STUDIES ON CUCURBIT VIRUSES IN MADRAS STATE*

II. Vector-Virus Relationships of the Bittergourd Mosaic Virus

BY K. NAGARAJAN** AND K. RAMAKRISHNAN,‡ F.A.SC.

Received February 16, 1970

ABSTRACT

Investigations carried out on the relationship of Bittergourd Mosaic virus and its vectors indicated that the pre-acquisition fasting threshold, acquisition threshold and inoculation feeding threshold were 15 minutes, 5 seconds and 60 seconds respectively for *Myzus persicae*; 15 minutes, 20 seconds and 5 minutes respectively for *Aphis gossypii*; 30 minutes 30 seconds and 5 minutes respectively for *Aphis nerii* and *Brevicoryne brassicae*; 30 minutes, 20 seconds and 5 minutes respectively for *A. malvae*. The optimum number of viruliferous aphids per plant for transmission was 15 for all the five aphid vectors; increasing the number of aphids above the optimum decreased the percentage of transmission. Persistence of the virus during fasting was 4 hours, 90, 60, 45 and 30 minutes respectively for *M. persicae*, *A. gossypii*, *A. malvae*, *B. brassicae* and *A. nerii* while persistence during feeding was 40, 30, 20, 15 and 10 minutes respectively for the same aphid vectors. Though both alate and apterous forms of the vectors transmitted the virus, the apterous forms were more efficient than the alate ones.

INTRODUCTION

AMONG the most important, most complex and most extensively distributed agents of plant virus vectors, aphids, leaf-hoppers, whiteflies and thrips have attracted world-wide attention and their relationships to the viruses they transmit have been extensively studied. Black (1959) pointed out that the vectors of any one plant virus are almost always restricted to one of the major taxa, such as the aphids, the leaf-hoppers, the whiteflies, the thrips or the nematodes and a plant virus is almost always transmitted by only one of the principal

---

* Submitted by the senior author as part of his Ph.D. Thesis to the Madras University.

** Sorghum Pathologist, The Rockefeller Foundation, Rajendranagar, Hyderabad–30.

‡ Dean, Agricultural College and Research Institute, Coimbatore–3, Tamil Nadu.

types of transmission, that is, either by the circulative, the stylet-borne, or the propagative type.

Aphids form the largest group of the vectors of plant viruses studied. Transmission by aphids has been reviewed, in recent years, by various authors, such as Bawden (1957), Broadbent and Martini (1959), Carter (1961), Kennedy (1960), Kennedy *et al.* (1962), Maramorosch (1963), Rochow (1961), Smith (1957, 1958), Sylvester (1958, 1962) and Watson (1960).

The mechanism of transmission of plant viruses, particularly non-persistent viruses, has interested many workers in the last few years. Smith (1931) wrote: "the question of the relationship of strains, biological races and varieties of insect vectors in regard to their ability to transmit viruses is a subject of great interest and one which up to the present has been much neglected." This statement seems still appropriate despite the passage of 35 years. Black (1954) pointed out that "our understanding of what acutally occurs during aphid transmission is far from complete".

Several theories of the mechanism of transmission of non-persistent viruses by aphid vectors have been put forth by numerous workers, important theories being "mechanical transmission hypothesis" (Hoggan, 1933); "inactivator-behaviour-theory" (Watson and Roberts, 1939, 1940); "mechanical inactivator-behaviour hypothesis" (Day and Irzykiewicz, 1954); "mechanical surface-adherence hypothesis" (Van der Want, 1954); and "mechanical inactivator-compatibility hypothesis" (Sylvester, 1954).

In recent years, a new virus disease on bittergourd (*Momordica charantia* L.) was found in and around Coimbatore. Preliminary studies on this virus disease have shown that the virus was not transmitted by sap or seed but transmitted by the aphid vectors. Among the aphid vectors tested, *Myzus persicae* Sulz., *Aphis gossypii* Glov., *A. malvae* Koch, *A. nerii* and *Brevicoryne brassicae* L. transmitted the virus while *A. croccivora* Koch., *A. maidis* F. and *Toxoptera citricidus* Kirk. failed to transmit the same. The results presented in this paper relate to the relationship of this Bittergourd Mosaic Virus to its five aphid vectors.

Methods

In the study of the vector-virus relationships of the Bittergourd Mosaic Virus, young healthy plants of *Cucurbita pepo* var. early white bush at 2-leaf stage were used as test plants. The methods adopted were the same as described by Nariani and Sastry (1962).

