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Evaluation of Different Citrus as Psorosis Disease Indicators and as Psorosis Virus Propagation Hosts

M. L. Garcia, N. B. Costa, C. M. Casafús and A. N. Sarachu

ABSTRACT. Indexing for psorosis is usually performed on Pineapple sweet orange. Partial purification of citrus psorosis virus (CPsV) was achieved from leaves of Pineapple sweet orange and Eureka lemon but infectivity on *Chenopodium quinoa* was usually low. We tested 14 citrus species and cultivars as psorosis indicators and as sources for CPsV purification.

Typical psorosis symptoms on seedlings inoculated with isolate 90-1-1 were recorded and infectivity assayed on *C. quinoa*, with the following results: 1) At 30 days post infection Temple mandarin, Cleopatra mandarin and Eureka lemon were as good as Pineapple sweet orange indicators of psorosis. Trifoliolate orange, Troyer citrange, King of Siam mandarin, rough lemon, Malvasio mandarin, Duncan grapefruit, sour orange, Rangpur lime, Madame Vinous sweet orange and Ellendale mandarin were poor indicators; 2) Leaf material from Temple mandarin and rough lemon was in general more infectious than that from Eureka lemon and Pineapple sweet orange. King of Siam mandarin, Duncan grapefruit, Cleopatra mandarin, sour orange and Rangpur lime consistently produced less infectious material; 3) Survival of Temple mandarin was shorter than rough lemon and Eureka lemon was longer than rough lemon.

Two conclusions were reached: a) Temple mandarin, Cleopatra mandarin and Eureka lemon could be used as Pineapple sweet orange for psorosis indexing; b) rough lemon could advantageously replace Eureka lemon and Pineapple sweet orange as source of material for CPsV propagation.

Psorosis is the most important viral disease in the main citrus-producing regions of Argentina, accounting at present for the loss of about 8% of orange and grapefruit trees per year (5). Pineapple sweet orange is the usual indicator plant for psorosis disease (6). In a previous report (7) we showed that Eureka lemon was also a good indicator for Isolate 90-1-1 from the citrus producing region of Concordia, Argentina, and as efficient as Pineapple sweet orange as starting material for purification of the disease agent. However, infectivity of extracts from either plant on *C. quinoa* was usually low, which has been a major limitation for the progress of our work on virus purification and characterization. Therefore, we undertook an evaluation of several available citrus and citrus relatives as psorosis disease indicators and as starting material to purify virus.

MATERIALS AND METHODS

Inoculation of seedlings and symptoms recording. Seedlings 12 to 24-months old were inoculated by grafting with two bark chips per seedling with psorosis isolate 90-1-1 (Concordia, Argentina) and cut back to 20

cm above the graft in order to force new growth. Symptoms (flecking, spots, shock, death) on the new flush were recorded periodically, from 30 to 534 days post inoculation (pi). The following citrus and citrus-relatives were included: trifoliolate orange (TO), Duncan grapefruit (DGF), sour orange (SO), Rangpur lime (RPL), Madame Vinous sweet orange (MVSwo), Temple mandarin (TM), Malvasio mandarin (MM), King of Siam mandarin (KSM), Ellendale mandarin (EM), Cleopatra mandarin (CM), Troyer citrange (TC), rough lemon (RL), Pineapple sweet orange (PASwo) and Eureka lemon (EL). Eight to 20 seedlings of each species were employed. Noninoculated PASwo seedlings were used as controls.

Statistical analysis. Symptoms at 30 and 48 days pi were analyzed statistically. The number of seedlings showing each symptom were divided by the number of inoculated seedlings (Table 1). The values obtained (fractions) were subjected to factor analysis, which gives the contribution of information of each variable (symptom) to the statistical analysis according to the contribution of the variances. Clustering by average method (8) in a four-di-

