Transmission of Citrus Tristeza Virus by Aphids (Homoptera, Aphididae) in Spain

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ABSTRACT. Transmission of citrus tristeza virus (CTV) by aphids was tried with two Spanish CTV isolates, T-300 and T-308. Isolate T-300 causes mild symptoms on Mexican Lime and is very widespread, while T-308 is very severe on Mexican Lime and has a limited distribution.

Transmission of the two isolates was tried using 200 aphids/plant of the most important aphid species found on citrus in Spain: *Aphis citricola, Toxoptera aurantii, Myzus persicae, Aphis gossypii, Aphis fabae* and *Aphis craccivora.* Two other aphid species, *Aphis nerii* and *Hyalopterus pruni* were tested, only with the T-300 isolate.

Isolate T-308 was transmitted only by *A. gossypii* with an efficiency of 28%, whereas the isolate T-300 was transmitted by *A. gossypii* (78%), by *A. citricola* (6%) and by *T. aurantii* (6%).

Tristeza was first detected in Spain at the Ribera Alta area in Valencia in 1957. Subsequently it spread to various citrus growing areas, and thus far, about 8 million trees have been killed by the disease and many thousands more are in different stages of decline.

At present, the following seven insect species from the Aphididae (Homoptera), have been established as CTV vectors in different counties: *Toxoptera citricidus* (Kirk.) (9), *Aphis gossypii* Glover (6), *Aphis citricola* Van der Goot (11), *Toxoptera aurantii* (Boyer de Fonsicolombe) (12), *Myzus* (*Nectarosiphon*) *persicae* (Lube.) (16), and *Aphis craccivora* Koch and *Uroleucon* (*Uromelan*) *jaceae* (Linnaeus) (15). Differences of transmission efficiency have been observed among the various aphid species and virus isolates used by different authors (1, 2, 4, 5, 12, 13, 14, 15, 16).

In previous work, the composition of the aphid fauna feeding on citrus was studied in areas where natural spread of CTV occurred (8). The following species were found: *A. citricola, T. aurantii, M. persicae, A. gossypii, Aphis fabae* Scopoli, *A. craccivora, Macrosiphum euphorbiae* (Thomas) and *Brachycaudus helichrysi* (Kaltenbach). Nevertheless, the species responsible for CTV spread in Spain remained undetermined with transmission experiments with this objective were begun.

The relative frequency of the different species was evaluated by means of yellow water traps (unpublished results) and since *M. euphorbiae* and *B. helichrysi* appeared in negligible quantities they were not included in this study. However, two other species were included: *Aphis nerii* Boyer de Fonscolombe and *Hyalopterus pruni* (Geoffroy). Neither have been found on citrus in Spain, and moreover the second one has never been reported on citrus. However, CTV could be detected in both of them by the immunoenzymatic ELISA method after feeding on CTV infected plants (3).

In this paper we report the first results of the transmission experiments carried out with several aphid species and some Spanish isolates of CTV.

MATERIALS AND METHODS

Transmissibility of CTV isolates was tested with the following aphid species: *A. citricola, T. aurantii, M. persicae, A. gossypii, A. fabae, A. craccivora, A. nerii,* and *H. pruni.* Two CTV isolates

coded as T-300 and T-308, from citrus growing areas of Spain were used.

Isolate T-300 induces mild symptoms on Mexican lime and it was obtained by exposing healthy Mexican lime seedlings to natural infection in the field. This isolate was representative of the commonest type of CTV in Spain and it was suspected to be highly transmissible in the field.

In contrast, isolate T-308 induces very severe symptoms on Mexican lime including stunting, vein clearing, leaf yellowing, vein corking and severe stem pitting. This isolate was obtained from a calamondin tree that was known to be infected for many years. Since none of the trees around became infected it was suspected to be a poorly transmissible isolate.

Virus source plants were prepared by graft-inoculating each CTV isolate on several potted Madame Vinous sweet orange seedlings. Plants were pruned periodically to produce young shoots for aphid feeding.

Some of the aphid species used in transmission experiments were collected from field trees and some others were reared in controlled environment growth chambers at 22 ± 1.5 C, 70 ± 10% relative humidity and a 15-hour photoperiod at 700 lux. *A. citricola* and *T. aurantii* were collected from field citrus trees, *A. nerii* from oleander plants (*Nerium oleander* L.) and *H. pruni* from peach trees (*Prunus persicae* L.). *A. gossypii* was reared on cotton and *A. craccivora, A. fabae* and *M. persicae* on broad bean plants in a controlled environment chamber.

Usually, 50 Mexican lime plants, about 30 cm high were used for each aphid species and virus isolate in transmission experiments. *A. nerii* and *H. pruni* were tested only with isolate T-300 using 40 and 17 Mexican lime plants, respectively. Negative controls consisted of three plants (six for *A. citricola*) in which aphids of the different species were fed without a previous acquisition feeding on virus-infected plants.

