Interference of Citrus Viroids with Cachexia Symptoms on Parson's Special Mandarin

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ABSTRACT. In the routine indexing of cachexia on Parson's special mandarin, irregularities and inconsistencies of symptom expression were found. In most cases these seemed to be associated with co-infection of the original explant with exocortis. Therefore the hypothesis of an interaction between exocortis and cachexia agents was considered. An experiment was carried out by co-inoculating on Parson's special mandarin a pure cachexia isolate (X-701) and three different exocortis sources, previously characterized as severe (E-117), moderate (E-111) and mild (E-140) according to their reaction on Etrog citron. The results indicated that E-117 and E-111 interfered with cachexia symptom expression. None of the plants inoculated with X-701 and E-117 or E-111 showed cachexia symptoms, whereas all plants inoculated with X-701 alone or in combination with E-140 gave positive reactions. These results indicate that certain citrus viroids or viroid combinations may mask symptom expression of cachexia.

In the Citrus Variety Improvement Program in Spain (6) routine indexing for cachexia is done by graft inoculation to Parson's special mandarin grafted on Rough lemon (11). Indicator plants are incubated at warm temperatures (27-32C) for one year and symptom expression is, in general, very good with very consistent positive reactions on the controls inoculated with pure cachexia sources. However, in some tests results were inconsistent because some of the inoculated plants did not show any symptoms and the severity of symptoms varied among indicators of a single bioassay. Mild and irregular cachexia symptoms were in most cases induced by source trees that also produced exocortis symptoms on Etrog citron.

The above observations, with the increasing evidence that cachexia was produced by a viroid, led us to initiate experiments to study the possible interference of different strains of exocortis with cachexia symptom expression on Parson's special mandarin. During the process of these experiments, it was demostrated that the cachexia was caused by a viroid (12) and that the exocortis syndrome as indexed on Extrog citron was associated with a complex of citrus viroids (2). In addition, there has been an increasing awareness of cross-protection or interference among viroids (1, 5, 8, 9).

MATERIALS AND METHODS

Disease sources. The cachexia isolate X-701 (kindly provided by Dr. Vogel and originally coded as Corsica 114) was used in the experiments. This source was free of tristeza, vein enation and exocortis.

The exocortis sources E-117, E-111 and E-140 were used. E-117 was obtained by mechanical transmission to Etrog citron from a stunted Troyer citrange seedling graft inoculated with Nules clementine buds. It induced severe symptoms on Etrog citron Arizona 861-S-1. The source E-111 was obtained by mechanical inoculation to Etrog citron from a Navelina orange and it induced moderate symptoms on Etrog citron Arizona 861-S-1. The source E-140 was segregated by shoot tip grafting in vitro (7) from a Etrog citron Arizona 861-S-1 with severe exocortis symptoms. The segregated source induced mild symptoms on Etrog citron Arizona 861-S-1.

Inoculation. Sixty Rough lemon seedlings were each grafted with a Parson's special mandarin bud. Twelve plants were left as negative controls and the others were graft inoculated at the same time with X-701.

Twelve of these plants were left as positive controls and groups of twelve plants were simultaneously coinoculated with E-117, E-111 or E-140. Half of the plants of each treatment were incubated in a relatively cool greenhouse (18-25C) and the other half in a warm greenhouse (27-32C) for one year. After this period bark of all plants was completely removed to observe gum-like deposits induced by the cachexia agent (13).

RESULTS AND DISCUSSION

Table 1 shows the reaction of Parson's special mandarin incubated at two different temperatures and coinoculated with a pure cachexia source and the three exocortis isolates used in this study. None of the plants coinoculated with X-701 and E-111 or E-117 showed cachexia symptoms, either at 18-25C or at 28-32C. All the plants incubated at 28-32C and inoculated only with X-701 or coinoculated wit X-701 and E-140 showed typical severe cachexia symptoms, whereas only two of the six plants inoculated with the same sources, but incubated at 18-25C showed mild symptoms.

The poor cachexia symptoms observed at the cooler temperature confirms previous evidence that indicated a better symptom development in plants incubated continuously at warm temperature (23-33C) for one year than in plants incubated for four months at warm temperatures and then transferred to a screenhouse and incubated for eight additional months (11).

Our results clearly indicate an interference of the severe (E-117) and moderate (E-111) exocortis sources with symptom development of the cachexia source X-701. The disease sources were further analyzed by nucleic acid extraction and sPAGE (2). It was found that isolate X-701 contained the cachexia viroid CV-IIb (CCaV) (12), isolate E-117 contained the citrus viroids CEV, CV-Ia, CV-IIa and CV-IIId, isolate E-111 contained CV-IIa and CV-IIIc, and isolate E-140 contained only CV-Ia. Therefore it could be concluded that CV-Ia was not involved in the interference and CEV was not necessary to produce interference with CCaV. The exocortis isolates producing interference have in common the presence of viroids CV-IIa and CV-III and consequently, one of these viroids or the combination of both were involved in the interference.

In fact CV-IIa and CCaV, previously reported as CV-IIb, have been demostrated to have similar physical and biological properties (2, 4), including a high degree of sequence homology (13).

The interference of the severe and moderate exocortis isolates with cachexia symptom development could be due to complete lack of replication of the CCaV or to a reduction of the replication rate of the CCaV. In the later case, symptoms could have developed with a longer incubation period. The recommended time for

TABLE 1
REACTION ON PARSON'S SPECIAL MANDARIN OF A PURE CACHEXIA ISOLATE COINOCULATED WITH DIFFERENT EXOCORTIS SOURCES

Inoculum		Incubation at 18-25C		Incubation at 27-32C	
Cachexia	Exocortis	No. positive/ no. inoculated[z]	Average symptom severity[y]	No. positive/ no. inoculated[z]	Average symptom severity[y]
X-701	—	2/6	1	6/6	2.8
X-701	E-140	2/6	1	6/6	3.2
X-701	E-111	0/6	0	0/6	0
X-701	E-117	0/6	0	0/6	0
—	—	0/6	0	0/6	0

[z]Data taken one year after inoculation.
[y]Symptom severity rated from 0 = no symptoms to 4 = very severe symptoms in each plant.

cachexia indexing on Parson's special mandarin is one year, but it is known that sources inducing a mild reaction on Parson's special mandarin may require a longer time for symptom development (10). In addition, symptoms do not appear in all inoculated plants (10). Since many cachexia isolates also carry other citrus viroids (12), it is possible that some of the problems found in the cachexia biological indexing could be caused by some interference between CCaV and other citrus viroids.

In our experiments, all inoculated plants were eliminated after one year of incubation for a careful symptom reading and could not be further analyzed. The findings presented in this paper have very important implications for citrus certification programs. In some cases negative cachexia indexing results on Parson's special mandarin may not necessary imply that the original source is free of the disease. The results reinforce the recommendation of using several positive mild reacting pure cachexia isolates in each test and to finish it only when the positive mild controls show symptoms (10). However, it has to be pointed that most of the well characterized mild reacting cachexia sources are known to be also infected with other citrus viroids. The establishment of a cachexia indexing method based on nucleic acid extraction and sPAGE (3) may help to solve the problems encountered in cachexia detection.

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