SHORT COMMUNICATIONS

Estimation of the Number of *Citrus tristeza virus*-Viruliferous Aphids Landing on Individual Citrus Seedlings and Viral Incidence in Different Citrus Rootstocks in Spain

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ABSTRACT. The number of aphid species landing, from January to June 2004, on alemow, Cleopatra mandarin and Carrizo citrange grown in experimental nursery blocks in the same area in the north of the Valencian Community, were estimated by counting the number of young shoots/plant and the number of aphids trapped on sticky shoots. The proportions of different aphid species represented by captured individuals (10,569) were: *Aphis gossypii* (34.9%), *Myzus persicae* (12.7%), *A. spiraecola* (8.6%), *A. craccivora* (2.4%), *A. fabae* (0.8%), *Toxoptera aurantii* (0.8%) and others (39.9%). *C. macrophylla* was the most visited citrus species with estimated 1,656 aphids landing/plant during the test period. May was the month with higher aphid populations. The number and percentage of aphids carrying *Citrus tristeza virus* (CTV) was determined by a validated nested-PCR method in a single closed tube using extracted RNA from previously trapped aphids that were squashed on paper. Viral RNA was detected in 13.3% of tested *A. gossypii* individuals. CTV infection rates assessed by tissue print-ELISA in experimental plots located in the same area were proportional to the number of aphids landing on each susceptible host. Average annual CTV infection incidence ranged from approximately 4% for alemow and *Volkamer lemon* to 2% in Cleopatra mandarins. Nevertheless, despite the number of CTV-viruliferous vectors visiting Carrizo citrange, sour orange and citrumelo, CTV incidence was only 0.6%, 0.2% and 0%, respectively, suggesting some resistance to natural (aphid-vectored) infection in these particular citrus seedlings.

The Spanish citrus industry has a high economic value and annual production is about 6 million metric tones from *ca*. 300,000 ha. Spain is the leading country exporting fresh citrus and the fourth most important producer in the world. Citrus is located along the Mediterranean coast mainly in the Valencian Community (VC) which is one of the main citrus growing areas in the world. *Citrus tristeza virus* (CTV) was probably introduced into Spain in the 1930s and subsequently has caused the death of more than 40 million trees grafted on sour orange (3). A systematic replacement of the trees affected by tristeza disease has occurred since the beginning of the 1980s. More than 120 million certified pathogen-free citrus trees grafted on tristeza tolerant rootstocks which are produced in regulated nurseries have been planted, comprising approximately 85% of the Spanish citrus industry. The production of nursery plants (approximately 7 million per year) is mainly located in the North of the VC and in the South of Tarragona (in the province of Catalonia), in areas where CTV was non-existent or very low when the nurseries were established. CTV incidence is increasing in these same areas, increasing the risk of natural infection of nursery plants grown in open field blocks.

The main goal of this work was to design strategies based on CTV epidemiology to avoid or reduce the risk of viral incidence in nurseries. For this purpose the most important

citrus rootstocks grown in Spain (Carrizo citrange, Cleopatra mandarin, alemow, Volkamer lemon, sour orange and citrumelo) were studied for susceptibility to natural infection with CTV. In addition, the number of aphid species and the number of CTV-viruliferous *Aphis gossypii* individuals (the main CTV vector in Spain) visiting or landing on different citrus seedlings in the same area was estimated during the first 6 mo of 2004.

Natural incidence of CTV, from 1999 to 2004, was assessed by tissue print-ELISA (2, 9) using a commercially available kit (PlantPrint Diàgnostics) which uses CTV-specific monoclonal antibodies 3DF1 and 3CA5. A total of 35,319 seedlings grown in open field blocks in the same area were tested. The CTV incidence was: alemow (4.8%), Volkamer lemon (3.7%), Cleopatra mandarin (1.9%), Carrizo citrange (0.7%), sour orange (0.2%) and citrumelo (0%). Based on this data, alemow showed the highest susceptibility to natural CTV infection. Three experimental blocks of 10,000 plants each of alemow, Cleopatra mandarin and Carrizo citrange (representative of high, medium and low natural CTV incidence, respectively) were established to elucidate the reasons for this different susceptibility to natural infection. Aphids were monitored by the sticky shoot method (1, 3) (Fig. 1). This method is the most efficient to estimate the real number of aphids landing on the plants or visiting young shoots and leaves (4, 6, 8). A total of 1,080 standard sticky shoots (one shoot per plant, 60 plants of each of the three citrus species monitored during 6 mo) were analyzed. The sticky shoots remained on the plants during the last ten days of each month, from January to June 2004. Trapped winged adult aphids were identified and counted to separate the aphid species previously found on citrus in Spain (5) from any other aphid spe-

Fig. 1. Aphid species were monitored by the sticky-shoot method. Young shoots and leaves were sprayed with a sticky substance. The captured aphid species were recovered, identified and counted. This method is the most efficient to estimate the real number of winged adult aphids landing on the plants.

