves from the previous and subsequent tests.

(ii) *Laboratory methods.*—The sap source for physical property tests was cucumber leaves harvested approximately 10-12 days after inoculation. Leaf tissue was ground with a mortar and pestle and squeezed through terylene

cloth. Inoculations were performed by finger after carborundum powder was dusted on the cotyledons or primary leaves. Excess inoculum was washed off with tap water.

Thermal end points were conducted using 1 ml thin-walled glass ampoules suspended in a thermostatically controlled water-bath with forced circulation. The heated preparations were cooled quickly and inoculated at once.

In other mechanical inoculations, leaves were ground with an approximately equal weight of buffer. A pH 7, 0·1M phosphate buffer with 0·1% sodium sulphite was used in most inoculations, but in some work a pH 7·2 phosphate buffer to which 0·1% thioglycollic acid had been added was sometimes substituted. Plants tested in host range work were assayed back to cucumber seedlings after a period which was predetermined as the most suitable for each virus.

Purification work was accomplished using a "Servall" SSI centifuge for low-speed work and a "Spinco" model 'L' for high-speed runs. The purification source varied with the virus but in general tobacco seedlings were used for CMV, squash seedlings for WMV and cucumber cotyledons for SMV. Some variations from this are discussed later.

(iii) *CMV purification method.*—A preliminary experiment was carried out by mechanically inoculating Turkish tobacco and subsequently assaying the virus content on Blackeye cowpea primary leaves. Under similar conditions to the test inoculations, wooden trays of tobacco seedlings were then inoculated and leaves harvested on the day when the highest virus infectivity was expected. Some midrib tissue was removed and the remainder of the leaves chilled and then ground, using a cold mortar and pestle. About 2/3 w/v of 0·1M, pH 7·9 phosphate buffer with 0·1% thioglycollic acid was added. The juice was then squeezed through terylene cloth, the fibre re-extracted with buffer, and the second squeezing added to the first.

The extract was then chilled and 1/5 vol. cold chloroform added. This was emulsified by shaking for 20–30 sec in a measuring cylinder. The emulsion was broken by centrifuging at 5,000 r.p.m. for 8 min and the clarified extract decanted and again chilled. Two cycles of differential centrifugation followed—the first high-speed at 30,000 r.p.m. for 90 min, a low speed for 10 min at 10,000 r.p.m. after resuspension in 0·02M, pH 8·2 phosphate buffer, and a further cycle of 39,000 r.p.m. for 60 min and 5,000 r.p.m. for 10 min. The final pellet was also resuspended in 0·02M, pH 8·2 phosphate buffer.

(iv) *WMV purification method.*—Several preliminary experiments were carried out on methods of clarification of WMV. Although van Regenmortel (1961) found that very little infectivity was lost using chloroform solvent with South African isolates, considerable losses appeared to occur with Queensland isolates when tests were made and assayed on *Chenopodium amaranticolor.* However, no satisfactory alternative presented itself and a modification of van Regenmortel's (1961) chloroform and acid precipitation method was used.

Squash leaves harvested at a time of high virus concentration were ground with weak borate buffer, ·005M, pH 8·5. The extract was squeezed through terylene cloth and then clarified by chloroform as described for CMV. The clarified extract was precipitated by dropwise addition of 2% v/w acetic acid, using a magnetic stirrer and pH meter to check when a pH of 4·9–5·0 had been reached. The precipitate was collected by centrifuging for 10 min at 8,000 r.p.m. The pellet was rinsed lightly with water and then resuspended in borate buffer pH 8·5 until a final pH of 7·0 was reached. At this pH the pellet was vigorously dispersed and allowed to stand in a refrigerator at 0–4°C for 2 hr. The suspension was then subjected to another low-speed centrifuging and the supernatant spun in a Spinco 30 rotor at 29,000 r.p.m. for 90 min. The high-speed pellet was resuspended in ·005M, pH 8·7 borate buffer before the final low-speed run to remove insolubles.

(v) *SMV purification method.*—Squash mosaic virus was purified using the method described by Steere (1956) for TRSV, with minor modifications. The virus source was cucumber cotyledons harvested 5–10 days after inoculation and ground with a pestle and mortar. The tissue was also re-extracted after the first squeezing through terylene cloth. The rest of the procedure followed that described by Steere except that most of the final pellet was resuspended in borate buffer, ·005M and pH 8·7. This was found to maintain infectivity better during storage.

(vi) *Electron microscopy.*—Electron microscope grids were viewed in a Siemens Elmiskop IA. Grids were coated with cellulose nitrate and backed with carbon. Most were either sprayed or covered with a "blotted drop" of virus suspension in 1% neutralized phosphotungstic acid. The "Tauchmethode" (Brandes 1964) was used to prepare grids from cut-leaf exudates.

(vii) *Serology.*—Cucumber isolates were checked serologically in gel-diffusion tests (Ball 1961; Crowle 1961), using various arrangements of 5 mm and 7 mm diam wells cut in 0·75% agar by cork borers. The gel was prepared from Ionagar No. 2 with 0·85% sodium chloride and ·02% sodium azide added. Tests were done using 12 ml of agar poured into a 9 cm diam plastic petri dish. Two different CMV antisera were obtained. One was prepared from a Californian isolate (Grogan and Kimble 1962) and the other from a capsicum isolate (Francki 1964), originally forwarded from Queensland. Both crude infective sap and partially purified preparations were used as antigens in the diffusion tests.

Partially purified preparations of WMV resuspended in ·005M borate buffer were injected at the rate of 1 ml of suspension and 1 ml of Freund's complete adjuvant into the leg muscle of a rabbit. A second injection was given 1 week later and a test bleeding made after a further 2 weeks. Using this antiserum, microprecipitin tests were performed using drops on a plastic petri dish covered with paraffin oil as described by van Slogteren (1954*a*) and gel-diffusion tests in 0·75% agar were used to check host antigens (cf.

van Regenmortel 1961). Virus preparations of crude infected sap as well as clarified sap and resuspended acid precipitates were tested in 4 mm tubes by the ring precipitin test against an antiserum prepared by Dr. R. Grogan.

Most serological work was involved in tests and diagnosis of SMV. This virus is particularly suitable for use in serological work since the particle morphology makes the gel-diffusion test suitable (van Slogteren 1954*b*) and the virus is easily purified for the preparation of antisera.

Partially purified preparations of SMV were injected in a similar manner to that described for WMV except that three injections at weekly intervals were used. The rabbit was bled 2 weeks after the last injection. The first suspension injected was in phosphate buffer and the last two in ·005M borate buffer.

Antiserum was frozen at –20°C or stored in 1:1 glycerol at the same temperature. It was diluted in 0·85% saline for use in gel diffusion tests. Wells of equal size in parallel lines were used for comparison of two antisera and a central 7 mm antiserum well with six peripheral 5 mm diam antigen wells used for general diagnostic purposes. Methods of interpreting the reactions of heterologous antisera were described by Grogan, Taylor, and Kimble (1964). Test antigens varied from purified virus to crude infective sap. Even the latter was found to give consistently good reactions and was used extensively in diagnosis.

(viii) *Vector transmission.*—Aphid transmission experiments were conducted with virus-free colonies reared in cages. Representative individuals were checked for species identification by Mr. T. Passlow, Senior Entomologist, Department of Primary Industries, Toowoomba. The aphids were also checked before each experiment according to published details of each species (Stroyan 1952, 1961; Cottier 1953).

Myzus persicae (Sulz.) were reared on swede turnip; *Macrosiphum euphorbiae* (Thomas) and *Aphis gossypii* Glov. were bred on squash and *Malva parviflora* L. Aphids for experiments were removed by aspiration and starved in ventilated tubes for 30–60 min before being fed in groups of five or six on the source plant. Those insects which settled to feeding within a reasonable time were allowed one probe of 15–45 sec. This was terminated artificially, if necessary, before the aphids were transferred to test plants. Those aphids which did not feed readily or probed irregularly were rejected. Either five or six aphids per plant were used for the infection feed.

