Recent Experiences and Studies on the Type of Psorosis from Concordia, Argentina

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ABSTRACT. The type of psorosis that spreads naturally in Concordia is the most harmful virus affecting citrus in that area. It produces severe damage to oranges, grapefruit and to some mandarin varieties. It has spread to most of the citrus trees grown in that district and varieties not showing the characteristic bark symptoms are also carriers of the disease. This report describes the reaction of a number of citrus species and varieties to this local form of psorosis, compares it with psorosis in other citrus growing areas, reports its relationship with other viruses and its distribution in different parts of the affected trees. The aim of this paper is to contribute to a better knowledge and characterization of the disease.
Index word. Ringspot.

In 1966 Pujol and Beñatena (7) and in 1968 Pujol (5) reported on the behaviour and characteristics of psorosis-affected trees at Concordia, Entre Ríos, Argentina, which are different from those cited for psorosis A in other citrus regions of the world. The most striking differences found were the severity of the symptoms, which resemble those of psorosis B, and the possible natural spread of psorosis at Concordia. Transmission of the Concordia-type psorosis through seed was demonstrated using Troyer citrange (6) and trifoliate orange (2). However, seed transmission in trifoliate orange is too low to account for the great diffusion of psorosis in Argentina. Timmer and Beñatena (8) observed leaf flecking in sweet orange seedlings, but transmission of the bark scaling agent through seed has not been proved. These authors reported that symptoms induced by Concordia-type psorosis on citrus plants are similar to those provoked by citrus ringspot virus (CRSV). Recently, Fischer *et al.* (3) obtained mechanical transmission of the Concordia-type psorosis to herbaceous plants.

Pujol (5) reported that 15 to 20% of the sweet orange trees showed bark scaling in the Concordia citrus region. The trees ranged from 15 to 20 years old. Two hundred and sixty four sweet orange trees and 160 grapefruit trees were planted in the mother blocks at the Concordia Experimental Station in 1962. Foliar and cortical symptoms appeared on 46.5% of the sweet orange trees and 63.1% of the grapefruit trees (4).

This work reports on recent experiments that contribute to the knowledge of Concordia-type psorosis.

MATERIALS AND METHODS

The seedlings used in the five experiments reported in this paper were grown in plastic pots in a greenhouse. The potting soil received an initial fertilization and additional fertilization was added periodically by irrigation. Insecticides and fungicides were applied weekly.

Inoculations were made using bark chip grafting and two bark chips were grafted to each indicator seedling. The seedlings were cut 20 cm above the graft in order to force new growth. Symptoms on the new flush were recorded periodically.

Experiment 1. Reactions on citrus species and varieties induced by the virus associated with Concordia type psorosis. Two isolates

were used: RF-10,4 (virulent strain) and ringspot "Los Paraísos". The source of RF-10,4 was a Común tangerine showing bark scaling and ringspot "Los Paraísos" came from a Valencia sweet orange tree with leaf symptoms similar to citrus ringspot virus (CRSV) but with no bark symptoms. Seedlings of Común sweet orange, sour orange, Duncan grapefruit, Pineapple sweet orange, Común tangerine and Key lime were inoculated. Greenhouse temperature averaged 36°C maximum and 18°C minimum.

Experiment 2. A comparison of the Concordia type psorosis with isolates from Spain. The sources of isolates from Spain were: No. 1: P250-2 A (psorosis B); No. 2: Concave gum; No. 7: P 102 (psorosis A); No. 8: P 100 (psorosis A). The source of isolates from Concordia, Argentina were: No. 3: a tree with oak leaf foliar symptoms with no bark scaling from "Los Paraísos" grove; No. 4: a tree with oak leaf foliar symptoms with bark scaling from "Los Paraísos" grove; No. 5: a tree with foliar symptoms similar to ringspot from "Los Paraísos" grove; No. 6: RF-10,4 (the isolates 5 and 6 were used in the Experiment 1); No. 9: RF-10,3 (similar to RF-10,4); No. 10: RP 12 (a B type psorosis kept in greenhouse by A. R. Pujol). The indicator seedlings Común sweet orange, Duncan grapefruit and sour orange were inoculated with isolates 1 to 6 while isolates 7 to 10 were inoculated in Común sweet orange only. Greenhouse temperatures averaged 36°C maximum and 19°C minimum.