cies. The number of shoots per citrus species was also estimated by counting the number of shoots on 20 1-yr-old plants grown in nursery blocks. The average number of shoots/plant were: 6 in alemow, 2.5 in Cleopatra mandarin and 1.5 in Carrizo citrange. The total number of aphids that landed on an adult plant was estimated for each rootstock species by multiplying the number of captured aphids/shoot by the number of shoots/plant. In all 10,569 individual aphids were captured and identified. The percentages of different aphid species captured were: 34.9% (*A. gossypii*), 12.7% (*Myzus persicae*), 8.6% (*A. spiraecola*, 2.4% (*A. craccivora*), 0.8% (*A. fabae*), 0.8% (*Toxoptera aurantii*) and 39.9% other species. *A. gossypii* was the most abundant species visiting citrus seedlings during the monitored period, in agreement with previously reported data (7, 8).

Table 1 shows the number of *A. gossypii* identified among aphids captured on different citrus rootstocks. Close to 33% of the captured individual aphids were *A. gossypii*. May was the month with the higher aphid population and alemow was the most visited citrus rootstock species tested. The percentage of *A. gossypii* aphids captured was very high in winter (from January to March). After identification, 10 *A. gossypii* from the number captured each month were squashed on paper as previously described (8) and RNA was extracted (10, 12) and analyzed by nested RT-PCR in a single closed tube to detect CTV (9, 11). The amplification products were assessed by gel electrophoresis and their identity was confirmed by molecular hybridization (13). The percentage of *A. gossypii* which yielded a positive amplification was 13.3% (24 positives out of 180 individuals analyzed) ranging from 30.0% in January to 3.3% in May (data not presented).

Table 2 shows an estimation of the number of total aphids/plant [1656, 320, 186], the number of *A. gossypii*/plant (545, 98, 63) and the number of viruliferous *A. gossypii*/plant (77, 18, 6), which landed on alemow, Cleopatra mandarin and Carrizo citrange seedlings respectively during the indicated period. Table 2 also shows the actual CTV incidence in the experimental nursery blocks used for monitoring aphids. The number of competent CTV vectors visiting seedlings is consistent with the incidence and spread of the virus. CTV infection rates seem proportional to the number of viruliferous aphids landing on alemow or Cleopatra mandarin seedlings (i.e., proportional to the number of young actively growing shoots). Nevertheless, despite the number of

TABLE 1
NUMBER OF *APHIS GOSSYPII* PER TOTAL NUMBER OF APHIDS CAPTURED BY THE STICKY SHOOT METHOD[a] ON DIFFERENT CITRUS ROOTSTOCKS GROWN IN NURSERY BLOCKS IN THE SAME AREA

Citrus species	January	February	March	April	May	June	Total tested period
Alemow	30/35[b]	4/10	30/46	63/141	1337/3876	427/1413	1891/5521
Cleopatra mandarin	19/29	3/3	35/45	31/93	542/1571	187/821	817/2562
Carrizo citrange	22/26	4/14	32/42	32/82	578/1676	203/646	786/2486
Total	71/90	11/27	97/131	126/316	2457/7123	817/2880	3579/10569
A. gossypii (%)	78.8	40.7	72.9	39.8	34.5	28.4	33.8

[a]Aphids were captured using one shoot/plant and 60 plants per citrus species. Sticky shoots remained on the plant the last 10 days of each month from January to June, 2004.
[b]Number of *A. gossypii* per total number of aphids captured on sticky shoots.

TABLE 2
ESTIMATION OF CTV INCIDENCE, THE TOTAL NUMBER OF APHIDS AND THE NUMBER OF CTV-VIRULIFEROUS *APHIS GOSSYPII* LANDING ON CITRUS ROOTSTOCK PLANTS IN EXPERIMENTAL NURSERY BLOCKS MONITORED FOR A SIX MONTH PERIOD

Citrus species	Number of aphids per plant	Number of *A. gossypii* per plant	Viruliferous *A. gossypii* per plant	Actual CTV incidence in the experimental blocks[a]
Alemow	1656	545	77	4.6%
Cleopatra mandarin	320	98	18	2.0%
Carrizo citrange	186	63	6	0.0%

[a]CTV incidence in each experimental block of 10,000 plants as evaluated by tissue print-ELISA.

viruliferous aphids visiting not only Carrizo citrange, but sour orange and citrumelo, very low or no CTV incidence was found in these seedlings, suggesting some resistance to natural infection by aphids.

Seedlings of the most susceptible citrus species, alemow, Cleopatra mandarin and even Volkamer lemon, must be protected against natural CTV infection from their initial planting (including winter time) in Mediterranean countries in which *A. gossypii* is the predominant aphid species. To achieve this, different conventional and non-conventional strategies are under evaluation.

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