TABLE 1
NUMBER OF SEEDLING OF CITRUS SPECIES AND CULTIVARS WITH PSOROSIS SYMPTOMS

Species	Days Postinoculation									
	30		48		78		149		534	
	F	SPSH D	F	SPSH D	F	SPSH D	F	SPSH D	F	SPSH D
TO(20) ^y	0	11 0 0	0	3 1 0	0	5 1 0	0	2 0 0	0	8 4 0
DGF(20)	1	11 13 0	0	6 13 3	1	2 1 0	0	1 1 9	0	10 2 10
SO(20)	2	18 2 0	1	11 0 0	2	7 2 2	0	8 1 6	0	8 0 12
RPL(89)	1	3 1 0	4	8 1 0	1	7 2 0	1	6 0 0	0	8 4 0
MVSWO(10)	0	3 3 0	1	6 5 0	1	1 3 1	0	5 2 2	0	4 3 3
TM(8)	2	6 2 0	0	2 2 0	1	4 2 2	0	1 0 4	0	0 0 7
MM(8)	0	5 5 0	1	6 1 0	1	8 4 0	0	5 0 0	0	4 5 1
KSM(10)	1	4 1 0	2	3 2 0	1	2 1 3	1	1 0 4	1	5 0 5
EM(20)	3	11 1 0	6	15 1 0	7	16 2 0	0	11 0 0	0	15 10 2
PASwO(20)	7	14 15 0	4	10 17 0	6	14 15 0	0	15 0 0	1	13 12 1
EL(20)	8	15 14 0	3	16 16 0	3	13 3 1	0	8 0 7	0	8 2 12
CM(8)	3	6 4 0	1	7 6 0	2	6 3 0	0	5 9 0	1	4 5 1
TC(8)	0	5 0 0	0	1 3 0	0	2 2 0	0	0 0 0	0	3 2 0
RL(10)	0	3 10 0	3	9 10 0	0	9 9 0	0	6 0 1	0	6 7 2
HPASwO(10) ^x	0	0 0 0	0	0 0 0	0	0 0 0	0	0 0 0	0	0 0 1

^yNumber of seedlings showing flecking (f), spots (SP), shock (SH), or were dead (D).

^xNumber of initial seedlings.

^zHealthy Pineapple sweet orange seedlings.

mensional space (four symptoms) was used to discriminate different classes of citrus as psorosis indicators. The Karhunen-Loeve Expansion method (3) was applied to the four symptoms to reduce them to one dimension giving an index. The indexes were subjected to cluster analysis in one dimension.

Partial purification and infectivity assays. Leaves from inoculated seedlings were collected at different times pi, ground in mortar and homogenized with 7 ml/g of homogenization buffer (HB: 0.050 M Tris-HCl pH 8; 0.1% cysteine; 0.1% ascorbic acid; 0.5% 2-mercaptoethanol). The homogenate was filtered or centrifuged at low speed (10,000 x g, 10 min), plant debris was reextracted with 3 ml/g of HB and supernatants were combined and clarified with 8% (v/v) CCl₄ for 10 min with gentle stirring. After low speed centrifugation, the aqueous phase (crude extract: CE) was subjected to high speed centrifugation (300,000 x g, 1 hr); the pellet was resuspended in HB (P₅₄). P₅₄ was inoculated with spatula onto 6 leaves of *Chenopodium quinoa* predested with carborundum (15 μl per leaf).

Necrotic lesions were counted 6 to 8 days after inoculation.

RESULTS

Four typical psorosis symptoms were considered to evaluate the species as psorosis indicators: flecking, shock, spots and death of the seedlings. According to the factor analysis, flecking and spots carry discriminatory information in 91% and 78% at 30 and 48 days pi, respectively, indicating that the rest of symptoms do not contribute significantly. After 48 days these symptoms have less discriminatory power.

The four symptoms were subjected to a cluster analysis at 30 and 48 days pi. The results indicated that at 30 days pi PASwO, TM, EL and CM belong to the same class and the rest of citrus to another class. At 48 days pi two different classes were obtained: DGF was separated from all the other 12 citrus. The indexes obtained by the transformation of Karhunen-Loeve (Table 2) and the clustering analysis confirmed these results. At 30 days pi the class of PASwO, TM, EL and CM remained

TABLE 2
EVALUATION OF CITRUS SPECIES AND
CULTIVARS AS PSOROSIS INDICATOR
HOSTS

Species	Days Postinoculation	
	30	48
TO	0.25 ^z	0.11 ^z
DGF	0.51	0.23
SO	0.49	0.39
RPL	0.28	1.00
MVSwoO	0.25	0.53
TM	0.55	0.20
MM	0.51	0.59
KSM	0.27	0.35
EM	0.34	0.69
PASWO	0.76	0.58
EL	0.79	0.73
CM	0.71	0.76
TC	0.28	0.13
RL	0.51	0.92
H-PASwo ^y	0.00	0.00

^zPsorosis indicator index.

^yHealthy Pineapple sweet orange seedlings.

in the same class which also included RL, DGF, SO and MM. At 48 days pi more species behaved as PASwo and only RPL and RL were not included in this class.

