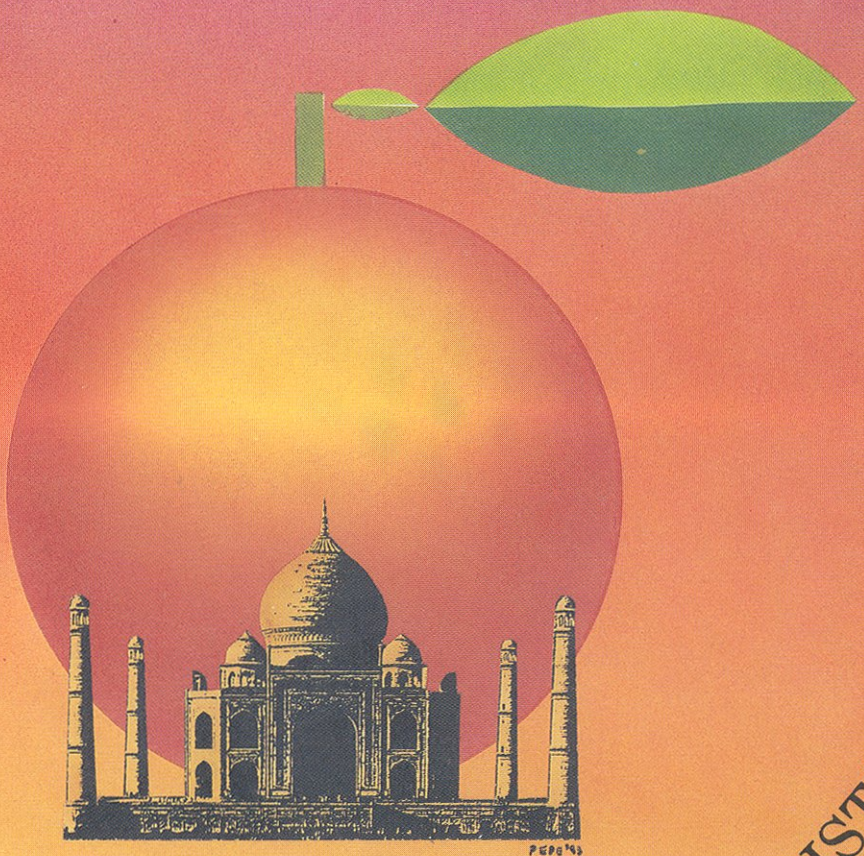
PROCEEDINGS OF THE 12th CONFERENCE OF THE INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

P. Moreno, J. V. da Graça,
and L. W. Timmer, editors

IOCV – 1993



Persistent Transmission of Citrus Vein Enation Virus by *Aphis gossypii* and *Myzus persicae*

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ABSTRACT. Citrus vein enation virus (CVEV) is widely distributed in Spain and circumstantial evidence suggests a high rate of natural spread. No experimental transmission was achieved in extensive trials with several aphid species using a 2-day acquisition period and a 2-day inoculation period. In further experiments, using a 5-7 day acquisition period and a 9-16 day inoculation period, a 95% transmission efficiency was obtained with *Aphis gossypii* and 10% with *Myzus persicae*. Furthermore, a 10% transmission efficiency was achieved with viruliferous *A. gossypii* maintained on healthy plants for a period of up to 14 days after acquisition then given an 11-day inoculation access feed. These results show that CVEV is persistently transmitted by *A. gossypii* in Spain, thus explaining the high rate of natural spread in the field.

Citrus vein enation virus (CVEV) was found in Spain in 1976 by indexing on Mexican lime (9). It is presently widely distributed in the country, and there is indirect evidence of rapid natural spread of the virus. CVEV has been experimentally transmitted by several aphid species, namely *Toxoptera citricidus* (8), *Myzus persicae* (10), *Aphis gossypii* (4), and *T. aurantii* (7), although the mode of transmission was not indicated in any of these studies. However it was recently reported that CVEV is persistently transmitted by *T. citricidus* in South Africa (5, 6). In order to explain the spread of the CVEV in the field in Spain, several experiments on transmission of the virus by aphids present here were carried out.

MATERIALS AND METHODS

Transmission experiments of CVEV. In 1984, several experiments were carried out to transmit CVEV following the methodology previously described for semi-persistent transmission (1). Pineapple sweet orange seedlings, inoculated with the pure CVEV isolates from the IVIA collection VE-205, VE-206, VE-209, and VE-211, were used as donor plants. Transmission was attempted with *A. gossypii* reared on cotton plants growing in a greenhouse at 18-25 C, *M. persicae* reared on *Vicia faba* plants growing in the greenhouse, and *Aphis spiraecola* and *T. aurantii* collected from field orange trees. These were the most abundant aphid species in Spanish citrus orchards at that time (2).

Aphids were fed for 2 days on the donor plants and then transferred to healthy Mexican lime seedlings for a 2 day inoculation access period. About 200 aphids were used in each transmission, and ten receptor Mexican lime seedlings were inoculated with each aphid species-virus isolate combination.