EXPERIMENTAL RESULTS

(*a*) *Effect of Preliminary Fasting on the Efficiency of the Aphid Vectors to Acquire the Virus*

The results are presented in Table I. It is evident from the table that for successful transmission of the Bittergourd Mosaic Virus a preliminary fasting was essential for all the five species of aphid vectors. In the case of *Myzus persicae* and *Aphis gossypii* a fasting of 15 minutes prior to acquisition was sufficient for virus transmission while in the case of *A. malvae*, *A. nerii* and *Brevicoryne brassicae* a preliminary fasting of 30 minutes was required to effect transmission. With a progressive increase in the preliminary fasting period, the precentage transmission of the virus by the five aphid vectors increased progressively up to 90 minutes which was found to be the optimum fasting period for all the five aphid vectors. No appreciable increase in the

TABLE I

Effect of pre-acquisition fasting period of the vectors on the transmission efficiency of the Bittergourd Mosaic Virus

(Acquisition feeding period: 5 minutes)

S. No.	Pre-aquisition fasting period	*Myzus persicae*		*Aphis gossypii*		*Aphis malvae*		*Aphis nerii*		*Brevicoryne brassicae*	
		No. of plants infected out of 10	Percentage trans-mission	No. of plants infected out of 10	Percentage trans-mission	No. of plants infected out of 12	Percentage trans-mission	No. of plants infected out of 10	Percentage trans-mission	No. of plants infected out of 10	Percentage trans-mission
1	No fasting	..	..	..	..	..	..	..	..	..	..
2	5 min.	..	..	..	..	..	..	..	..	..	..
3	15 ,,	3	30·00	2	20·00	..	..	..	..	..	..
4	30 ,,	5	50·00	2	20·00	3	25·00	2	20·00	3	30·00
5	45 ,,	5	50·00	4	40·00	5	41·66	5	50·00	5	50·00
6	60 ,,	7	70·00	6	60·00	9	75·00	6	60·00	6	60·00
7	90 ,,	7	70·00	7	70·00	9	75·00	7	70·00	6	60·00
8	2 hrs.	6	60·00	5	50·00	8	66·66	5	50·00	4	40·00
9	6 ,,	3	30·00	4	40·00	3	25·00	3	30·00	4	40·00
10	24 ,,	4	40·00	4	40·00	3	25·00	2	20·00	2	20·00

percentage transmission of the virus was noticed when the vectors were fasted over 90 minutes.

(*b*) *Acquisition Threshold of the Aphid Vectors*

The results presented in Table II indicated that the acquisition threshold varied from one vector to another. Among the aphid vectors tested, the acquisition threshold of *Myzus persicae* was as short as 5 seconds while it was 20 seconds in the case of *Aphis gossypii* and *A. malvae*. In the case of *A. nerii* and *Brevicoryne brassicae* the acquisition threshold was 60 and 30 seconds respectively. However, it was evident from the table that an optimum acquisition feeding of 5 minutes by all the five species of aphid vectors was necessary to get higher percentage transmission of the virus.

(*c*) *Inoculation Threshold of the Aphid Vectors*

From the results presented in Table III, it was observed that the minimum inoculation feeding period or the inoculation threshold was only 60 seconds

TABLE II

Effect of acquisition feeding period of the aphid vectors in the transmission of the Bittergourd Mosaic Virus

(Pre-acquisition feeding period: 90 minutes)

S. No.	Aquisition feeding period	*Myzus persicae*		*Aphis gossypii*		*Aphis malvae*		*Aphis nerii*		*Brevicoryne brassicae*	
		Infected/Inoculated	Percentage transmission	Infected/Inoculated	Percentage transmission	Infected/Inoculated	Percentage transmission	Infected/Inoculated	Percentage transmission	Infected/Inoculated	Percentage transmission
1	5 sec.	2/10	20·00	0/10	..	0/10	..	0/8	..	0/10	..
2	10 ,,	2/10	20·00	0/10	..	0/10	..	0/8	..	0/10	..
3	20 ,,	4/10	40·00	2/10	20·00	2/10	20·00	0/8	..	0/10	..
4	30 ,,	5/10	50·00	3/10	30·00	4/10	40·00	0/8	..	2/10	20·00
5	60 ,,	6/10	60·00	5/10	50·00	4/10	40·00	3/8	37·50	5/10	50·00
6	3 min.	8/10	80·00	6/10	60·00	5/10	50·00	6/8	75·00	6/10	60·00
7	5 ,,	8/10	80·00	8/10	80·00	7/10	70·00	6/8	75·00	6/10	60·00
8	10 ,,	6/10	60·00	5/10	50·00	5/10	50·00	4/8	50·00	5/10	50·00
9	15 ,,	4/10	40·00	3/10	30·00	4/10	40·00	4/8	50·00	5/10	50·00

