Interactions Among Citrus Viroids and *Phytophthora citrophthora*

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ABSTRACT. Reports from the laboratory of V. Rossetti in the early 1980's indicated that exocortis infected sweet orange and lemon displayed an ". . . induced greater resistance . . ." to lesion development by *Phytophthora citrophthora*. Severe strains of exocortis were described by Rossetti as having ". . . a disastrous effect on trees . . ." but little different from mild strains in the resistance response to *P. citrophthora*. Citrus materials containing selected variants of the more widely occurring citrus viroids CVd-Ia, CVd-IIa, and CVd-IIIb, known to induce only mild to moderate responses on *Poncirus trifoliata* rootstocks and citron were tested for *Phytophthora* lesion development on Washington navel sweet orange grafted on Carrizo citrange and viroid infected for 2 yr, rooted cuttings of Lisbon lemon from infected field trees infected with viroids for 10 yr and field trees of Valencia sweet orange on *P. trifoliata* infected with viroids for 20 yr. Data from greenhouse and field inoculations with *P. citrophthora* verified a significant reduction in lesion development in some viroid infected sweet orange but not in lemon. However, a specific and consistent relationship with a single citrus viroid could not be identified.

Among the most serious soilborne diseases of citrus are those caused by *Phytophthora* spp. Although most rootstocks are moderately tolerant, most scion tissues are susceptible to infection with sweet orange and lemon cultivars being especially affected (13). A now classical observation by Rossetti *et al.* (8) in 1980 reported that Hamlin sweet orange scions on Rangpur lime rootstocks infected with exocortis displayed resistance to *Phytophthora* infection. Although infection by severe strains of exocortis seriously inhibited the growth and vigor of trees, some "mild" strains that ". . . did not induce scaly bark symptoms . . ." also conferred a similar level of resistance to *Phytophthora*.

Further studies reporting that exocortis infection in Sicilian lemons reduced *P. citrophthora* lesions extended the initial observations and, in addition, correlated the reduced development of *Phytophthora* lesions with ". . . stunted trees . . ." (9). "Tolerance" to *P. citrophthora* was suggested (12, and unpublished data) as occurring more specifically in *Citrus exocortis viroid* (CEVd) infected citrus and was conjectured to be possibly related to the anti-fungal properties of the pathogenesis related (PR) protein P23. Investigations with transgenic citrus linked the *in vivo* activity of P23 against *P. citrophthora* and the possible development of systemic acquired resistance (SAR) (5).

When the initial observation of a relationship between exocortis disease and resistance or tolerance to *Phythophthora* infection was made (8), a comprehensive understanding of the complexity of the viroid population in citrus was not yet appreciated. At times, any moderation in exocortis-like symptoms both in susceptible rootstocks and the indexing host, Etrog citron, were interpreted as induced by "mild" or "moderate" strains of the exocortis disease agent. With the recognition of five distinct viroids as potential components of any viroid population in citrus, this relationship has been clarified (3).

Investigation of the development of *P. citrophthora* lesions in the presence of single citrus viroids as influenced by diverse experimental

conditions and plant materials is the subject of this report.

Earlier studies focused on citrus viroids with the potential for the improvement of citrus performance (1, 2, 6, 7, 10, 11) in the absence of viroid induced diseases of economic importance. Representative viroids known to induce only mild to moderate effects on two prime indicator hosts, *Poncirus trifoliata* and Etrog citron, include the well characterized citrus viroids Citrus viroid Ia (CVd-Ia), Citrus viroid IIa (CVd-IIa) and *Citrus viroid IIIb* (CVd-IIIb), which occur with wide frequency in citrus growing regions (4). Consistent with the observations of Rossetti *et al.* (8), the reduction of vegetative growth on scion cultivars grafted on *P. trifoliata* rootstock caused by these specific variants has also been documented (10, 11).

Host species highly susceptible to the citrus viroids and *P. citrophthora* were selected. These differed in age and were maintained under either greenhouse or field conditions (Table 1). Testing was accomplished over a 1-yr period. Experiments 1 and 2 were conducted during January through April under standard greenhouse conditions at UC-Riverside while Experiment 3 entailed a field study from March to June at the Lindcove Research and Experimental Center in central California.