Beetles for transmission experiments were collected from the field in an area where tests did not show any SMV incidence. They were then caged on healthy squash plants and shifted daily to new plants. None of these were infected and the insects were presumed to be free of virus. The beetles were given a 2-day acquisition feed on diseased squash plants with subsequent daily shifts for infection tests. Precautions were taken during these tests to ensure an approximately equal amount of feeding on each plant. Gel-diffusion serological tests were employed to check plants showing positive symptoms.

(ix) *Field observations and collections.*—Most collections were from south-eastern Queensland but specimens were forwarded for testing from other parts of the State as far north as Townsville. The pumpkin variety Queensland Blue constitutes the largest proportion of the area devoted to cucurbits but considerable areas of watermelon, cucumber and rockmelon are also grown.

Assessment of incidence and rates of infection were mainly carried out in the Lockyer Valley and Deception Bay areas of south-eastern Queensland. Field isolates were obtained from seven species in Cucurbitaceae and one species each in Malvaceae and Leguminosae.

A block approximately 1 ac in area was selected at the centre of one side of a total area of 12 ac of pumpkins at Deception Bay for detailed mapping of infection and aphid counts. These counts were made at intervals of 4 weeks by detaching three leaves of varying ages from each of 10 plants on diagonal traverses through the block. These leaves were placed in polythene bags and aphids later transferred to vials of alcohol for sorting and counting with the aid of a binocular microscope.

III. EXPERIMENTAL RESULTS

(a) Preliminary Separation of Isolates

Specimens collected from all the main cucurbit-growing areas of Queensland were transferred by mechanical inoculation to cucumber, *Chenopodium amaranticolor* and *Nicotiana glutinosa* L. Over 300 cucurbit samples were tested in this way. After some experience WMV could be diagnosed by the characteristic and slower reaction on cucumber. SMV and CMV produced a systemic reaction in cucumber within 3–5 days and showed vein chlorosis rather than the vein clearing and green mosaic caused by WMV. However, most specimens with SMV were also infected by WMV and further work was necessary on any isolate which showed the quick cucumber reaction or which reacted positively with SMV antiserum.

Isolates which contained WMV alone produced marked vein clearing 8–12 days after inoculation to cucumber. This was followed by a rugose light and dark green mosaic (Figure 1), often with some leaf distortion and raised dark green blisters. Chlorotic spots which expanded to necrotic red-margined lesions appeared on inoculated leaves of *C. amaranticolor* (Figure 2).

Isolates which contained SMV alone produced vein chlorosis 3–5 days after inoculation to cucumber followed by a systemic chlorotic mottle which tended to fade after a few weeks. A similar loss of symptom intensity in cucumber was described by Frietag (1956). When specimens were infected by both SMV and WMV the symptoms did not fade and gradually assumed the green blistered mosaic appearance typical of WMV. In this case the red lesions were also produced on *C. amaranticolor.*

Cucumber mosaic isolates caused a systemic mottle on *N. glutinosa* and a somewhat similar early reaction to SMV on cucumber. In this case, however, the mottle on cucumber persisted in a severe form and considerable stunting resulted.

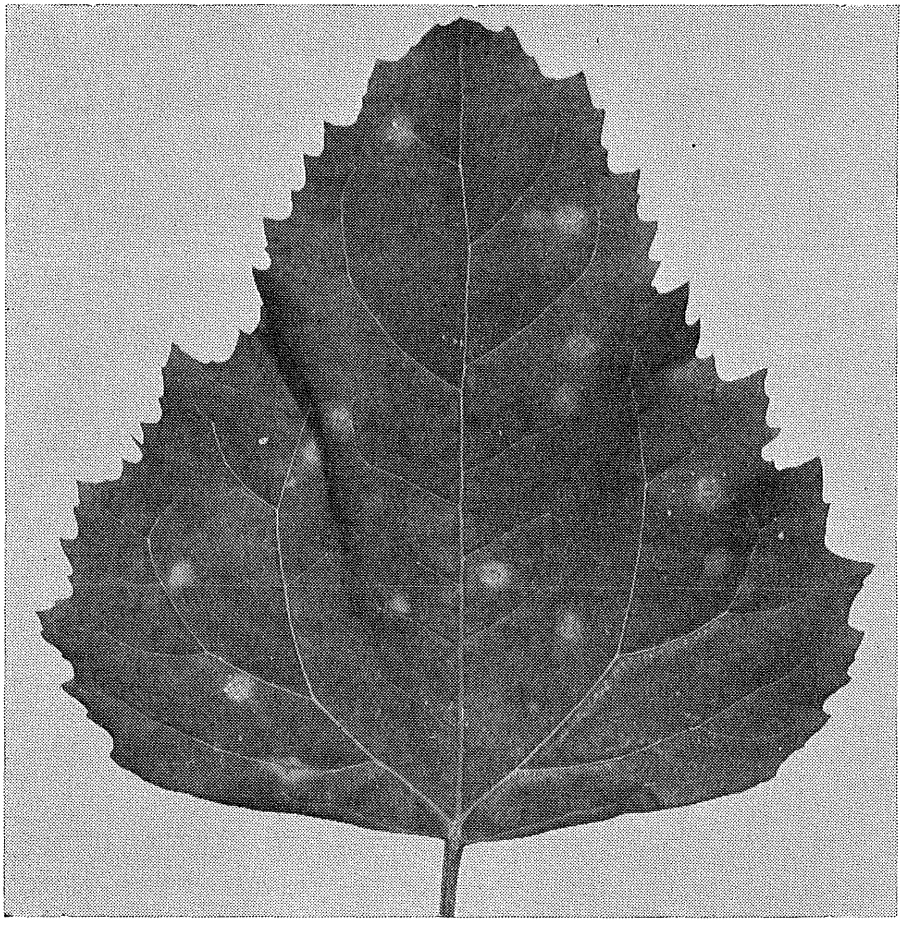

Fig. 2.—Typical watermelon mosaic lesions on *Chenopodium amaranticolor.*

(b) Watermelon Mosaic Virus

Except that none of the three CMV isolates was associated with WMV, all mosaic infected specimens examined were found to contain this virus.

(i) *Host range.*—All isolates produced local lesions on *C. amaranticolor.* This is characteristic of Webb and Scott's WMV type 2 and also of most melon mosaic isolates described in other countries (van Velsen 1960; Cohen

and Nitzany 1963; Schmelzer and Miličić 1966; van Regenmortel, Brandes, and Bercks 1962). WMV type 1 evidently does not give any reaction on this species (Webb and Scott 1965).

Sixty isolates were checked on the differential muskmelon 633–3 and all produced a systemic mosaic only. This fact, in combination with the *Chenopodium* reaction, would tend to indicate that WMV type 1 is not present in Queensland.

TABLE 1

RE-ISOLATION OF SEVEN WMV ISOLATES FROM A RANGE OF NON-CUCURBIT HOSTS

Host Species	Isolate Number						
	1	2	3	4	5	6	7
Chenopodium quinoa Willd.	+	+	+	+	+	−	−
Cyamopsis tetragonoloba (L.) Taub.	+L	+L	−	−	+L	−	−
Gomphrena globosa L.	+L	+L	+L	+L	−	+L	−
Gossypium hirsutum L.	+	+	−	+	−	−	−
Lathyrus odoratus L.	+	+	+	+	+	+	+
Malva parviflora L.	+	+	−	−	+	−	−
Medicago polymorpha L.	+	+	−	−	+	−	−
Nicotiana clevelandii Gray	+L	+L	+L	+L	+L	+L	+L
Phaseolus lathyroides L.	+	+	+	+	+	+	+
Phaseolus vulgaris L.	+L	+L	+L	+L	+L	+L	+L
Pisum sativum L.	+	+	+	+	+	+	+
Trifolium incarnatum L.	−	−	+	+	−	+	−

\+ = Virus re-isolated to cucumber from systemic infection.

+L = Virus re-isolated to cucumber from inoculated leaves only.

− = Not attempted.

Source of isolates:—1, pumpkin, Deception Bay; 2, cucumber, Home Hill; 3, marrow, Brisbane Market; 4, cucumber, Redland Bay; 5, pumpkin, Gatton; 6, pumpkin, Townsville; 7, watermelon, Bowen.