Transmission experiments with each aphid species were carried out in the following way: leaf pieces carrying about 200 aphids were caged in transparent rigid plastic tubes 8 cm long and 3 cm in diameter with one end closed by a screen of tergal or silk cloth. A young shoot or a leaf of the virus source plant was introduced into the cage and a strip of foam was wound around the shoot or leaf petiole to close the cage and to keep it attached to the source plant. The aphids moved from the leaf pieces to the young shoot or leaf of the source plant inside the cage. After 48 hours the shoot or leaf was cut off the plant, the cage with aphids inside was attached to a healthy Mexican lime plant as previously described and it was kept there for another 48 hours. During that time aphids moved from the source plant shoot to the Mexican lime receptors. Finally, the cages were removed and the aphids were killed by spraying with nicotine.

All these operations were carried out in the controlled environment chambers used for aphid rearing.

After aphid inoculation feeding, Mexican lime plants were transferred to a greenhouse with temperatures ranging from 18 C to 25 C. Plants were checked for foliar symptoms periodically over a 6 month period and after that period lime plants were cut, the bark was removed and the trunks were checked for stem pitting.

RESULTS

Results of transmission experiments are shown in table 1. Isolate T-308 was transmitted only by *A. gossypii* with a transmission rate

TABLE 1
TRANSMISSION OF TWO CTV ISOLATES FOUND IN SPAIN BY SEVERAL APHID SPECIES

Aphid species	CTV isolate T-300 Transmission[x]	(%)	T-308 Transmission	(%)	Controls Transmission	(%)
Aphis citricola	3/50	6	0/50	0	0/6	0
Toxoptera aurantii	3/50	6	0/50	0	0/3	0
Myzus persicae	0/50	0	0/50	0	0/3	0
Aphis gossypii	39/50	78	14/50	28	0/3	0
Aphis fabae	0/50	0	0/50	0	0/3	0
Aphis craccivora	0/50	0	0/50	0	0/3	0
Aphis nerii	0/40	0	—	—	—	—
Hyalopterus pruni	0/17	0	—	—	—	—

[x]Numerator = no. of infected plants; denominator = no. of inoculated plants.

of 28%. Isolate T-300 was transmitted by *A. gossypii* with high efficiency (78%) and by *A. citricola* and by *T. aurantii* with lower efficiency (6% in both cases). Complete symptom homogeneity was observed between the plants inoculated by aphids and the corresponding virus source plant.

DISCUSSION

In previous work (3), the ELISA technique enabled CTV detection in the eight aphid species used above after they were fed on CTV-infected plants. In the present transmission experiments only three of the eight species were able to transmit isolate T-300 and only one transmitted isolate T-308. This indicates that different aphid species can acquire CTV, regardless of their ability of transmit the virus to healthy plants.

The ability of different aphid species to transmit CTV was variable. Under our experimental conditions, *A. gossypii* was the most efficient vector for the common tristeza present in Spain. High vector efficiency of *A. gossypii* was found with CTV isolates from Florida (12), California (14), India (4, 16) and Israel (13), while low or no transmission was obtained in Brazil (2) and in the early years in California (7) and Florida (11). In California, increased transmission efficiency of *A. gossypii* was pointed out by Roistacher *et al.* (14), who suggested the virus mutated to strains readily transmissible by this aphid.

The ability of *A. citricola* to transmit the common tristeza in Spain is in agreement with results obtained in Florida (11, 12), Israel (13) and India (10). *T. aurantii,* which is able to transmit tristeza in Spain, was also reported as a CTV vector in Florida (12) and India (4), but it was unable to transmit two tristeza isolates in Israel (13).

The rest of the aphid species assayed in this work failed to transmit tristeza. Of these, only *A. craccivora* and *M. persicae* have been reported as CTV vectors in India (4, 15, 16). No transmission was achieved with *M. persicae* in Israel (13).

Vector efficiency was different with the two isolates assayed. Isolate T-308 could not be transmitted by *A. citricola* and *T. aurantii.* Variability of vector efficiency with different tristeza isolates has been observed elsewhere with *A. gossypii* (4, 13, 14) as well as with *A. citricola* (13) and *T. aurantii* (4).

Low transmissibility found with the severe isolate T-308 might explain reduced spread of severe CTV

isolates that may be present in Spain, compared with the important spread of milder isolates (similar to T-300).

In a previous survey (unpublished) it was determined that *A. citricola* is, by far, the most abundant species found in Spanish citrus areas. This finding and results presented here suggest these two vectors as mainly responsible for natural spread of tristeza in Spain: *A. gossypii,* more efficient but less abundant, and *A. citricola,* less efficient but more abundant.

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