In addition to recording symptoms, leaves were collected from inoculated seedlings and subjected to virus purification and infectivity assay on *C. quinoa*. Expression of the results of infectivity assays must take into account the following facts: a) for any time postinoculation, all the citrus leaves collected were used for purification and their weight varied from one species to another; b) the infectivity assay is intrinsically variable; c) citrus samples from different times pi were necessarily assayed on different *C. quinoa* plants; d) we were looking for a propagation host better than those routinely used (EL and PASwo). Therefore, results for any species were expressed as the ratio between the number of lesions per gram of leaves of that species and the number of lesions per gram of leaves of EL, assayed in parallel. Fig. shows the infectivity results for the 14 species and varieties included in this study.

Leaf material from Temple mandarin and rough lemon was consistently

as or more infectious than that from the reference species (Eureka lemon; Fig. 1 A), whereas infectivity of material from Pineapple sweet orange was in general similar to that of the reference (Fig. 1 B). Although death of the inoculated seedlings was taken as an indication of susceptibility to psorosis disease, it becomes important when a species is evaluated as virus propagation host. In this respect, the first death was recorded 78 days pi for EL (1 of 20 seedlings) and TM (2 of 8 seedlings), 149 days pi for RL (1 of 10 seedlings) and 534 days pi for PASwo (1 of 20 seedlings) (Table 1). At the end of our experiment (534 days pi) 12 EL (60%), 7 TM (87%), 2 RL (20%) and PASwo (5%) seedlings had died. Taken together, these results indicate that rough lemon is the best of the analyzed citrus hosts for propagation of isolate 90-1-1 of psorosis virus; infectivity and survival were higher for RL than for EL.

Infectivity of leaf material from the other 10 species fell into two groups. Malvasio and Ellendale mandarin (Fig. 1 C), Troyer citrange, trifoliolate orange and Madame Vinous sweet orange (Fig. 1 D) were highly variable in infectivity and no definitive conclusion could be drawn on their capability to support multiplication of the virus. Sour orange, Cleopatra mandarin (Fig. 1 E), Duncan grapefruit, King of Siam mandarin and Rangpur lime (Fig. 1 F) consistently behaved as poor psorosis virus multiplication hosts.

DISCUSSION

To the present, biological indexing has been the only method for psorosis diagnosis, which is important, particularly for propagation of healthy citrus material. Pineapple sweet orange has been usually employed as the indicator. Results presented here indicate that it is indeed one of the best psorosis indicators. Because it is important for a good biological indexing its rapidness, the results at 30 days pi would be more valuable than at 48 days pi. Thus, in the first flush, Eureka lemon,