A more extended host range test was performed on seven isolates. Results are set out in Table 1; they conform to the general pattern found by other workers except that no variation was found between isolates (Kamuro 1962; Grogan, Hall, and Kimble 1959; van Regenmortel, Brandes, and Bercks 1962). A number of new hosts were established (Table 2). Some are of diagnostic value only, as the infection is confined to inoculated leaves, and others appear to be characteristic of the Queensland isolates. Phasey bean (*Phaseolus lathyroides* L.) appears to be a new systemic host which could have some epidemiological significance.

The local lesions on *Dolichos uniflorus* Lam. are conspicuous and useful for assay work, although *Chenopodium* spp. are possibly more suitable for this purpose. The reaction on pea (*Pisum sativum* L.), sweet pea (*Lathyrus odoratus*

L.) and burr medic (*Medicago polymorpha* L. var. *vulgaris* (Benth.) Shin.) was a mild to moderate mottle which tended to fade on old growth or under conditions of slower growth.

In hosts belonging to the family Malvaceae the first systemically infected leaf usually showed vein chlorosis. This was particularly marked in okra (*Hibiscus esculentus* L.) and somewhat variable in cotton (*Gossypium hirsutum* L.). Virus could usually only be recovered from the inoculated leaves and the first one or two systemic leaves. This appeared to be due to a drop in virus concentration more than to inhibitors in the typically gummy sap, because results from aphid transfers paralleled those of mechanical inoculations back to cucumber.

TABLE 2

NON-CUCURBIT HOSTS OF WATERMELON MOSIAC VIRUS (MECHANICAL INOCULATION)

Host	Re-isolation from Inoculated Leaves	Re-isolation from Systemic Leaves
Leguminosae—		
Calopogonium mucunoides Desv.	+	0
Centrosema pubescens Benth.	+	0
Cyamopsis tetragonoloba (L.) Taub.	++	0
§*Dolichos uniflorus* Lam.	++	0
Lathyrus odoratus L.	++	++
Lupinus angustifolius L.	+	+
**Medicago polymorpha* L.	++	++
Melilotus alba Desr.	++	+
Phaseolus lathyroides L.	++	++
†*Phaseolus vulgaris* L.	++	0
‡*Pisum sativum* L.	++	++
Pueraria phaseoloides (Roxb.) Benth.	+	0
Malvaceae—		
Gossypium hirsutum L.	+	+
Hibiscus esculentus L.	+	+
**Malva parviflora* L.	++	+
Chenopodiaceae—		
Chenopodium album L.	++	0
Chenopodium amaranticolor Coste & Reyn.	+	0
Chenopodium quinoa Willd.	++	+
Amaranthaceae—		
Gomphrena globosa L.	++	0
Solanaceae—		
Nicotiana clevelandii Gray	++	0

++ = Good recovery of virus by re-inoculation to cucumber.
+ = Fair or occasional recovery of virus.
0 = Virus not re-isolated.
* = Natural field hosts.
† = Variety Bountiful.
‡ = Variety Greenfeast.
§ = Often referred to as *D. biflorus* L.

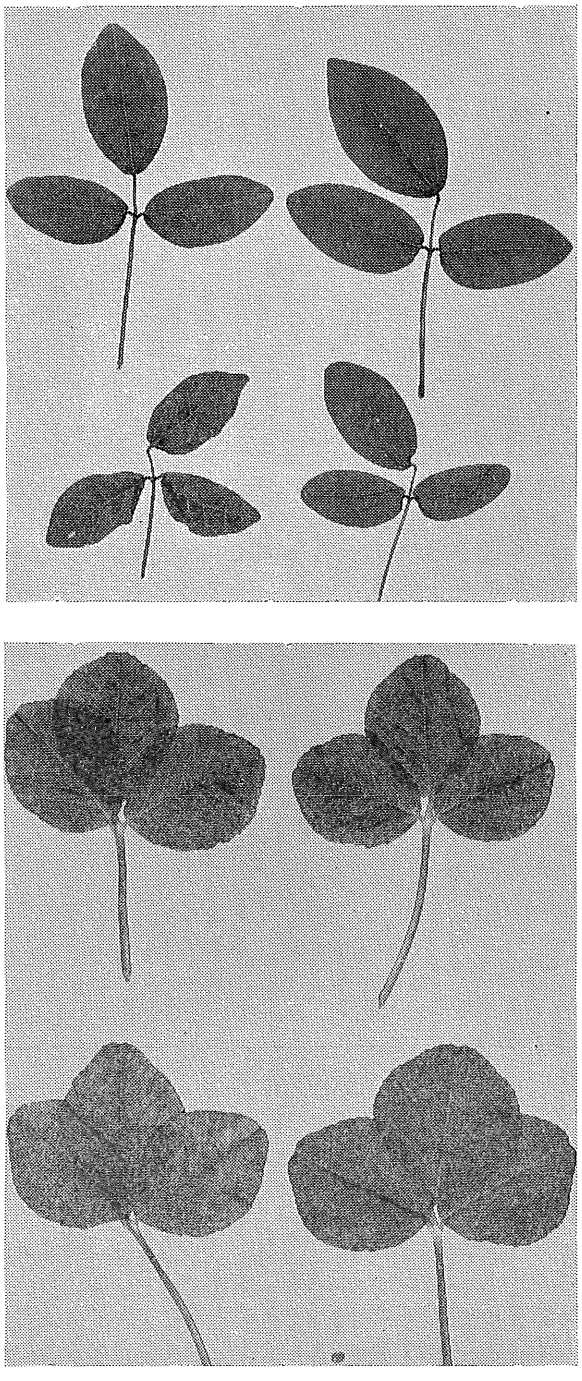

Fig. 3.—Top series, systemic mottle symptoms of watermelon mosaic virus on *Phaseolus lathyroides* compared with healthy leaves; bottom series, systemic mottle on new growth of *Medicago polymorpha* compared with a healthy leaf.

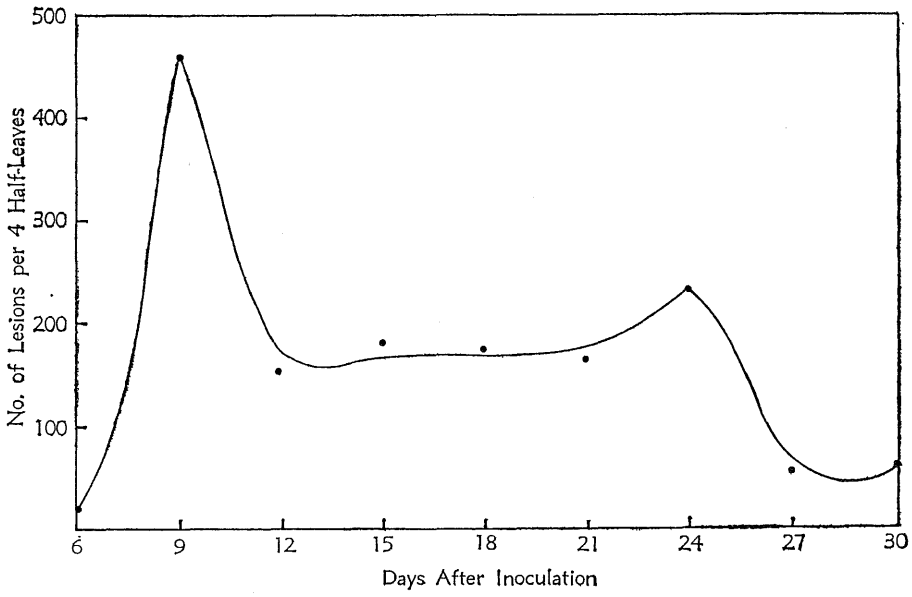

Fig. 4.—Watermelon mosaic virus infectivity from new growth of cucumber, indexed on *Chenopodium amaranticolor.*

The systemically infected legumes (Figure 3) appeared to be a more consistent virus source, but some considerable variation in concentration with time was evident in new growth even in cucumber (Figure 4). However, results of other assays on *Chenopodium amaranticolor* indicate that the concentration in the first leaves formed after inoculation remains high for a considerable time.

(ii) *Aphid transmission.*—Preliminary tests indicated that this virus was efficiently aphid transmitted by short feeding times and in a non-persistent manner. In experiments under standardized conditions, using freshly infected cucumber as a source and feeding to cucumber cotyledons, the transmission rates were as follows:—*Aphis gossypii,* 4 of 6 plants infected; *Macrosiphum euphorbiae,* 8 of 12; *Aphis craccivora* Koch, 9 of 12; and *Myzus persicae,* 10 of 12.

