OTHER VIRUSES

Defining Psorosis by Biological Indexing and ELISA

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ABSTRACT. In 1958, the Citrus Clonal Protection Program at the University of California at Riverside established a virus bank with collections of various graft-transmissible pathogens of citrus to be used primarily as positive controls in their indexing program. Included in this virus bank are a number of psorosis and concave gum isolates in sweet orange holding plants. An experiment was done testing 18 psorosis and concave gum isolates from this collection by indexing them to specific indicator plants and then applying cross protection for identifying the isolate as positive or negative for psorosis-A. The results showed: 1) distinct differences between psorosis-A and concave gum; 2) large variability in symptom expression among the various psorosis isolates; 3) a pronounced effect of temperature on symptom expression; and 4) the segregation of psorosis-A and non-psorosis sources by cross protection. Many of these psorosis isolates from our virus bank have been used worldwide as standards in indexing programs for defining psorosis-A or concave gum. Thirteen psorosis-like and three concave gum isolates were tested for psorosis by ELISA; 12 of the former were positive while the concave gum and a non-psorosis isolate, P-214, were negative.

Citrus psorosis virus (CPsV) is the oldest known graft-transmissible disease of citrus and remains as one of the more serious diseases of citrus, especially in those countries where indexing and certification are not practiced and where there is a strong indication of natural spread. The early history of psorosis was reviewed by Wallace (28, 29) and more recent developments by Roistacher (25) and by Timmer and Benateña (26). Fawcett and Klotz (11) showed that there are two symptomatic components of psorosis which they called psorosis-A and psorosis-B, and Wallace (28) showed that psorosis-A would protect a sweet orange seedling against a challenge from the more severe bark lesion forming psorosis-B.

New technology is now defining the nature of the psorosis virus (7, 15). The virus has been purified, antibodies obtained and ELISA indexing has accurately discriminated between psorosis-A and other leaf mottling diseases (1, 6, 8, 9, 12, 14, 15, 17). The international committee on citrus taxonomy have approved the name citrus psorosis ophiovirus (19).

At one time, concave gum, crinkly leaf, infectious variegation and blind pocket were all called psorosis because they induced leaf flecking symptoms on indicator plants (10, 29). Leaf flecking viruses such as infectious variegation (which has been well characterized as an ilarvirus), concave gum and Dweet mottle do not protect against a challenge with psorosis-B lesion inoculum and for this reason, plus distinct differences in symptomatology in field trees and indicator plants, they should not be classified as psorosis-A (4, 20, 21). Other leaf flecking diseases also should not be classified as psorosis (2, 3, 16, 18, 25, 29). The 48 KDa capsid protein associated with the CPsV is absent in plants infected with concave gum and two other fleck-inducing agents, cristacortis and impietratura (5). A citrus virus collection (virus bank) was established at the Citrus Clonal Protection Program at the University of California at Riverside (UCR) in 1958. It is an extensive collection

consisting of various citrus virus and virus-like pathogens which have been used for research but used primarily as positive controls during comprehensive indexing tests in the California Citrus Clonal Protection Program. Results on the indexing performance of 11 psorosis sources from the virus bank at UCR over a 27-yr-period have been reported (25). Many of these psorosis sources have been, and are being used by other research workers throughout the world as positive controls and also used to test the validity of new detection methods by ELISA or PCR, or for observing virus particles (7, 15).

This paper gives the history and background and the results of biological indexing of 14 psorosis and psorosis-like sources, plus four concave gum sources. All were obtained from the virus bank at UCR and their biological indexing is compared against indexing done by ELISA.

MATERIALS AND METHODS

Eighteen various known and unknown psorosis and concave sources present in the virus bank were indexed to specific indicator plants. The origin and background history of these psorosis and concave gum sources are given in Table 1. The methods of indexing and cross protection were based on the UC system of plant growth (24). Immunological testing of many of these isolates using ELISA, DAS-ELISA, TAS-ELISA and monoclonal antibodies (MA) have been described (1, 6, 8, 9, 12, 15, 17). All of these virus sources were maintained in holding plants of sweet orange in a screenhouse at the University of California Rubidoux facility in Riverside. Some of the sources were collected between 1938 through the 1960's by Drs. Fawcett, Wallace and Calavan and have been continually in use as positive controls over these many years. Many of these psorosis sources, numbered from P-200 to P-216, plus the concave sources numbered from C-301 to C-306 are used worldwide as positive controls for indexing and for testing new technologies. Five virus sources, collected from the field at the Citrus Research Center and listed by field, row and tree number were included in this study because, in previous index tests, they had shown definitive leaf patterns in sweet orange or Dweet tangor indicator seedlings and their status as a psorosis-A was not known.