Transmission attempts were conducted in a growth chamber at 20-24 C with 60-80% relative humidity and a 15-hr photoperiod of 4,000 lux. The Mexican lime plants were then transferred to a greenhouse set at 18-25 C and observed for 6 months for the appearance of vein enation symptoms.

In 1991, experiments were conducted to determine whether CVEV is persistently transmitted by *A. gossypii* and *M. persicae*. The virus isolate used was VE-211 and the donor and receptor plants and environmental conditions were the same as described above. Aphids were given a 5-day acquisition access feed on the infected donor plants and 16 days of inoculation access time on the healthy receptor plants. Ten Mexican lime plants with about 300 aphids each were used. This experiment was repeated, using only *A. gossypii*, with an acquisition access time of 7 days and an inoculation access time of 9 days on 20 Mexican lime plants.

Retention period of CVEV by *A. gossypii*. *A. gossypii* and the virus iso-

late VE-211 were used in the experiments to determine the retention period of CVEV by aphids. Aphids were given a 7-day acquisition access time on infected Pineapple sweet orange donor plants. The shoots of the donor plants with the aphids were then removed and placed in small cages on shoots of Mexican lime plants to allow a natural transfer of the aphids. Later aphids were serially transferred to four groups of healthy Mexican lime plants. The first two groups comprised 20 plants each and the latter two had 10 plants each. Some of the aphids were transferred from the first to the second group after 6 days, from the second to the third after 8 days (14 days after acquisition), and from the third to the fourth after 7 days (21 days after acquisition). Aphids that remained in each group of plants were given 11 days of inoculation time. There remained approximately 300 aphids per plant in the first group, about 250 in the second, about 200 in the third, and about 150 in the fourth. Environmental conditions for transmission and incubation of inoculated plants were as described above.

RESULTS AND DISCUSSION

None of the four CVEV isolates used in the experiments could be transmitted by *A. gossypii*, *M. persicae*, *T. aurantii*, or *A. spiraecola* semi-persistently, with 2 days for acquisition and inoculation access times (Table 1). However, CVEV was persistently transmitted by *A. gossypii* and *M. persicae*, with 5-7 days of acquisition access time and 9-16 days of inoculation access time (Table 1). *A. gossypii* had a 90-95% transmission efficiency compared to the 10% transmission efficiency of *M. persicae*.

Earlier works on the transmission of CVEV by *A. gossypii* (4), *M. persicae* (10), and *T. aurantii* (7), did not give details on the methodology. Recent works on transmission of CVEV with *T. citricidus* also indicate that the virus can only be transmitted in a persistent manner by this aphid species (5, 6).

The maximum retention period of CVEV by *A. gossypii* was 2 weeks under the experimental conditions studied (Table 2). A 10% transmission was obtained with aphids that fed on healthy

TABLE 1
TRANSMISSION OF CITRUS VEIN ENATION VIRUS (CVEV) BY APHIDS

Aphid species	Virus isolate	No. of aphids per plant	Acquisition time (days)	Inoculation time (days)	Transmission[z]
Aphis gossypii	VE-205	200	2	2	0/10
"	VE-206	200	2	2	0/10
"	VE-209	200	2	2	0/10
"	VE-211	200	2	2	0/10
"	"	300	5	16	9/10
"	"	300	7	9	19/20
Myzus persicae	VE-205	200	2	2	0/10
"	VE-206	200	2	2	0/10
"	VE-209	200	2	2	0/10
"	VE-211	200	2	2	0/10
"	"	300	5	16	1/10
Toxoptera aurantii	VE-205	200	2	2	0/10
"	VE-206	200	2	2	0/10
"	VE-209	200	2	2	0/10
"	VE-211	200	2	2	0/10
Aphis spiraecola	VE-205	200	2	2	0/10
"	VE-206	200	2	2	0/10
"	VE-209	200	2	2	0/10
"	VE-211	200	2	2	0/10

[z]Number of infected plants/number of inoculated plants.

TABLE 2
RETENTION PERIOD OF CITRUS VEIN ENATION VIRUS (CVEV) BY *APHIS GOSSYPII*.

Time after acquisition (days)	Transmission[z]
0	15/19
6	5/20
14	1/10
21	0/10

[z]Number of infected plants/number of inoculated plants.

plants for 14 days after the acquisition, as compared with 25% transmission with aphids 6 days after acquisition and 79% with the controls. No transmission was obtained with aphids 21 days after acquisition. This data was confirmed in an additional experiment comparing transmission of CVEV by aphids with zero and two weeks after acquisition. However, the aphids degenerated somewhat during the course of the experiments, causing a reduction in the number of aphids per plant. This may have affected the transmission rate. It is possible that under natural conditions the retention period of *A. gossypii* could be much longer than the two weeks obtained in these experiments. In *T. citricidus* it was found that the retention period of CVEV was at least 8 days (5).

The increasing populations of *A. gossypii* in Spanish citrus orchards (3) and the high transmission efficiency of CVEV by *A. gossypii* could explain the quick spread of the virus in Spain.

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