(iii) *Epidemiology.*—Assessment of vector populations on cucurbits in south-east Queensland indicate that in general only a few *M. persicae* can be found, mostly during late summer. The population of *A. gossypii* is highest in autumn and this aphid is probably the chief vector in areas where a summer-autumn crop is grown. *M. euphorbiae* is most numerous in spring, when high populations may be present. At this time, and with a type of crop in which neighbouring vines intertwine, it is considered that wingless forms could be significant vectors, particularly as *M. euphorbiae* has a prodigious walking ability. Since the figures indicate a high ratio of wingless to winged aphids (Table 3) this assumption would appear to be reasonably based. Also, the higher numbers of new infections correspond to an increase in wingless populations rather than the

TABLE 3

APHID POPULATION OF *Cucurbita maxima* DUCHESNE PER 30 LEAVES, DECEPTION BAY, 1963

Month	*Macrosiphum euphorbiae*		*Aphis gossypii*	
	Alates	Wingless	Alates	Wingless
July	1	56	37	65
August ..	2	88	44	23
September ..	7	215	2	1
October ..	30	2,290	0	0

relatively static number of alates present (Figure 5), but allowance must be made for the increasing inoculum source. The uncultivated land in the area where the counts were made is mainly eucalypt forest and pumpkins constituted almost the only crop grown at this time of the year. Because of this and the method of sampling used, itinerant aphid species such as *M. persicae* (Dickson *et al.* 1949) were rarely found and have been neglected. No cucurbit virus other than WMV was isolated in this area (Table 4).

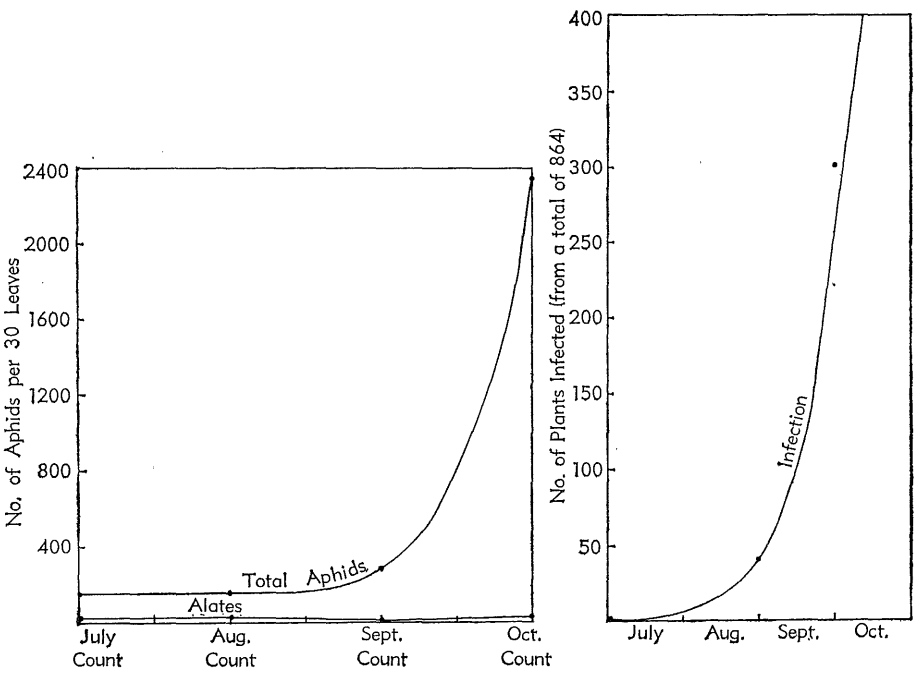

Fig. 5.—Aphid population of *Cucurbita maxima,* Deception Bay, 1963.

TABLE 4

SQUASH MOSAIC VIRUS INCIDENCE IN VARIOUS DISTRICTS OF QUEENSLAND IN RELATION TO TOTAL CUCURBIT MOSIAC INCIDENCE

District	No. of Collections Tested	No. Positive for SMV*
Upper Burnett	20	0
Lockyer Valley	40	9
Deception Bay	44	0
Rochedale	20	2
Bowen	40	0

* Based in the first instance on serological tests of random samples of plants showing mosaic symptoms and subsequently on further tests after mechanical isolation.

A plant-by-plant mapping of infection spread indicated that there was no initial (i.e. seedborne) infection. Some seedlings showed abnormal early growth but indexing of these indicated that the symptoms were evidently physiological in origin. The first virus infection appeared as three widely spaced plants, and although subsequent infections appeared to group around these, the irregular spacing of plants along the rows would make statistical analysis very difficult. Plotting of individual plants became increasingly difficult as the vines spread, but by the time harvesting was completed all plants appeared to be infected.

In the Lockyer Valley, observations of WMV infection showed that in one case an interseason crop of infected cucurbits had been allowed to persist within the main pumpkin crop. The infection percentage at the time plants began to run was five times as high (20%) on this farm and adjacent crops as in the rest of the district generally. It would thus appear to be important to eliminate such sources in areas where only one main cucurbit crop is grown. The relative importance of species of aphid vectors is probably different in the Lockyer Valley to Deception Bay. Large areas of potatoes in the former district often are hosts to high populations of *M. persicae* and alates of this species would be important vectors in these circumstances (cf. Dickson *et al.* 1949).

The virus was recovered from only two naturally infected non-cucurbit species during these investigations. A typical isolate was obtained from *Malva parviflora,* which was also colonized by *A. gossypii.* Several isolates were obtained from burr medic which showed a faint mottling of younger leaves. It is possible that this species could act as an initial source of virus in the Lockyer Valley, but because of the relatively mild climate in Queensland, it is considered that "backyard" plots of cucurbits are probably a more significant interseason source. Burr medic is a good host of *A. craccivora,* which is an efficient vector of WMV. Phasey bean is a good systemic host of WMV but eight suspected field specimens were indexed and yielded only three isolates of common bean mosaic

virus. WMV was isolated from the weed species *Cucumis anguria* L. growing in proximity to commercial cucurbit plantings in the Bowen district of north Queensland.

(iv) *Seed transmission.*—Field observations of considerable areas of young pumpkin seedlings did not show any evidence of seed transmission of WMV. Glasshouse plantings of 200 seeds each from infected marrow, pumpkin and cucumber fruit produced no infected seedlings.

(v) *Physical properties.*—The physical properties of Queensland isolates of WMV are in general accord with those determined elsewhere. The thermal end point of infection is between 55 and 60°C for a 10 min exposure. The dilution end point was found to be close to 10^{-3}, when inoculated back to cucumber cotyledons. The virus was not resistant to ageing and even as a desiccated leaf culture was uninfective when tested after 2 weeks' storage. Similarly prepared CMV cultures were still highly infective after 4 years under similar conditions. Results from a number of other viruses in desiccated leaf culture are given by McKinney, Silbur, and Greeley (1965).

(vi) *Purification.*—Assays of infectivity at each stage showed considerable loss in clarification and also a large proportion of virus remaining insoluble after acid precipitation and attempted resuspension. This appeared to be the critical step and even close control of pH did not seem to enable attainment of consistent results. However, if leaf tissue of high infectivity was used as a source, the amount of virus resuspending was usually adequate to give final material suitable for electron microscopy. Infectivity was not well maintained in storage and the number of lesions produced on *C. amaranticolor* had dropped to 1/10 by the end of a week in an ice-bath in a refrigerator.

(vii) *Serology.*—An attempt to produce an antiserum by two intramuscular injections of a partially purified preparation with adjuvant added was not successful. There was no consistent reaction when the serum was tested in microprecipitin drops. Some reaction to host material was indicated in gel-diffusion but the main line was not excessively dense and compared reasonably well with a similar test on a WMV antiserum produced in California. There was no definite or repeatable reaction of clarified or partially purified Queensland virus extracts with the American antiserum when used in small-tube precipitin and ring precipitin tests with adequate controls.