All scion cultivars were derived from disease-tested sources from the Citrus Clonal Protection Program (CCPP) at UC-Riverside. However, preparation of test plants required varied treatment and time for propagation. Materials for Experiments 1 and 3 were prepared as budded seedlings and graft inoculated. Rooted cuttings were made for Experiment 2 from field grown Lisbon lemon that had been infected with the respective individual viroids for 10 yr, requiring two additional years of greenhouse culture to reach a size suitable for *Phytophthora* inoculation. Even though both sweet orange and lemon have been characterized as excellent reservoirs for systemic viroid infection, all sources were verified for the presence of the respective viroids. For greenhouse tests, 9-14 replicates were employed while the field experiment included 8-12 replicate inoculations.

P. citrophthora (R. E. Sm. & E. H. Sm.) Leonian (Isolate M189 from a citrus nursery in San Diego, California) was used for the inoculations after growing on clear V8 juice agar for 4 days. Two to six hours prior to inoculation, 4 mm and 5 mm discs of agar containing hyphae (for greenhouse and field inoculations respectively) were transferred to distilled water to stimulate sporangial formation. Bark flaps of a size equal to the agar discs were cut and folded back for both viroid free (controls) and viroid treated plants. An agar disc was inserted between the bark and the wood and the bark was replaced. Moistened cheesecloth covering the inoculation site was sealed with Parafilm and held in place with duct tape. The cheesecloth was kept moist for approximately 48 h to ensure the production and release of the infectious zoospores from sporangia.

Approximately 1.5-3 mo post inoculation, the bark was stripped to

TABLE 1
EXPERIMENTAL SYSTEMS EMPLOYED IN THE STUDY OF THE INTERACTION OF CITRUS VIROIDS AND *PHYTOPHTHORA CITROPHTHORA*

Exp.	Scion	Rootstock	Age	Location
1.	Washington navel sweet orange	Carrizo citrange	4 yrs	Greenhouse
2.	Lisbon lemon	Rooted cuttings from 10-yr-old trees	2 yrs.	Greenhouse
3.	Valencia sweet orange	*Poncirus trifoliata*	20 yrs.	Field

allow tracing of the lesion areas. Representative lesions pictured in Fig. 1 were induced by *P. citrophthora* on sweet orange or lemon in the greenhouse (Fig. 1A) and on sweet orange in the field (Fig. 1B). The tracings were scanned and lesion areas calculated using an Image-Pro Plus 3.0 (Media Cybernetics, Inc.) program. Means were separated using the Waller-Duncan *k* ratio t-test after appropriate ANOVA analysis indicated significant differences greater than P = 0.05.

Reduction of lesion development in citrus scion tissues was statistically significant in Exp. 1 and Exp. 3 (Table 2). In both cases, the cultivars were sweet orange. However, the most significant reduction was obtained in plants containing CVd-IIIb in Exp. 1 and CVd-Ia in Exp. 3. Test plants in these experiments were subject to similar propagation protocols. However, they exhibited the most extreme difference in tree age (4 and 20 yr) prior to inoculation with *P. citrophthora*. Variations inherent in distinctions between greenhouse and field experimentation must also be considered as a possible factor in this differential response.

No significant difference in lesion development associated with the presence of viroids was observed with rooted lemon cuttings as seen from Exp. 2 (Table 2). Nevertheless, viewing only the viroid treatments as a group, reduction in lesion size of the CVd-IIIb containing plants was greater than the other viroid treatments and thereby might be considered as the most effective viroid treatment. Although admittedly tenuous, this relationship supports the statistically significant values obtained for lesion reduction of the CVd-IIIb containing sweet orange in Exp. 1 and Exp. 3.