Experiment 3: Distribution of the Concordia type psorosis in psorosis-infected citrus trees. The inoculum source was a nucellar Pineapple sweet orange grafted to rough lemon showing bark lesions. The tree was planted in the foundation plot at the INTA Concordia Experimental Station. Común sweet orange seedlings were used as indicators. Greenhouse temperatures averaged 32°C maximum and 15°C, minimum.

RELATIONSHIP WITH OTHER VIRUSES

Experiment 4. Effect of the Concordia type psorosis + citrus tristeza virus (CTV) vs. the Concordia type psorosis. Común sweet orange and Key lime seedlings were inoculated with canopy bark from a Valencia sweet orange grafted to trifoliate orange infected with psorosis + CTV and bark from a shoot of the same tree from which CTV had been filtered through the trifoliate rootstock. The test was performed to check if synergism exists between CTV and this type of psorosis. CTV infection was determined by enzyme-linked inmunosorbent assay (ELISA) and by sensitive Key lime tests. The ELISA tests were conducted by the double antibody sandwich method (1). Greenhouse temperatures. averaged 32.5°C maximum and 15.3°C minimum.

Experiment 5. Effect of the Concordia type psorosis + infectious variegation (CVV). A strain of CVV from Concordia that showed positive reaction with antiserum against CVV from Florida (S. Garnsey, personal communication), and the virulent strain of psorosis, RF-10.4 (used in experiments 1 and 2), were used in this test to inoculate Pineapple sweet orange seedlings. Greenhouse temperatures averaged 32.5°C maximum and 15.3°C minimum.

RESULTS

Experiment 1. Except sour orange and Común tangerine, all inoculated seedlings showed symptoms with both virus isolates. Pineapple sweet orange, Duncan

grapefruit and Key lime showed the most striking reactions. Sour orange and Común tangerine showed small leaves with nutrient deficiencies, flecking and spots.

Experiment 2. The isolates from Concordia developed strong symptoms in Duncan grapefruit and Común sweet orange. Some of them caused the death of Duncan grapefruit seedlings (Table 1). On the other hand, the strains from Spain did not develop strong symptoms. Only the No. 10 inoculum of B type psorosis caused strong symptoms. However, after one year, the seedlings inoculated with both B isolates showed bark lesions.

Experiment 3. The inoculum taken from all sides of the source tree produced symptoms of flecking in the leaves of the Común sweet orange indicator seedlings. However, they showed different symptoms of shock or leaf spotting. In only one case, when inoculum from bark lesions was used, were the indicator seedlings killed (Table 2).

Experiment 4. Joint inoculation with psorosis and CTV did not increase symptoms of Concordia type psorosis, like shock, leaf spots or death of the seedlings. However, the number of indicator seedlings of Común sweet orange with flecking, nutritional deficiencies and small leaves increased. On the contrary, in Key lime these symptoms increased when psorosis was inoculated alone (Table 3).

Experiment 5. Seedlings inoculated with both viruses simultaneously showed a more pronounced wrinkling and spotting of the leaves than seedlings inoculated with either virus alone. No cross protection against RF-10,4 strain was obtained using CVV.

DISCUSSION

Pineapple sweet orange, Duncan grapefruit and Key lime are the most sensitive indicator plants

TABLE 1
A COMPARISON OF THE CONCORDIA TYPE PSOROSIS WITH ISOLATES FROM SPAIN

	Isolates									
	S*	S	C†	C	C	C	S	S	C	C
Symptoms	1	2	3	4	5	6	7	8	9	10
Duncan grapefruit										
Death	—	—	+	+	+	—				
Shock	—	—	+	+	+	+				
Crinkly leaf variegation	—	—	—	—	+	+				
Común sweet orange										
Shock	—	—	—	+	+	+	—	—	+	+
Spots	+	—	—	+	+	+	—	—	+	+
Rings	—	—	—	—	+	—	—	—	—	—
Flecking	+	—	—	—	—	—	+	+	—	—
Crinkly leaf variegation	—	—	—	—	—	—	—	—	—	—
Sour orange										
Spots	—	—	+	—	—	—				
Crinkly leaf variegation	—	—	—	—	+	—				

* S = source Spain.
† C = source Concordia.