(viii) *Electron microscopy.*—No particular trouble was experienced with aggregation. In fact, when tobacco mosaic virus suspension was added for measurement comparisons, more aggregation occurred with these particles than with the WMV. This is in contrast to WMV aggregation difficulties experienced by van Regenmortel, Brandes, and Bercks (1962).

Figure 6 shows particles of WMV from a partially purified preparation. In contrast to work by van Regenmortel, Brandes, and Bercks (1962), cut leaf exudate "dip" preparations were relatively unsuccessful with the Queensland virus.

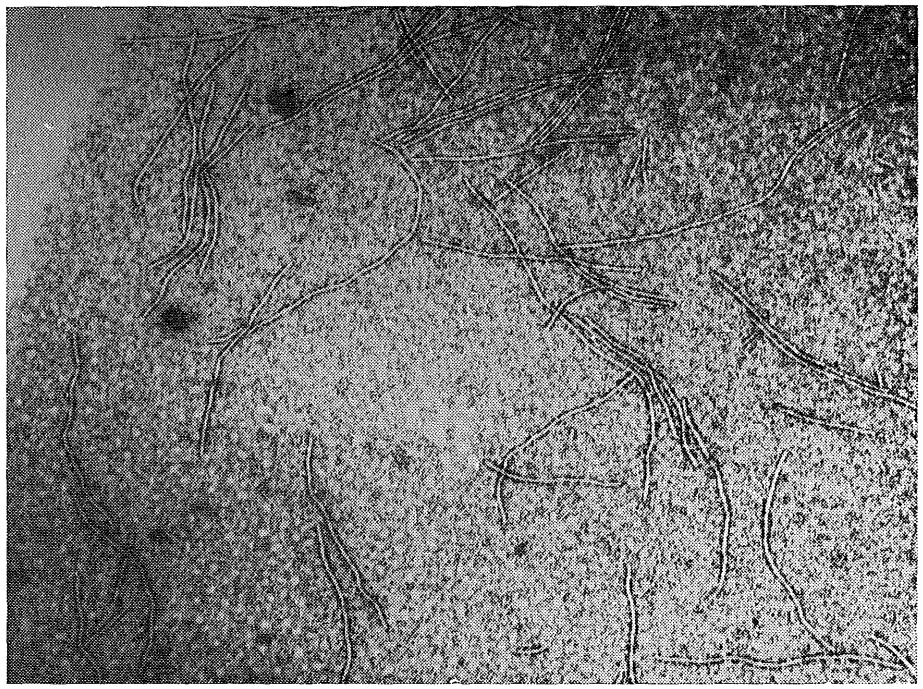

Fig. 6.—Electron micrographs of particles of watermelon mosaic virus from a partially purified preparation negatively stained with potassium phosphotungstate.

Most fields from this type of preparation could show only a single flexuous rod. Particle lengths were relatively consistent. The most frequent length calculated, based on comparison with TMV at 300 mμ, was 760 mμ.

(c) Cucumber Mosaic Virus

(i) *Host range.*—Only three isolates of this virus were recovered despite attempts to concentrate isolations on species likely to carry this virus. Early experiments in this work showed that the commonly grown Queensland Blue pumpkin is immune to systemic infection by CMV. Inoculation to cotyledons or leaves produces local lesions but no systemic spread of the virus follows (Figure 7). This was checked at a range of temperatures from 20 to 32°C.

CMV produces a different type of local lesion on *Chenopodium amaranticolor* to WMV, although the characteristics of both lesions were found to vary with temperature. Those from CMV increased in size at moderate temperatures while WMV lesions increased in size at higher temperatures.

Some host reactions of CMV isolates are shown in Table 5. The cucurbit CMV isolates from Queensland appeared to be similar in host range and physical properties and serologically related to QCMV, which was described by Francki (1964) and Francki *et al.* (1966).

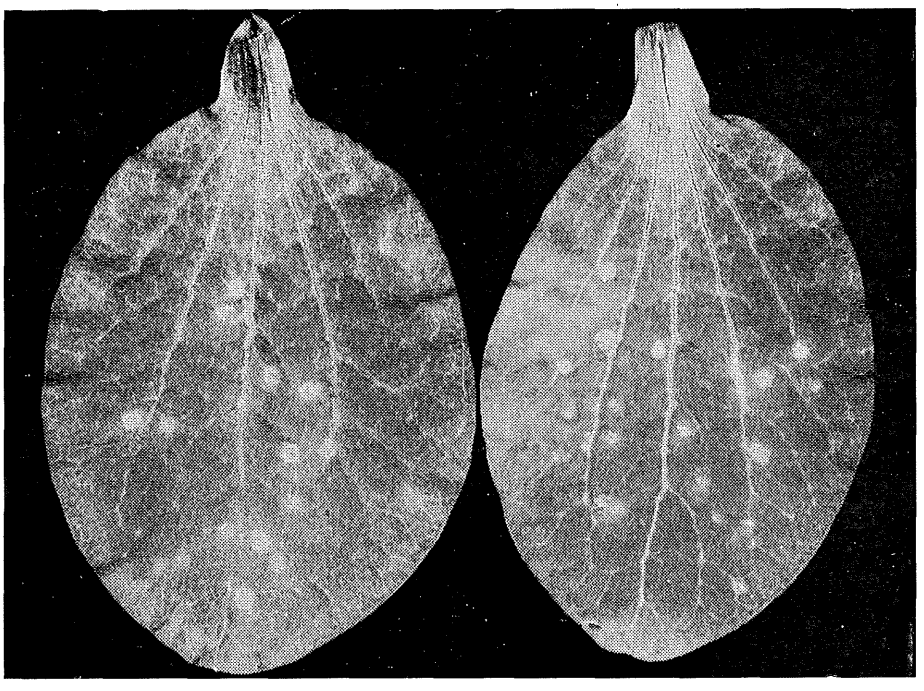

Fig. 7.—Local lesions produced by mechanical inoculation of cucumber mosaic virus on Queensland Blue pumpkin (*Cucurbita maxima*) cotyledons.

(ii) *Epidemiology.*—Aphid transmission by *Macrosiphum euphorbiae* and *Myzus persicae* was demonstrated, but detailed work on transmission was not attempted.

The only CMV isolates obtained were one from cucumber from North Queensland and two from Zucchini marrow in the south-east of the State. The virus commonly infects solanaceous plants in this area (Simmonds 1966). The marrow isolates were obtained close to tomatoes infected by fern-leaf disease from which CMV was also isolated.

(iii) *Purification and electron microscopy.*—Partial purification was accomplished by an uncomplicated chloroform clarification of an extract in phosphate buffer and 0·1% thioglycollic acid. After two cycles of differential centrifugation the product was highly infectious and reacted well in gel-diffusion with antisera to QCMV and a Californian isolate. This purification method probably involves considerable loss of virus (Scott 1963).

Difficulty was experienced in obtaining satisfactory electron micrographs (cf. Francki *et al.* 1966; Murant 1965). Formalin stabilized preparations sprayed on grids with 1% sodium phosphotungstate did not show any clearly discernible particles. Grids sprayed with stabilized virus and shadowed with platinum-palladium showed numerous particles but these did not have an entirely satisfactory uniformity of size.

(d) Squash Mosaic Virus

(i) *Identification of isolates.*—Mixed infections of SMV and WMV are not easily detected by a small differential host range. The more rapid systemic reaction on cucumber can be detected after some experience because the vein chlorosis appears in approximately half the time that is required before vein clearing from WMV infection can be seen. Suspected isolates can then be separated into components by aphid transmission of WMV, and either beetle transmission or ageing employed to obtain the SMV component. However, the presence of SMV is best checked serologically.

Of 175 mosaic-infected cucurbit specimens serologically tested for SMV, 11 were found to be infected. In addition, 3 isolates which had been maintained from earlier collections because of the quick cucumber reaction described above were also found to react positively with SMV antiserum.