Indicator plants used for indexing were Madam Vinous sweet orange and Eureka lemon seedlings plus 861-S-1 citron grafted on a rough lemon rootstock. Dweet tangor was substituted for the Eureka lemon as the indicator for the four concave gum sources. All plants were grown individually in 15 cm by 15 cm deep tapered plastic containers at the Rubidoux glasshouse. Soils, fertilizers and plant care was based on the U.C. system of plant growth (24). After inoculation, the Madam Vinous plants were divided into two groups; one held at relatively cool temperatures of 24° to 27°C maximum day and 18° to 21°C minimum night and the other held at warm temperatures of 28° to 38°C maximum day and 25° to 27°C minimum night. Plants were inoculated in February and periodically observed for symptoms beginning 4 wk after inoculation through mid-June. Four and one half months after initial inoculation, cross protection was done by challenging the infected Madam Vinous seedlings with psorosis-B lesion bark inoculum by the method described by Roistacher (24). Final readings for cross protection were made two months after challenge inoculation.

Citron plants were kept in the cool temperature room for the first 3 mo and during this time the emerging leaves were observed for leaf

TABLE 1
THE ORIGIN OF THE PSOROSIS AND CONCAVE GUM SOURCES USED IN THIS EXPERIMENT

Code	Species	Year collected	Location[z]	Viroids[y]	Notes
P-200	Sweet orange	1958	CRC 11G-6-18	CVd-IIa?	Severe bark scaling.
P-201	Citrus species	1963	CRC 8A-33-16	Neg	Imported from Hupeh, China, early 1900's; No bark scaling in the field tree.
P-202	Sweet orange	1959	CRC 11G-6-18	CEVd	Severe bark scaling.
P-203	Kao Panne pummelo	1966	CRC 8A-13-18	Neg	Imported from Thailand in early 1900's. No bark scaling.
P-205	Valencia orange	1966	Sespe 4-5-12	Neg	From Ventura County. A 50 year old tree showing no bark scaling but whose progeny showed over 60% bark scaling.
P-209	Navel orange	1971	Stuttsman grove	Neg	From Tulare county showing severe bark scaling.
P-211	Robertson navel	1975	Tulare County	CEVd	No bark scaling but showed leaf symptoms in the field tree.
P-212	Prior Lisbon lemon	1970	Hardison ranch	CVd-IIa,b	From Ventura County. A mechanically transmitted psorosis with mild reacting CVd.
P-213	Grapefruit seedling	1978	CRC 8C-2-19	Neg	A ringspot type psorosis collected by Wallace and Drake.
P-214	Imperial variegated	1982	CRC 7C-51-13	—	A sweet orange from Florida, severely stunted, severe reaction in Dweet tangor, sweet and sour orange. No shock in sweet.
10A-21-14	Robertson navel	1979	CRC 10A-21-14	—	Severe bark scaling in the field tree.
14-18-1	Valencia orange	1979	CRC 14-18-1	—	Severe bark scaling in the field tree.
7B-15-14	Selecta orange	1979	CRC 7B-15-14	—	Tree in decline on Carrizo citrange. Original import was CRC- 1045 from Brazil; introduced in the early 1900's. Indexed positive in Dweet tangor with a leaf flecking.
7B-38-7	Chinese pummelo	1979	CRC 7B-38-7	—	Tree in decline in the field; no bark lesions - initial strong reaction in Dweet tangor. Original import as CR-325 and introduced in 1914.
7B-40-1	Howell grapefruit	1979	CRC 7B-40-1	—	Introduction from Florida in 1914. Symptomless in the field. Index to Dweet tangor shows OLP's.
C-301	Sweet orange	1960	CRC 11G-9-4	Neg	Originally from Fawcett #571. Concavities in the trunk and consistently induced OLP in indicator plants.
C-302	Valencia orange	1962	CRC 11E-3-19	Neg	A Valencia orange collected by Dr. Fawcett in October, 1938 showing concavities on the trunk at Rancho Santa Ana, California. Fawcett No. 516.
C-303	Sweet orange	1973	CRC 14A-20-2	CVd-IIa?	Original tree showed concavities. Tristeza removed by passage through Troyer citrange.
C-306	Sweet orange	1977	CRC 14A-21-1	Pos	Original tree showed concavities. Tristeza removed by passage through Troyer citrange.