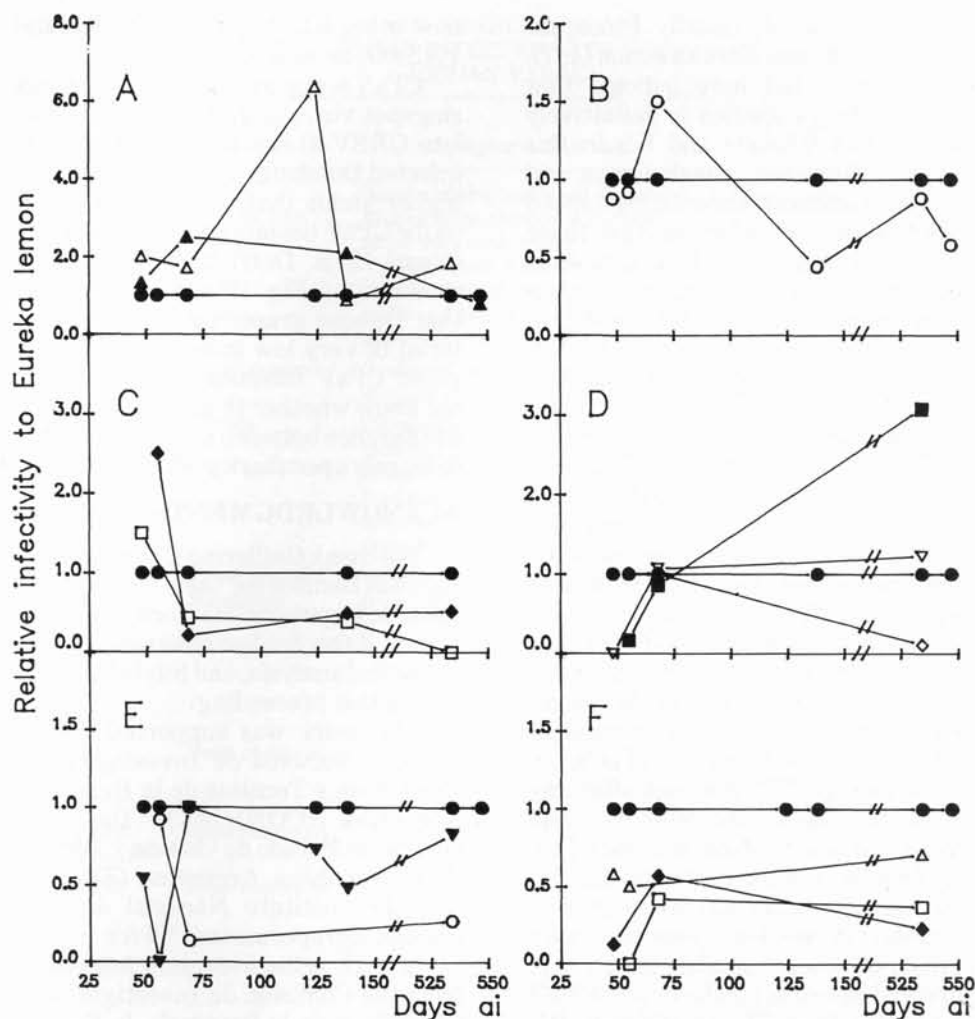


Fig. 1. Level of ineffectivity recovered from various citrus at different times after inoculation. Values for Eureka lemon (●) were taken as 100%.

1A: Temple mandarin (▲); Rough lemon (Δ). 1B: Pineapple sweet orange (○). 1C: Malvaiso mandarin (□); Ellendale mandarin (◆). 1D: Trifoliate orange (▽); Madam Vinous sweet orange (◇); Troyer citrange (■). 1E: Sour orange (▼). Cleopatra mandarin (○). 1F: King of Siam mandarin (Δ); Duncan grapefruit (□); Rangpur lime (◆).

Cleopatra mandarin and Temple mandarin are also reliable indicators. On the other hand, the index obtained could be used to classify new species in future statistical analyses.

In short, we believe that, at 30 days pi, any of the four species belonging to the class of PASwO could be used for routine psorosis indexing. The choice will depend on available or affordable facilities and on personal preferences. Indexing for psorosis, and probably for any disease, in a given region should

take into account not only the generally recommended specifications (indicators, symptoms, etc.) but also the experience in that particular region. In our case, the factor analysis indicated that spots and flecking are the symptoms that give the major information to analyze the psorosis indicators from Argentina.

Purification and characterization of CPsV has progressed slowly until now. One of the suspected reasons is the low virus concentration in plants used as

starting material, usually Pineapple sweet orange and Eureka lemon (4, 7). Results reported here indicate that none of the 14 species is definitively better than PASwO and EL in this respect. However, rough lemon and Temple mandarin rendered high speed pellets as or more infectious than those obtained from EL, which, in turn, were similar or slightly more infectious than those from PASwO (Fig. 1A and 1B). Nevertheless, two additional considerations deserve attention: a) for the first three flushes, material from RL and TM was consistently more infectious on *C. quinoa* than that from EL (RL was 2, 1.7 and 6.4 times and TM was 1.3, 2.5 and 2.1 times higher than EL); at longer times pi these differences became negligible (Fig. 1A); b) a high percentage of infected Temple mandarin and Eureka lemon seedlings died during the experiment, whereas most rough lemon and Pineapple sweet orange seedlings survived psorosis infection up to 534 days pi (Table 1). Consequently, TM does not offer any significant advantage over EL and PASwO as source of material for CPsV purification. On the contrary, RL appears as a good alternative for the same purpose: it renders material with higher, or at least similar, infectivity, survived infection similarly to PASwO and better than EL. In addition, RL is more vigorous than EL and PASwO, so that more starting material for CPsV purification can be obtained per infected seedling. Therefore, we are

now using RL, together with EL and PASwO, as source of virus.

CPsV is apparently similar to citrus ringspot virus (1, 2, 4, 7). CRSV (isolate CRSV-4) has been purified from infected Duncan grapefruit, with much higher yields than those obtained by us for CPsV (isolate 90-1-1) on any host (1 and K. S. Derrick, personal communication). Fig. 1F and Table 1 show that Duncan grapefruit produced material of very low infectivity and survived CPsV infection poorly. We do not know whether these results reflect a difference between CPsV and CRSV or merely a peculiarity of the isolates.

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This paper is dedicated to the memory of Alberto N. Sarachu.

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