TABLE 5

COMPARATIVE REACTIONS OF 20 SPECIES TO THREE CUCURBIT VIRUSES

Species	CMV	WMV	SMV
Citrullus vulgaris Schrad.	LL occasionally systemic	SM	LL
Cucumis anguria L.	LL chlorotic	SM	SM vein extensions
Cucumis melo L.	SM	SM	SM mild
Cucumis sativus L.	SM chlorotic	SM green	SM fading
Cucurbita maxima Duchesne ..	LL	SM	SM
Cucurbita moschata Duchesne ..	SM mild or latent	SM	SM mild
Cucurbita pepo L.	SM chlorotic	SM green	SM vein extentions
Lagenaria siceraria (Mol.) Standl.	LL chlorotic	LL green rings	0
Luffa cylindrica Roem.	SM or lethal	Symptomless, local	Symptomless, local
Mamordica charantia L.	SM	Symptomless	SM
Sechium edule Sw.	0	0	0
Trichosanthes anguina L.	SM variable	SM	Symptomless
Chenopodium amaranticolor Coste & Reyn.	LL	LL	Symptomless, local
Chenopodium quinoa Willd. ..	LL	SM variable	Symptomless, local
Dolichos uniflorus Lam.	—	LL rusty rings	LL faint
Hibiscus esculentus L.	LL chlorotic	Poorly systemic	LL
Lathyrus odoratus L.	LL spreading	SM mild	SM mild
Phaseolus lathyroides L.	LL	SM	—
Phaseolus vulgaris L.	Symptomless, local	LL chlorotic	LL fine necrotic
Pisum sativum L.	Systemic, necrotic	SM mild	SM variable

LL = Local lesions
0 = No infection
SM = Systemic mottle
— = Not attempted

(ii) *Host range and symptoms.*—Some relative host reactions are set out in Table 5. In general, the Queensland isolates conform to Frietag's host range findings. Pea and sweet pea were the only good systemic hosts found outside

Cucurbitaceae. Several other species were locally infected by mechanical inoculation. Virus was recovered from *Phaseolus vulgaris* L., *Chenopodium quinoa* Willd., *Dolichos uniflorus, Carica papaya* L., *Hibiscus esculentus* and *Trifolium incarnatum* L. The symptoms on garden pea were found to be variable, with a proportion of plants developing a slightly necrotic terminal reaction from which they later recovered with new growth. The virus concentration in pea and sweet pea appeared to be quite high by estimation from the number of lesions on cucumber cotyledons. These lesions were mostly indistinct and not particularly suitable for assay purposes. Lesions on *D. biflorus* were less distinct than those produced by WMV. None of the Queensland SMV isolates systemically infected watermelon.

(iii) *Beetle transmission.*—No previously recognized vectors of SMV have been recorded in south-eastern Queensland. The beetle *Epilachna chrysomelina* (F.), which has been shown to be a vector in Israel (Cohen and Nitzany 1963), has been recorded in North Queensland.

The two most frequently encountered leaf-eating beetles on cucurbits in this State are the pumpkin beetle (*Aulacophora hilaris* (Boisd.)) and the 28-spotted ladybird (*Henosepilachna vigintioctopunctata* (F.)). The latter was shown in these experiments to be an efficient vector of SMV. Vector tests were not extensive but a 2-day acquisition feed and daily transfers of a group of 10 beetles to a fresh pot of squash seedlings demonstrated daily transmission up to the seventh day. The experiment was then discontinued. Of the 27 plants used in this test 20 were infected, and this confirmed the 28-spotted ladybird as a new vector of SMV. This beetle is most active during the summer months and is low in numbers during winter and early spring. Aphid transmission of this virus using *M. persicae* was attempted but none of the 12 cucumber plants was infected.

(iv) *Seed transmission.*—Two hundred seeds from infected rockmelon (cv. Rocky Ford) and the same number from infected cucumber (cv. Supermarket) were planted in trays in the glasshouse and the seedlings examined for symptoms. Approximately half of these were also tested serologically in groups of five, in case symptoms were not obvious. One hundred pumpkin (cv. Queensland Blue) seeds were also tested. No infected seedlings were found. Only five viable squash seeds (cv. White Scallop) were produced by an infected plant. One of these produced an infected seedling. Although this indicates that the rate of seed transmission of local isolates of SMV is low in some cucurbits, squash and marrow require further testing.

(v) *Infection sources and distribution.*—Leppik (1964) implicated seed transmission as initial infection sources of SMV in Iowa. However, it would appear from extensive field observations of pumpkins in Queensland that this is not an obvious factor here, although seed transmission in other species could provide a source ready for the summer build-up of vectors.

Incidence of SMV varied considerably between districts. Tests showed 9 of 40 pumpkin isolates positive in the Lockyer area but 0 of 44 positive in the Deception Bay area. No SMV isolates were obtained from 49 virus infected specimens from North Queensland.

(vi) *Physical properties.*—In squash sap the virus survived 65°C but not 70°C and the dilution end point from *C. pepo* sap was 10^{-4}. The virus was stored frozen without great loss of infectivity.

(vii) *Purification.*—SMV was easily purified by Steere's TRSV method, which produced a small opalescent final pellet with very high infectivity. In later work the method used by Hollings (1965) for Anemone necrosis virus was employed and produced a satisfactory preparation. Cucumber cotyledons were found to be a better source than pumpkin leaves, which produced a good deal more host material in the final pellet. It was evident from electron microscope grids made from the same preparation at different times that the virus tends to crystallize in phosphate buffer. This problem appeared to be largely overcome by the use of borate buffer and consequently those preparations which were to be stored were resuspended in ·005M, pH 8·7 borate buffer.

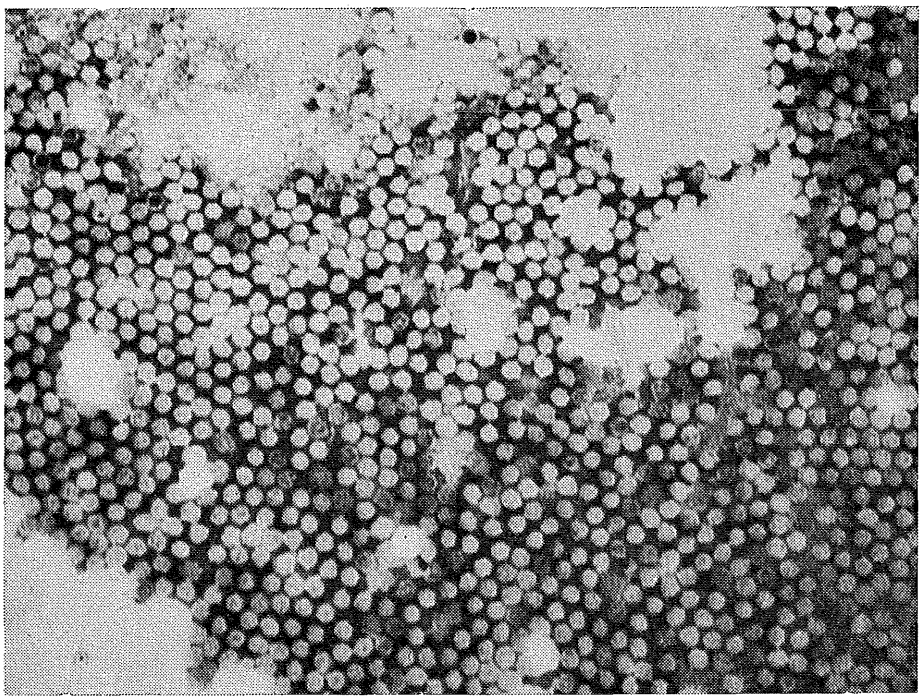

Fig. 8.—Electron micrographs of particles of squash mosaic virus from a purified preparation negatively stained with potassium phosphotungstate.

(viii) *Electron microscopy.*—Electron microscope grids prepared by stabilizing the diluted virus preparation with 1% formaldehyde for a few minutes and then spraying onto the grids with an equal volume of 1% sodium phosphotungstate were examined at 40K and 60K magnification. There was a marked tendency to aggregation in thin regular plates, with particles arranged to give a honeycomb appearance. Where particles were separate, a hexagonal outline was not quite so obvious. "Empty" and "full" particles could be seen together (Figure 8). Smaller particles were described by Nelson and Milbrath (1965), but although some small particles were also present in Queensland preparations, no evidence of their relationship was obtained. Assuming a general value of 300 mμ as the average length of intact TMV particles, a diameter of 30 mμ was calculated for the Queensland SMV particles. This was done by photographing the viruses on consecutive plates at the same instrument setting and comparing the measurements.