in the case of *M. persicae* and 5 minutes in the case of *A. gossypii*, *A. malvae* *A. nerii* and *B. brassicae*. However, the percentage of transmission was lower at the minimum inoculation feeding period. An inoculation feeding period of 1 to 2 hours on the test plant resulted in an appreciable increase in the percentage transmission of the virus. However, inoculation feeding over 2 hours did not result in any increase in the transmission percentage.

TABLE III

Inoculation threshold of the aphid vectors in the transmission of the virus

(Preliminary fasting period: 90 minutes)
(Acquisition feeding period: 5 minutes)

S. No.	Inoculation feeding period	*Myzus persicae*	*Aphis gossypii*	*Aphis malvae*	*Aphis nerii*	*Brevicoryne brassicae*
1	30 sec. ..	0/10	0/10	0/8	0/8	0/10
2	60 ,, ..	2/10	0/10	0/8	0/8	0/10
3	5 min. ..	4/10	2/10	2/8	2/8	3/10
4	10 ,, ..	6/10	5/10	5/8	4/8	5/10
5	20 ,, ..	6/10	5/10	5/8	5/8	6/10
6	30 ,, ..	5/10	6/10	4/8	5/8	6/10
7	60 ,, ..	7/10	6/10	5/8	6/8	7/10
8	2 hrs. ..	7/10	7/10	5/8	5/8	6/10
9	4 ,, ..	6/10	5/10	5/8	3/8	5/10
10	8 ,, ..	5/10	4/10	3/8	3/8	4/10
11	24 ,, ..	4/10	4/10	4/8	3/8	5/10

Numerator : No. of plants infected.
Denominator : No. of plants inoculated.

(*d*) *Relation of Number of Viruliferous Aphids per Plant to Percentage Transmission*

The data presented in Table IV show that even a single viruliferous aphid in the case of *M. persicae* transmitted the virus whereas in the case of *A. malvae*, a minimum number of three viruliferous aphids was found necessary for successful transmission. In the other three aphid vectors, *viz.*, *A. gossypii*, *A. nerii* and *B. brassicae* the minimum number for transmission was five. However, it was observed that an optimum number of 15 aphids per plant would result in higher percentage of transmission. Further, it was noticed that larger number of aphids above the optimum level decreased the transmission percentage.

TABLE IV

Relation of number of viruliferous aphids per plant to percentage transmission of the virus

(Preliminary fasting : 90 minutes)
(Acquisition feeding : 5 minutes)

S. No.	No. of aphids per plant	*Myzus persicae*	*Aphis gossypii*	*Aphis malvae*	*Aphis nerii*	*Brevicoryne brassicae*
1	One ..	2/10	0/8	0/8	0/6	0/8
2	Three ..	3/10	0/8	1/8	0/6	0/8
3	Five ..	5/10	2/8	2/8	1/6	2/8
4	Ten ..	7/10	6/8	5/8	5/6	5/8
5	Fifteen ..	7/10	6/8	6/8	5/6	6/8
6	Twenty ..	6/10	6/8	6/8	4/6	5/8
7	Above twenty	5/10	5/8	5/8	2/6	4/8

Numerator : No. of plants infected.
Denominator : No. of plants inoculated.

(*e*) *Persistence of the Virus in the Aphid Vectors During Fasting*

The effect of post-acquisition fasting of the viruliferous aphid vectors on the efficiency of transmission of the virus was determined and the results are presented in Table V. The results indicated the retention of the virus in the five aphid vectors during fasting varied. It was found that persistence of the virus during fasting was 4 hours in *M. persicae,* 90 minutes in *A. gossypii,* 45 minutes in *A. malvae* and *B. brassicae* and 30 minutes in *A. nerii.*