TABLE 2
DISTRIBUTION OF CONCORDIA TYPE PSOROSIS IN PSOROSIS INFECTED CITRUS TREES

Location in the tree where inoculum was taken	Reaction			
	Shock	Flecking	Spot	Death
root	0/4*	2/4	0/4	0/4
rootstock shoot	0/4	1/4	0/4	0/4
bark lesion	2/4	3/4	2/4	2/4
0.50 m from the lesion	0/4	4/4	1/4	0/4
1.00 m from the lesion	0/4	1/4	2/4	0/4
at skirt height	1/4	2/4	2/4	0/4
1.50 m from the soil surface	0/4	1/4	2/4	0/4
2.00 m from the soil surface	0/4	3/4	1/4	0/4
canopy shoot starting to show shock symptom	1/2	1/2	1/2	0/2
negative control	0/4	0/4	0/4	0/4

* No. plants positive/No. plants inoculated.

for indexing psorosis from Concordia in our experience. Local isolates of psorosis are more virulent than psorosis isolates from Spain.

Inocula taken from all parts of the infected tree induced foliar symptoms on indicator seedlings. However, shock symptoms were obtained only when inoculation was done with plant material taken from trunk lesions, the tree skirt, and canopy shoots showing initial symptoms of shock. Pujol and Beñatena (7) reported that plant tissues taken from asymptomatic parts of diseased trees induced no symptoms when inoculated into sweet orange seedlings. Timmer and Garnsey (9) also found uneven distribution of CRSV in diseased host trees.

If psorosis from Concordia is a complex of components, the shock symptom must be induced by one component and foliar symptoms by another one. Also, the distribution of the components in the infected trees must vary with the foliar symptom component predominant over the shock symptom component. Furthermore, if the shock symptom is due to a higher concentration of the Concordia type psorosis agent, different concentrations of the virus should be found in different parts of the infected trees. A possibility exists that the Concordia type psorosis agent is mixed with the psorosis A type with the A type better distributed in the trees. The psorosis A type would provoke foliar symptoms and the Concordia type the shock symptoms.

Concordia type psorosis when combined with CTV seemed to increase the symptoms of flecking and nutrient deficiency, and to reduce the leaf size. However, the shock symptom, death or size of the seedlings was not affected. A lower number of shock symptoms and seedling death was observed in Key lime. Even though synergism is dubious this experiment should be repeated to corroborate these results.

Trees infected with the Concordia type psorosis show foliar symptoms as rings, spots, variegation, deformation, and wrinkling which appear by December or January, on the second flush of growth of the season. Flecking and oak leaf symptoms are seen in September on the first flush of growth. Shock symptoms were induced on indicator seedlings when inoculum from trees showing oak leaf symptoms was used. This indicates that

TABLE 3
EFFECT OF PSOROSIS (PSO) + TRISTEZA (CTV) AND PSOROSIS ALONE

Inoculum	Shock	Flecking	Spot	Reaction: Nutrition deficiency	Little leaf	Plant death	Vein clearing
Común sweet orange							
pso. + CTV*	2/4†	3/4	2/4	3/4	3/4	0/4	0/4
pso. — CTV	2/4	0/4	2/4	0/4	1/4	0/4	0/4
healthy control	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Key lime							
pso. + CTV	3/4	0/4	2/4	0/4	0/4	2/4	2/4
pso. — CTV‡	4/4	0/4	0/4	0/4	0/4	4/4	0/4
healthy control	0/4	0/4	0/4	0/4	0/4	0/4	0/4

* CTV infection determined by ELISA test.
† No. plants positive/No. plants inoculated.
‡ No test for CTV was done because the plants died after psorosis inoculation.

the trees were infected with both "concave gum" (CGV) and Concordia type psorosis.

Seed transmission of psorosis virus reported by Pujol (6) and Campiglia (2) was done with seeds from trees that, possibly, were infected with more than one virus. It is not known if the Concordia type psorosis virus was present in the seed source trees.

The observations and tests done in this work indicate that the Concordia type psorosis shows similarities with the citrus ringspot virus in symptomatology, natural diffusion (5, 7), host range and mechanical transmission to herbaceous plants (3).

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