(ix) *Serology.*—The antiserum prepared reacted well in gel-diffusion down to a dilution of 1/64. Host lines became indiscernible at $\frac{1}{4}$ to $\frac{1}{8}$ dilution, depending on the spacing of the wells.

A frozen dried and reconstituted antiserum prepared in California was used in conjunction with the local antiserum to check the identity of the virus (Figure 9).

The precipitation line at low dilution tended to be very close to the virus well, but with antiserum dilutions of $\frac{1}{8}$ to 1/64 it moved further away. Closely spaced wells (2 or 3 mm) produced a quicker reaction at higher dilutions than wider patterns, but good reactions could otherwise be obtained with spacings up to 3 cm.

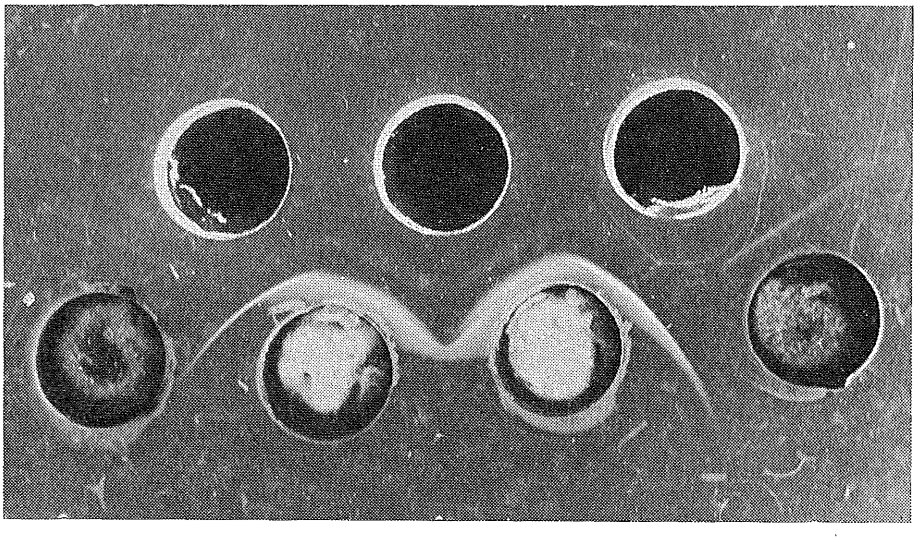

Fig. 9.—Agar gel-diffusion serology plate showing reaction between a Queensland isolate of squash mosaic virus, its homologous antiserum (centre top well) and an antiserum to a Californian isolate (two outer top wells).

Experiments showed that leaf tissue from cucumber plants which had been infected for 3 weeks could be ground with 10 times the weight of uninfected tissue and the composite extract still give a good serological reaction. This fact was used in testing seedlings for seed transmission in groups of five.

IV. DISCUSSION

(i) *Types of virus present.*—Only three viruses were definitely identified on cucurbit crops. Two others were collected—one resembled TRSV and another showed symptoms suggestive of tomato big bud virus infection. It is possible that further viruses will be isolated but except for possible new introductions these should be of minor importance.

An extensive check for a latent cucurbit virus such as described by Webb and Bohn (1961) failed to show that any was present. Typical isolates producing lesions on *Chenopodium amaranticolor* were transferred back from this species to cucumber by aphids or by passing through *C. quinoa* in order to minimize the inhibitory effect of *C. amaranticolor* sap. All produced normal WMV symptoms, indicating that the lesions were indeed caused by this virus. Re-isolations from five other non-cucurbit hosts still produced typical *C. amaranticolor* lesions.

The occurrence of CMV on solanaceous crops in Queensland is not uncommon and it may at first glance be difficult to explain an apparent rarity of this virus on cucurbits. However, the actual infection frequency is similar in both cases. This is obscured by the very high incidence of WMV in cucurbits, in which CMV infection is relatively unimportant and almost impossible to assess by symptoms alone.

(ii) *Host range and symptoms.*—Doolittle (1920) presented considerable experimental work on mosaic diseases of cucurbits but was evidently not able clearly to distinguish the viruses involved. With further work by Price (1934), Ainsworth (1935) and others, the separation of the type of mosaic which produces a systemic mottle in Solanaceae, CMV, became more definite.

No variation in host range between local isolates of WMV was found. However, with the poorer hosts (Table 2) it was sometimes necessary to repeat the test using high infectivity inoculum and re-isolating at an appropriate time after inoculation. The hosts with only localized infection are not well documented in the literature. Lucerne (*Medicago sativa* L.) could not be shown to be a host of our virus.

Mosaic isolates were recovered from all economic crop species of cucurbits grown in Queensland except choko (*Sechium edule* Sw.). This species appeared to be immune to all three viruses, and no natural infections were noted.

Particle length and serological comparisons are not yet conclusive, but there are sufficient host range differences to suspect that Queensland isolates of WMV are not identical with Webb and Scott's type 2, although they resemble it.

No extensive host range work on SMV appears to have been published since Frietag (1956) named five non-cucurbit hosts in three plant families. The demonstration of systemic infection of watermelon by a strain of SMV (Nelson, Matijka, and McDonald 1965) again indicates that a small differential host range is not by itself sufficient to separate these viruses. Symptoms of Queensland isolates of SMV on most cucurbits appear to be milder than those described elsewhere. The small dark-green areas on inoculated squash were not usually so obvious as the raised dark-green blisters caused by WMV. The final reaction of SMV on cucumber was often quite mild and serological checking of transfers to this species was mostly necessary. There was a tendency for this virus to cause vein extensions round the lamina margins of most cucurbits and occasionally enations formed under leaves of infected pumpkin (*C. maxima*), but neither of these was a satisfactory diagnostic symptom.

(iii) *Vectors.*—It would appear from the results of transmission tests conducted for this study that there is no great difference in efficiency of transmission of WMV by any of the four aphid species used, when conditions for proper feeding are assured. There could, however, be considerable differences in field importance of these vectors due to feeding preferences and behaviour patterns.

Aphis gossypii and *Macrosiphum euphorbiae* appear to be well adapted to cucurbit hosts. The former crawls among the hairs while feeding but the latter can stand above most hairs and feed between them. *M. euphorbiae* was usually the most common species on pumpkin (Table 3) and squash but did not appear to favour watermelon.

The pumpkin beetle (*Aulacophora hilaris*) was not tested as an SMV vector. *Epilachna chrysomelina* would not appear to be very common even in North Queensland where the only records of this species were made. The 28-spotted ladybird (*Henosepilachna vigintioctopunctata*) is evidently an efficient persistent vector of SMV, but the relationships could be further investigated.

(iv) *Particle size.*—There does not appear to be any experimental difference which could allow a correlation of the particle lengths of 728 mμ for South African WMV (van Regenmortel, Hahn, and Fowle 1964) and approximately 760 mμ for the Queensland virus. Although errors in electron microscope calibration are common, both these lengths were calculated by direct reference to a similar type of virus particle as a standard of length. Purcifull and Edwardson (1967) have recently reported a length of 760 mμ for WMV type 1.

The SMV isolated here appears to be of the commonly accepted dimensions. The particle appears to be an interesting subject for sedimentary fractionation. Preliminary work showed three separate light-scattering bands after purified virus was centrifuged in sucrose density-gradient columns.

(v) *Serology.*—Webb and Scott (1965) were unable to prepare an antiserum to WMV type 2. Limited efforts in Queensland were also unsuccessful, but further attempts will be made when more experimentation with purification methods is possible.

SMV is a very satisfactory virus for serological work. The agar double-diffusion reaction is an analytical technique ideally suited to this virus. The strength of the reaction makes it a far better proposition for identification of field specimens than CMV antisera, which had a relatively weak reaction.

(vi) *Control and infection sources.*—Some control of SMV might be obtained by ensuring that all commercial seed is free of the virus. Further data on seed transmission will be necessary for this purpose, but districts which have little or no SMV infection are available for seed production (Table 4).

From the results presented here it is concluded that the Queensland isolates of WMV, like their Californian counterparts (Grogan, Hall, and Kimble 1959) are not regularly seed transmitted. Because no place free of aphid vectors and suitable for growing cucurbits was available, it was not possible to set out a field experiment under natural conditions to test the effect of WMV on yield. Control measures for stylet-borne, aphid-transmitted viruses are difficult, but there is some evidence from observations in this State that attention to removal of small infected interseason cucurbit plantings before the main crop is sown could be well worthwhile.