(*f*) *Persistence of the Virus in Aphid Vectors During Continuous Feeding*

The results indicated that variation in persistence of the Bittergourd Mosaic Virus was noticed not only among the five aphid vectors but also between continuous feeding on test plant and continuous fasting on petri plates. It was found that *M. persicae* retained the virus for 35 to 40 minutes after acquisition when they were fed continuously on healthy plants. In the case of *A. gossypii* the persistence of the virus was 30 minutes after acquisition while it was 15–20 minutes in *A. malvae,* 10 minutes in *A. nerii* and 10–15 minutes in *B. brassicae.*

TABLE V

Persistence of the Bittergourd Mosaic Virus in the vectors during fasting

(Preliminary fasting : 90 minutes)

(Acquisition feeding : 5 minutes)

S. No.	Post-acquisition fasting	*Myzus persicae*	*Aphis gossypii*	*Aphis malvae*	*Aphis nerii*	*Brevicoryne brassicae*
1	No fasting ..	8/10	6/8	6/8	6/8	6/8
2	10 min. ..	8/10	6/8	5/8	3/6	5/8
3	30 ,, ..	6/8	5/8	5/8	2/6	5/8
4	45 ,, ..	6/8	5/8	3/8	0/6	2/6
5	60 ,, ..	3/8	2/8	0/8	0/6	0/6
6	90 ,, ..	3/8	2/8	0/6	0/6	0/6
7	2 hrs. ..	2/8	0/6	0/6	0/6	0/6
8	4 ,, ..	1/6	0/6	0/6	0/6	0/6
9	6 ,, ..	0/6	0/6	0/6	0/6	0/6
10	24 ,, ..	0/6	0/6	0/6	0/6	0/6

Numerator : No. of plants infected.

Denominator : No. of plants inoculated.

(g) *Relationship of Different Forms of the Aphid Vectors to the Percentage Transmission of the Virus*

The efficiency of both alate and apterous forms of the aphid vectors in the transmission of the Bittergourd Mosaic Virus was determined and the results are presented in Table VI.

The results indicated that both alate and apterous forms of the five species of the aphid vectors tested transmitted the Bittergourd Mosaic Virus. However, variation in the percentage transmission of the virus between the alate and apterous forms was observed, the percentage transmission being higher in the apterous forms than the alate forms of the vectors.

DISCUSSION

Detailed studies on the relationship of the Bittergourd Mosaic Virus to its five aphid vectors revealed some interesting results. It has been observed

TABLE VI

Comparative efficiency of alate and apterous forms of the vectors in virus transmissicn

(Preliminary fasting : 90 minutes)
(Acquisition feeding : 5 minutes)

S. No.	Aphid victors	Alate forms			Apterous forms		
		No. of plants		Percentage infection	No. of plants		Percentage infection
		Inoculated	Infected		Inoculated	Infected	
1	*Myzus persicae* ..	12	5	41·66	12	10	83·33
2	*Aphis gossypii* ..	12	7	58·33	12	9	75·00
3	*Aphis malvae* ..	12	5	41·66	12	6	50·00
4	*Aphis nerii* ..	12	2	16·66	12	5	41·66
5	*Brevicoryne brassicae*	12	6	50·00	12	9	75·00

that preliminary fasting increased the efficiency of the vectors; shorter the preliminary fasting lesser was the percentage transmission. The optimum period of preliminary fasting to get maximum transmission of the virus was found to be 90 minutes in all the cases. It was also observed that the length of the preliminary fasting period varied inversely with the efficiency of transmission. When no preliminary fasting was given none of the vectors acquired the virus. The reason for this may be attributed to the fact that in non-fasted aphids the salivary inhibitor is present in sufficiently high quantities as to inactivate the virus taken up during feeding and, secondly, during fasting the secretion of the inhibitor is reduced or even completely stopped. It is also possible that the rate of production of inhibitor during fasting is very low with the result that the rate of inactivation of the virus in fasting insects is low. These observations are similar to those reported by Watson (1936), Watson and Roberts (1939), Bradley (1952), Sylvester (1954), Day and Irzykiewicz (1954) and Nariani and Sastry (1962).

The experiments on the acquisition feeding period have shown that the Bittergourd Mosaic Virus can be acquired by the most efficient vector, *Myzus persicae* with an acquisition feeding period as short as 5 seconds and the least efficient vector, *Aphis nerii*, acquired the virus in 60 seconds, thus indicating that the acquisition threshold varies inversely as the efficiency of transmission. The optimum feeding period which produced maximum number of infections was found to be 5 minutes for all the five species of the vectors. Increasing the acquisition feeding period above the optimum decreased the percentage transmission. It has been reported by Watson and Roberts (1939), Day and Irzykiewicz (1954), Sylvester (1954), Bradley (1954, 1959), Nariani and Sastry (1962) that longer the aphid fed on the diseased plant, the less efficient it became as a vector, the reason being the production of inhibitors in insects during feeding. The differences in acquisition threshold could also be explained by postulating differences in quantity of inhibitor secreted - the least efficient vector secreting larger quantities than the most efficient vector and, therefore, larger quantities of the virus are required to neutralize the inhibitor leaving a residue of transmissible virus.