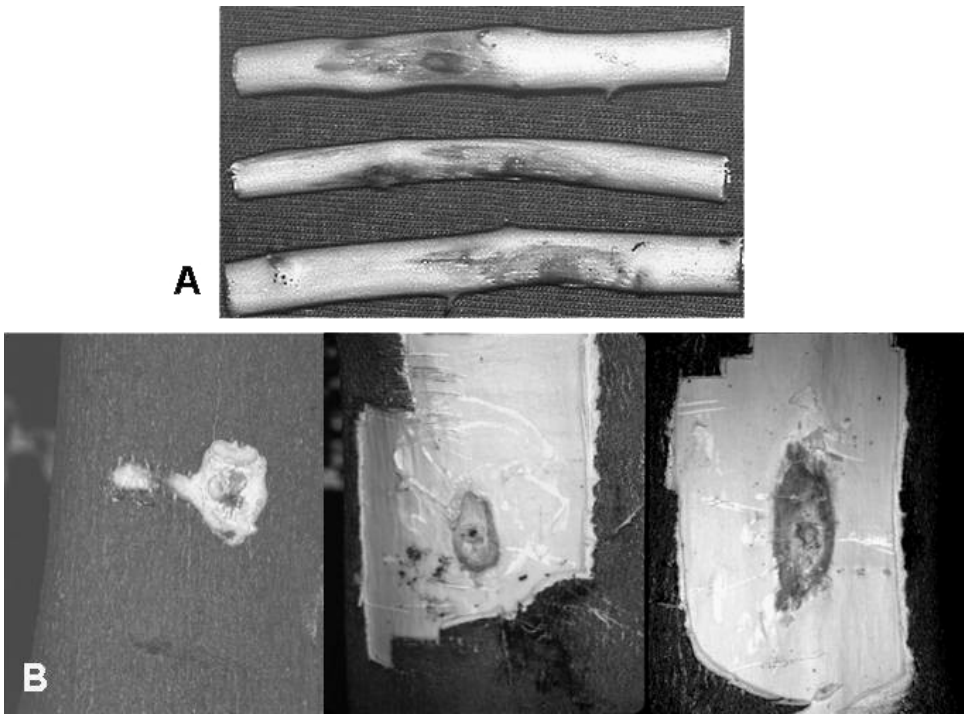

Fig. 1. Representative lesions induced by *Phytophthora citrophthora* infection of sweet orange scion tissue or lemon rooted cuttings in the greenhouse (A) or on Valencia sweet orange scions in the field (B).

TABLE 2
PHYTOPHTHORA CITROPHTHORA LESION DEVELOPMENT IN THE PRESENCE OF SINGLE CITRUS VIROID INFECTION OF SWEET ORANGE AND LEMON SCION TISSUES

	Lesion area (cm^2)[1]				
Treatment	Exp. 1		Exp. 2	Exp. 3	
Control	2.17 a		1.02 a	7.66 a	
+ CVd-Ia	2.21 a		4.06 a	2.77 b	(-64%)
+ CVd-IIa	2.40 a		3.42 a	6.12 ab	
+ CVd-IIIb	1.30 b	(-40%)	1.83 a	4.33 a	(-43%)

[1]Means were separated by the Waller-Duncan *k* ratio t-test after appropriate ANOVA analysis indicated significant differences for $P \leq 0.05$.

Exp. 3 presents the most distinct experimental system in this study due to the age of the trees (20 yr), as well as the field environment. CVd-Ia clearly inhibited lesion development most significantly. The reduction in lesion size in CVd-IIIb containing plants (~40%) equates favorably with the values obtained by Rossetti *et al.* (8, 9), and is similar to the reduction in lesion development relative to controls in CVd-IIIb containing tissues noted in Exp. 1.

These studies, in general, offer experimental support for the insightful observation of Rossetti *et al.* (8) for the moderating effect of viroid infection on *P. citrophthora* disease development. Further, it has been established here that citrus viroids other than CEVd (the causal agent of exocortis disease), are competent in inducing this response. Although it was not been possible to attribute the reduction in lesion size to a specific citrus viroid, the differential responses of tissues containing either CVd-Ia or CVd-IIIb are both pronounced with those with CVd-IIIb being the most consistent. With the results presented above, a case can be made that CVd-IIIb induces tolerance to *P. citrophthora* in sweet orange tissues. The significant dwarfing of scions on *P. trifoliata* rootstock induced by CVd-IIIb (1, 7, 10, 11) may also be relevant to the suggestion of Rossetti *et al.* (9) that ". . . development of *Phytophthora* spp. is reduced in stunted trees, or in trees submitted to stress due to different causes . . ."

Although originally designed to test a range of plant materials and conditions, this study may have suffered from that diversity. In addition, the proposition should be considered that different citrus viroids might vary in effectiveness in inducing tolerance to *P. citrophthora* damage in various citrus tissues and/or under different conditions. Implicit in the suggestion by Rossetti *et al.* (9) of a relationship of viroid induced tolerance to *P. citrophthora* in "stunted trees", is that the tolerance observed might also be the result of a "triggered" host response that may not exclusively be a response to viroid infection or any other specific agent, but to a more generalized physiological alteration in host tissues accompanying reduction in vegetative growth.

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