The results of field isolations from non-cucurbit hosts indicate that these are not very important as alternative hosts of WMV. In most cases investigated it appeared more probable that out-of-season cucurbits represented a greater danger than possible carry-over of virus in species of other families. Weed species of cucurbits could be important alternative hosts in the Bowen area, where a reservoir of WMV infection is present in *Cucumis anguria.*

Marrow and squash fruit are more severely distorted when formed soon after WMV infection. However, observations suggest that the most severe effect on pumpkins is the marked stunting of plants that results from early infection. Any control procedure which delays the onset of infection would thus be of considerable value in this case.

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REFERENCES

AINSWORTH, G. C. (1935).—Mosaic diseases of the cucumber. *Ann. Appl. Biol.* 22:55-67.

BALL, E. M. (1961).—Serological tests for the identification of plant viruses. (The American Phytopathological Society, Arendoville).

BRANDES, J. (1964).—Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologisher Grundlage. Mitt. Biol. *BundAnst. Ld-u. Forstw.* 110:1-130.

COHEN, S., and NITZANY, F. E. (1963).—Identity of viruses affecting cucurbits in Israel. *Phytopathology* 53:193-6.

COTTIER, W. (1953).—Aphids of New Zealand. *Bull. N.Z. Dep. Scient. Ind. Res.* No. 106.

CROWLE, A. J. (1961).—"Immunodiffusion." (Academic Press: New York).

DICKSON, R. C., SWIFT, J. E., ANDERSON, L. D., and MIDDLETON, J. T. (1949).—Insect vectors of cantaloupe mosaic in California's desert valleys. *J. Econ. Ent.* 42:770-4.

DOOLITTLE, S. P. (1920).—The mosaic disease of cucurbits. *Bull. U.S. Dep. Agric.* No. 879.

FRANCKI, R. I. B. (1964).—Inhibition of cucumber mosaic virus infectivity by leaf extracts. *Virology* 24:193-9.

FRANCKI, R. I. B., RANDLES, J. W., CHAMBERS, T. C., and WILSON, S. B. (1966).—Some properties of purified cucumber mosaic virus (Q strain). *Virology* 28:729-41.

FRIETAG, J. H. (1956).—Beetle transmission, host range, and properties of squash mosaic virus. *Phytopathology* 46:73-81.

GROGAN, R. G., HALL, D. H., and KIMBLE, K. A. (1959).—Cucurbit mosaic viruses in California. *Phytopathology* 49:366-76.

GROGAN, R. G., and KIMBLE, K. A. (1962).—Serological identification of cucumber mosaic virus in crude extracts from infected plants. (Abs.) *Phytopathology* 52:734.

GROGAN, R. G., TAYLOR, R. H., and KIMBLE, K. A. (1964).—Effect of placement of reactants on immunodiffusion precipitin patterns. *Phytopathology* 54:163-6.

HOLLINGS, M. (1965).—Anemone necrosis, a disease caused by a strain of tobacco ringspot virus. *Ann. Appl. Biol.* 55:447-57.

HOLLINGS, M., STONE, O. M., and BRUNT, A. A. (1964).—Cucumber mosaic virus. In *Rep. Glasshouse Crops Res. Inst.* 1963:88.

KAMURO, Y. (1962).—Virus diseases of cucumber and watermelon caused by melon mosaic virus. *Ann. Phytopath. Soc. Japan* 27:31-6.

LEPPIK, E. E. (1964).—Some epiphytotic aspects of squash mosaic virus. *Pl. Dis. Reptr* 48:41-2.

LINDBERG, G. D., HALL, D. H., and WALKER, J. C. (1956).—A study of melon and squash mosaic viruses. *Phytopathology* 46:489-95.

MCKINNEY, H. H., SILBER, G., and GREELEY, L. W. (1965).—Longevity of some plant viruses stored in chemically dehydrated tissues. *Phytopathology* 55:1043-4.

MURANT, A. F. (1965).—The morphology of cucumber mosaic virus. *Virology* 26:538-44.

NELSON, M. R., MATEJKA, J. C., and MCDONALD, H. H. (1965).—Systemic infection of watermelon by a strain of squash mosaic virus. *Phytopathology* 55:1362-4.

NELSON, M. R., and MILBRATH, G. M. (1965).—Heterogeneity of particle size in preparations of squash mosaic virus. (Abs.) *Phytopathology* 55:1069.

NITZANY, F. E., and FRIEDMAN, S. (1963).—Application of the half-life concept to some plant viruses. *Phytopathology* 53:548-51.

PRICE, W. C. (1934).—Isolation and study of some yellow strains of cucumber mosaic. *Phytopathology* 24:743-61.

PURCIFULL, D. E., and EDWARDSON, J. R. (1967).—Watermelon mosaic virus: tubular inclusions in pumpkin leaves and aggregates in leaf extracts. *Virology* 32:393-401.

SCHMELZER, K., and MILIČIĆ, D. (1966).—Zur Kenntnis der Verbreitung des Wassermelonen-mosaik-Virus in Europa und seiner Fähigkeit zur Bildung von Zellinschlusskörpern. *Phytopath. Z* 57:8-16.

SCOTT, H. (1963).—Purification of cucumber mosaic virus. *Virology* 20:103-6.

SIMMONDS, J. H. (1966).—"Host Index of Plant Diseases in Queensland." (Queensland Department of Primary Industries: Brisbane).

STEERE, R. L. (1956).—Purification and properties of tobacco ringspot virus. *Phytopathology* 46:60-9.

STROYAN, H. L. G. (1952).—The identification of aphids of economic importance. *Pl. Path.* 1:42-8.

STROYAN, H. L. G. (1961).—Identification of aphids living on Citrus. *Pl. Prot. Bull. F.A.O.* 9:45-65.

TOBA, H. H. (1962).—Studies on the host range of watermelon mosaic virus in Hawaii. *Pl. Dis. Reptr* 46:409-10.

TOBA, H. H. (1963).—Vector-virus relationships of watermelon mosaic virus and the green peach aphid, *Myzus persicae* (Sulz.). *J. Econ. Ent.* 56:200-3.

TOMLINSON, J. A., SHEPHERD, R. J., and WALKER, J. C. (1959).—Purification, properties and serology of cucumber mosaic virus. *Phytopathology* 49:293-9.

VAN REGENMORTEL, M. V. H. (1961).—Purification of a watermelon mosaic virus. *S. Afr. J. Agric. Sci.* 4:405-14.

VAN REGENMORTEL, M. V. H., BRANDES, J., and BERCKS, R. (1962).—Investigations on the properties of watermelon mosaic virus. *Phytopath. Z.* 45:205-16.

VAN REGENMORTEL, M. V. H., HAHN, J. S., and FOWLE, L. G. (1964).—Internal calibration of electron micrographs with an orchid virus. *S. Afr. J. Agric. Sci.* 7:159-64.

VAN SLOGTEREN, D. H. M. (1954*a*).—Serological microreactions with plant viruses under paraffin oil. *Proc. Conf. Potato Virus Dis.* 2:45-51.

VAN SLOGTEREN, D. H. M. (1954*b*).—Serological analysis of some plant viruses with the gel-diffusion method. *Proc. Conf. Potato Virus Dis.* 2:51-4.

VAN VELSEN, R. J. (1960).—A strain of melon mosaic *virus on Cucurbita moschata* Duchesne in New Guinea. *Papua New Guin. Agric. J.* 13:36-8.

WEBB, R. E., and BOHN, G. W. (1961).—A virus latent in some cucurbits. *Pl. Dis. Reptr* 45:677-9.

WEBB, R. E., and SCOTT, H. A. (1965).—Isolation and identification of watermelon mosaic viruses 1 and 2. *Phytopathology* 55:895-900.

WINGARD, S. A. (1928).—Hosts and symptoms of ringspot, a virus disease of plants. *J. Agric. Res.* 37:127-53.

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The author is an officer of the Plant Pathology Section, Queensland Department of Primary Industries, and is stationed at the Sciences Laboratory, Indooroopilly.