[z]CRC = Citrus Research Center at Riverside with Field-Row and tree number.
[y]CEVd = citrus exocortis viroid; CVd = Citrus viroid.

symptoms. The citron plants were then transferred to the warm room for detection of citrus viroids.

Details for the technology used for testing by ELISA are given by Djelouah and D'Onghia (8) and D'Onghia et al. (6) using the antiserum developed by Garcia et al. (12) which was produced against Florida psorosis isolate CtRSV-4 (13). Also, monoclonal antibodies developed against an Italian psorosis source by Djelouah et al. (9) was used to test our numbered psorosis "P" sources. The resulting values were based on the mean absorbance for the two wells. Each of the various psorosis "P" sources or the concave gum "C" sources were tested by ELISA a minimum of at least ten times, and the range of values obtained are given in the results.

RESULTS AND DISCUSSION

Table 2 summarizes the results of indexing for the 18 various virus sources. Shown are shock reaction in the emerging young shoots, young leaf symptoms, oak leaf patterns (OLPs) and leaf mottle or crinkling. Also given is the viroid reaction in citron and the positive or negative cross protection reactions after challenging the sweet orange with psorosis-B lesion inoculum. A negative reaction indicates protection and a positive reaction indicates no protection to the challenge inoculation.

The results obtained confirm previous findings. Psorosis induced shock reaction in many indicators, and leaf mottle and yellows in citron under cool conditions; concave gum induced none of these symptoms. The Howell grapefruit source (7B-40-1) produced strong concave gum associated-OLPs, and flecking in several indicators, and displayed cross protection against psorosis-B, indicating it was infected by both viruses.

The leaf mottle induced by different psorosis isolates varied considerably with each isolate from a mild general mottle in the young flush to intense chlorotic spotting and variegation in mature leaves, confirming what has been previously reported (23, 24).

The importance of temperature for symptom expression was clearly evident, especially for the shock reaction (Table 2). Leaves tended to develop spotting at warm temperatures rather than a general mottle and vein clearing (23, 24). The OLPs of concave gum were also more striking under cool conditions. Temperature also appear to effect virus concentrations; Djelouah (unpublished data) has found that ELISA on field samples gives lower readings than for samples from trees in a cool greenhouse. We emphasize that leaf symptoms alone do not define psorosis, and neither does the absence of bark scaling. Isolate 7B-38-7 from a Chinese pummelo showed no bark lesions in the field tree, but induced shock reaction and leaf mottle in indicators, and displayed cross protection against psorosis-B (Table 2). There are isolates of the ringspot form of psorosis that do not induce bark scaling, e.g. Florida ringspot-4b (13). The Selecta orange isolate (7B-15-14) also had no field symptoms, but induced interveinal flecking under cool conditions in Madam Vinous and Eureka lemon. It gave no cross protection against a psorosis-B challenge. It would appear to be infected by an unknown pathogen, sharing some of the chacteristics of non-psorosis agents reported elsewhere by Navarro et al. (16) and Powell et al. (18). Isolate P-214 similarly was shown to be a non-psorosis agent.

As shown in Table 3 all of the virus bank psorosis-A "P" sources indexed positive by ELISA with the exception of P-214, which, as mentioned, had been shown by symptomatology and cross protection in previous experiments, as not related to psorosis-A. Also, the three con-