The experiment on the inoculation threshold indicated that the most efficient vector, *Myzus persicae,* can transmit the virus in a short feeding period of 60 seconds, compared to the least efficient vector, *Aphis nerii* which required 5 minutes of feeding on healthy test plants. The percentage transmission of the virus, however, increased with the increase in the test plant feeding period up to 60 minutes. It is presumed that the more efficient vector carried a larger quantity of virus in its mouth parts and at every probe a larger charge of virus is delivered into the suscept leaf than in the case of less efficient vector. This may be the reason why a longer inoculation threshold is required in the less efficient vector. Nariani and Sastry (1962) working on the chilli mosaic virus observed similar results and the explanation given by them was that most of the aphids, that can cause infections, do so within the first hour and further increase in the duration of test plant feeding does not seem to increase the number of infections significantly.

Studies on the relationship of number of viruliferous aphids to the percentage transmission have revealed that even a single viruliferous aphid (*Myzus persicae*) is capable of transmitting the virus. In the case of other vectors, a minimum number of five viruliferous aphids was found necessary to incite infection. However, the percentage transmission of the virus was very low. Similar results have been obtained by Severin and Freitag (1938), Freitag and Severin (1945), Severin and Thompkins (1945, 1950) and Nariani and Sastry (1962) working with aphid transmitted non-persistent viruses. Another interesting observation made was that when a large number of viruliferous aphids, above an optimum, was used, there was a decrease in the transmission percentage.

Persistence of the virus in the vectors was longer during fasting than feeding. This corroborates earlier studies on CMV (Doolittle and Walker, 1928; Bhargava, 1951), PVY (Smith, 1931; Watson and Roberts, 1940), pea mosaic virus (Osborn, 1937), Henbane mosaic virus (Watson, 1938; Watson and Roberts, 1940). Day and Irzykiewicz (1954) observed that the duration of persistence of infectivity of aphids has a bearing on the inactivator hypothesis. During fasting, the viruses would have less opportunity of coming in contact with a salivary inhibitor. During feeding some virus is wiped off the stylets but the very short survival time of viruses during feeding indicates that they are subjected to an additional inhibiting action. The results obtained in the present studies seems to lend support to the inactivator hypothesis. It was further observed that in the efficient vector, *Myzus persicae*, the persistance of the virus during fasting as well as feeding was always longer than in the inefficient vector, *Aphis nerii*. This seems to indicate that there are inherent differences between aphid species in the quantities of the postulated inhibitor secreted.

Acknowledgements

The authors express their sincere thanks to the University of Madras for having permitted the publication of this research work.

References

Bawden, F. C. .. "The transmission of plant viruses by insects," *Biological Aspects of the Transmission of Diseases*, Horton, Smith, C., Ed., Oliver and Boyd, Edinburgh, Scotland-184, 1957, pp. 87–93.

Bhargava, K. S. .. "Some properties of four strains of Cucumber Mosaic Virus," *Ann. Appl. Biol.*, 1951, **38**, 377–88.

Black, L. M. .. "Arthropod transmission of plant viruses," *Exptl. Parasitol.*, 1954, **3**, 72–104.

——— .. "Biological cycles of plant viruses in insect vectors," *The Viruses*, 1959, **2**, 157–85.

Bradley, R. H. E. .. "Studies on the aphid transmission of a strain of Henbane mosaic virus," *Ann. Appl. Biol.*, 1952, **39**, 78–97.

——— .. "Studies on the mechanism of transmission of PVY by the green peach aphid, *Myzus persicae* Sulz., (Homoptera: Aphididae)," *Canadian J. Zool.*, 1954, **32**, 64–73.

——— .. "Loss of virus from the stylets of aphids," *Virology*, 1959, **8**, 308–18.

Broadbent, L. and Martini, C. "The spread of plant viruses," *Advances in Virus Research*, 1959, **6**, 93–135.

Carter, W. .. "Ecological aspects of plant virus transmission," *Ann. Rev. Entomol.*, 1961, **6**, 347–70.