TABLE 2
RESULTS OF INDEXING OF VARIOUS VIRUS SOURCES ON INDICATOR PLANTS HELD AT COOL AND WARM TEMPERATURES

Virus source	Madam Vinous sweet orange				Citron			Eureka lemon		Madam Vinous Challenged with psorosis B lesion inoculum
	Cool		Warm		Cool		Warm	Cool		
	Shock	Young lf	Shock	Young lf	Shock	Young lf	Viroid[z]	Shock	Young lf	
P-200	-	-	-	-	-	stunt	+	++	++	-
P-201	++	++	-	++	total[y]		-	-	-	-
P-202	-	mot++	+	++	total	-	++	-	+++; crlf+++	-
P-203	++++	++++	+++	++++	total	++++	+++	+++	crlf+++	-
P-205	++++	++	-	++	++	mot++	-	++++	++++; crlf++	-
P-209	++++	++	-	+++	total	sev stunt	-	+++	mot++	-
P-211	++	+++	-	+++	-	++++	+++	-	+++	-
P-212	++++	++	-	++[x]	-	-	+	-	++	-
P-213	+	mot++	++	+++[x]	total	stunt	-	-	+++	-
10A-21-14	++	yel++	+	++	total	yel+++	-	++++	++ (dead)	-
7B-38-7	+++	yel+++	-	spots[x]	-	+	-	-	++	-
7B-40-1	-	++[w]	-	+[w]	-	mot+++	++	-	OLP++; young lf+	-
14-18-1	++	crlf	-	+	-	yel++	++	-	+++; crlf++	-
7B-15-14	-	+[v]	-	-	-	-	-	-	+[w]	+ (not a Ps-A)
								Dweet tangor		
C-301	-	OLP+++	-	OLP+	-	-	-	-	OLP+++	+ (CG)
C-302	-	OLP++	-	OLP+	-	-	-	-	OLP+++	+ (CG)
C-303	-	OLP++	-	OLP+	-	-	+++	-	OLP+	+ (CG)
C-306	-	OLP++	-	OLP+	-	-	+++	-	OLP+	+ (CG)

[z]Citron plants removed from cool room to the warm room after 3 mo for viroid evaluation.
[y]Total = Complete shock with death of all the young developing shoots.
[x]Spotting in MV under warm conditions is different than under cool conditions.
[w]Mild psorosis like mottle and interveinal clearing - psorosis-like but indefinite.
[v]Interveinal clearing, no OLP, psorosis-like reaction, but indefinite.
[u]Abbreviations: lf = leaf; yel = yellow leaf; crlf = crinkly leaf; mot = mottle; OLP = oak leaf patterns; Ps-A = psorosis-A; CG = concave gum. Symptoms -negative; + mild; ++ moderate; +++ severe; ++++ very severe.

TABLE 3
DAS-ELISA TESTS ON VARIOUS PSOROSIS AND CONCAVE SOURCES[z]

Virus source	Mean absorbance values	
	Infected	Negative control
P-200	0.240-0.270	0.025-0.035[y]
P-201	0.120-0.130	0.020-0.035
P-203	0.100-0.130	0.020-0.035
P-203M[x]	0.230-0.250	0.025-0.045
P-205	0.100-0.140	0.030-0.040
P-208	0.120-0.170	0.030-0.040
P-209	0.350-0.400	0.030-0.045
P-213	0.225-0.280	0.030-0.045
P-214	0.020-0.035	0.025-0.045
P-215M	0.150-0.180	0.025-0.045
P-216	0.180-0.210	0.025-0.045
P-216M	0.250-0.280	0.025-0.045
P-250	0.210-0.250	0.025-0.045
C-301	0.020-0.035	0.025-0.045
C-302	0.020-0.035	0.025-0.045
C-306	0.020-0.035	0.025-0.045

[z]These sources originated at the University of California, Riverside virus bank. The best results were obtained in the cooler months from September to May. These results reflect an average of over 10 tests for each source used as positive controls against other suspect sources in Southern Italy.
[y]The range of a number of readings.
[x]M = Mechanically transmitted source.

cave gum sources C-301, C-302 and C-306 did not react serologically with antiserum derived from a psorosis source. Similarly, in a number of experiments by Garcia et al (12), Djelouah and D'Onghia (8), and D'Onghia et al. (6) they reported that all of our psorosis 'P' sources reacted positively with antibodies raised against two separate psorosis virus sources. One, from a Florida source (6, 8, 12) and the other from an Italian source (9). The low absorbance values found for some sample in ELISA can be explained by low titer, or as shown in recent work using MAs (9) the presence of these sources of different virus strains that are serologically related but not identical to the citrus psorosis virus strain used to raise the antiserum employed. Recently, Alioto et al. (1) and Potere et al. (17) reported new improvements to the ELISA protocol giving positive recognition to psorosis sources reported here and also to six sources from Argentina, seven from Italy, 10 from Spain and one from Uruguay. Recent studies (unpublished) also recognized Palestinian, Egyptian and Maltese psorosis sources. The results of ELISA indexing of our "P" psorosis sources correlates well with biological indexing which affirms that our "P" sources are true psorosis-A as defined by Fawcett and Klotz (11) and confirmed by cross protection (28).

In conclusion, the correct use of biological indicators to define psorosis isolates as those that induce shock reaction, various leaf symptoms and cross protection against psorosis-B was confirmed on several isolates by ELISA. Unknown isolates, suspected to be psorosis should be inoculated onto indicators under cool conditions, and then preferably subjected to ELISA and, if possible, PCR. Confirmation by cross protection should then be done.

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