Day, M. F. and Irzykiewicz, H. "On the mechanism of transmission of non-persistent phyto-pathogenic viruses by aphids," *Aust. J. Biol. Sci.*, 1954, 7, 251–73.

Doolittle, S. P. and Walker, M. N. .. "Aphid transmission of cucumber mosaic," *Phytopathology*, 1928, **18**, 143.

Freitag, J. H. and Severin, H. H. P. "Poison-hemlock-ring spot virus and its transmission by aphids to celery," *Hilgardia*, 1945, **16**, 389–410.

Hoggan, I. A. .. "Some factors involved in aphid transmission of CMV to tobacco," *J. Agric. Res.*, 1933, **47**, 689–704.

*Kennedy, J. S. .. "The behavioural fitness of aphids as field vectors of viruses," *Rept. Commonwealth Entomol. Conf.*, 1960, pp. 165–68.

*———, Day, M. F. and Eastop, V. F. A. *A Conspectus of Aphids as Vectors of Plant Viruses*, Commonwealth Inst. Entomol., London, 1962, pp. 114.

Marmorosch, K. .. "Arthropod transmission of plant viruses," *Ann. Rev. Entomol.*, 1963, **8**, 369–414.

Nariani, T. K. and Sastry, K. S. M. "Studies on the relationship of chilli mosaic virus and its vector, *Aphis gossypii* Glov.," *Indian Phytopath.*, 1962, **15**, 173–83.

Osborn, H. T. .. "Studies on the transmission of pea virus 2 by aphids," *Phytopathology*, 1937, **27**, 589–603.

Rochow, W. F. .. "A strain of barley yellow dwarf virus transmitted specifically by the corn leaf aphid," *Ibid.*, 1961, **51**, 809–10.

Severin, H. H. P. and Freitag, J. H. "Western celery mosaic," *Hilgardia*, 1938, **11**, 494–558.

——— and Tompkins, C. M. .. "Aphid transmission of cauliflower mosaic virus," *Ibid.*, 1948, **18**, 389–404.

——— ——— .. "Aphid transmission of severe mosaic virus of annual stock," *Ibid.*, 1950, **20**, 93–106.

*Smith, K. M. .. "Virus diseases of plants and their relationship with insect vectors," *Biol. Rev.*, 1931, **6**, 302–44.

*——— .. "Arthropods as vectors and reservoirs of phytopathogenic viruses," *Handb. Virusforsch.*, 1957, **4**, 143–76.

*——— .. "Transmission of plant viruses by arthropods," *Ann. Rev. Entomol.*, 1958, **3**, 469–83.

Sylvester, E. S. .. "Aphid transmission of non-persistent plant viruses with special reference to *Brassica nigra* virus," *Hilgardia*, 1954, **23**, 53–98.

*——— .. "Aphid transmission of plant viruses," *Proc. Intern. Congr. Entomol. Montreal*, 1956, 10th, 1958, **3**, 195–200.

*——— .. "Mechanisms of plant virus transmission by aphids," *Biological Transmission of Disease Agents*, 1962, pp. 11–31.

*Van der Want, J. P. H. .. "Onderzoekingen over Virusziekten van der Boon (*Phaseolus vulgaris* L.)," Veenman and Zonen, Wageningen 1954, pp. 6.

Watson, M. A. .. "Factors affecting the amount of infection obtained by aphid transmission of the Virus HY III," *Roy. Soc. London Phil. Trans.*, 1936, **B 226**, 457–89.

———— .. "Further studies on the relationship between Hyoscyamus virus 3 and the aphid, *Myzus persicae* Sulz., with special reference to the effects of fasting," *Proc. Roy. Soc. London*, 1938, **B 125**, 144–70.

——— and Mulligan, T. "The manner of transmission of some barley yellow dwarf viruses by different aphid species," *Ann. Appl. Biol.*, 1960, **48**, 711–20.

——— and Roberts, F. M. .. "A comparative study of the transmission of Hy. virus 3, PVY and cucumber virus 1 by the vectors, *Myzus persicae* Sulz., *M. circumflexus* Buckton., and *Macrosiphum gei* Koch.," *Proc. Roy. Soc. London*, 1939, **B 127**, 543–76.

——— ——— .. "Evidence against the hypothesis that certain plant viruses are transmitted mechanically by aphids," *Ann. Appl. Biol.*, 1940, **27**, 227–33.

*Originals not seen.