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Authors
Semancik, J. S.
Bash, J.
Gumpf, D. J.

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Induced Dwarfing of Citrus by Transmissible Small Nuclear RNA (TsnRNA)

J. S. Semancik, J. A. Bash, and D. J. Gumpf

ABSTRACT. Selected citrus viroids that induce no diseases of economic impact to citrus production have been investigated for the ability of a transmissible agent to confer an economic advantage in citrus performance. When introduced for these purposes, the term transmissible small nuclear RNA (TsnRNA) has been substituted for the designation "citrus viroid" (CVd). A field trial of Valencia sweet orange on *Poncirus trifoliata* rootstock established in 1984 with TsnRNA-IIa or TsnRNA-IIIb was monitored for yield in ten successive harvests and fruit size distribution for the most recent four years. Trees containing TsnRNA-IIa produced a 10-40% increase in total yield in five years while fruit from trees with TsnRNA-IIIb were noted with an increase in size and a very homogeneous distribution. The mild bark cracking of the trifoliate rootstock containing TsnRNA-IIa was accompanied by a new symptom of green streaking of the wood in the pattern of the cracking. Neither symptom affected the overall appearance or vigor of the tree other than a reduction in canopy of about 20%. No persistence of the previously reported "finger imprint" symptom or appearance of the gum pocket disorder was observed on trifoliate rootstocks containing TsnRNA-IIIb.

Citrus, as a single plant group, harbors a diverse collection of five viroid species (1). Although variants of each viroid express symptoms in the indexing host, citron, only two diseases of economic impact to citrus production, exocortis and cachexia, have been attributed to viroids (Table 1). This relationship facilitated the consideration of citrus viroids as two classes; those that induce diseases and thus impede agricultural performance, the **viroids**, and those that are either intrinsically or under defined conditions not harmful to crop productivity and may provide an economic benefit. To emphasize this functional distinction, the latter class has been termed **transmissible small nuclear RNA (TsnRNA)** (11) reflecting a possible regulatory role as elements affecting host genome expression resulting in a modification of crop performance.

The association of reduced vegetative growth of scions on *Poncirus trifoliata* with the presence of *Citrus viroid III* (CVd-III) variants (6, 9, 12) has progressively gained significance in many citrus growing regions (2, 3, 4, 7, 8). Citrus viroid IIa (CVd-IIa) has been detected as the sole viroid in high performance old-line selections of the Parent Washington navel source maintained in Riverside, California. From this perspective, performance

TABLE 1
CITRUS VIROIDS AND PROMINENT BIOASSAY SYMPTOMS AND DISEASE RELATIONSHIPS

Viroid	nts	Family	Citron bioassay	Citrus reactions
CEVd	369-375	PSTVd	Severe	Exocortis
CVd-I	318-330	ASSVd	Bent mid-vein	Mild pitting on *P. trifoliata*
CVd-II	295-302	HSVd	Tip browning Petiole necrosis	Cachexia Bark cracking on *P. trifoliata*
CVd-III	291-296	ASSVd	Leaf droop	Finger imprint on *P. trifoliata* Gum pocket on *P. trifoliata*?
CVd-IV	284	CCCVd	Mild-moderate CEVd-like	NR*

*Not reported.

studies of trees with different degrees of induced dwarfing have focused on the action of these molecular species under the terminology TsnRNA-IIa and TsnRNA-IIIb on scions grafted to trifoliate rootstock.

Valencia sweet orange on trifoliate rootstock over a period of about 10 yr has indicated a consistent increase in yield per canopy volume from trees reduced in size by about 20% with TsnRNA-IIa or 50% with TsnRNA-IIIb (11). The yield from trees with TsnRNA-IIa was never significantly lower than control trees and in six harvests, yields exceeded control trees by 10-40% (Fig. 1). Trees with TsnRNA-IIIb of half the size of controls consistently yielded less, however, when adjusted for yield/canopy, all dwarfed trees with either TsnRNA-IIa or TsnRNA-IIIb were superior in production (11). This is in the absence of any additional factor such as spacing or the increased numbers of trees of reduced size that may be accommodated in a given area.

More unexpectedly, has been the consistent production from trees with TsnRNAs of fruit not only larger in size but also of a more homogeneous distribution than controls. Some annual variation is evident as presented in Fig. 2, however, in the 4 yr analyzed by standard packinghouse methods, trees with reduced canopies produced fruit of greater commercial value. This response was especially pronounced in fruit from trees containing TsnRNA-IIIb.

The relationship of TsnRNA-IIa and TsnRNA-IIIb with symptoms previously reported to occur on *P. trifoliata* as a possible response to the presence of citrus viroids must remain a consideration for the commercial application of transmissible induced dwarfing. Mild bark cracking has been attributed to CVd-IIa (10) and an association between gum pocket (5) and "finger imprint" (10) with CVd-IIIb. Extended field trials of Valencia on trifoliate have verified the persistence of mild bark cracking on trifoliate rootstocks with TsnRNA-IIa. However, other than a 20% reduction in tree size, no other negative effects on tree vigor and performance has been observed in 17 yr. Since symptoms on trees with CVd-IIIb that had displayed "finger imprint" are no longer evident nor has this disorder ever reoccurred in other similar plantings, cultural practices and/or environmental conditions must also be considered as a possible cause to that of a specific reaction to CVd-IIIb or a transmissible agent.

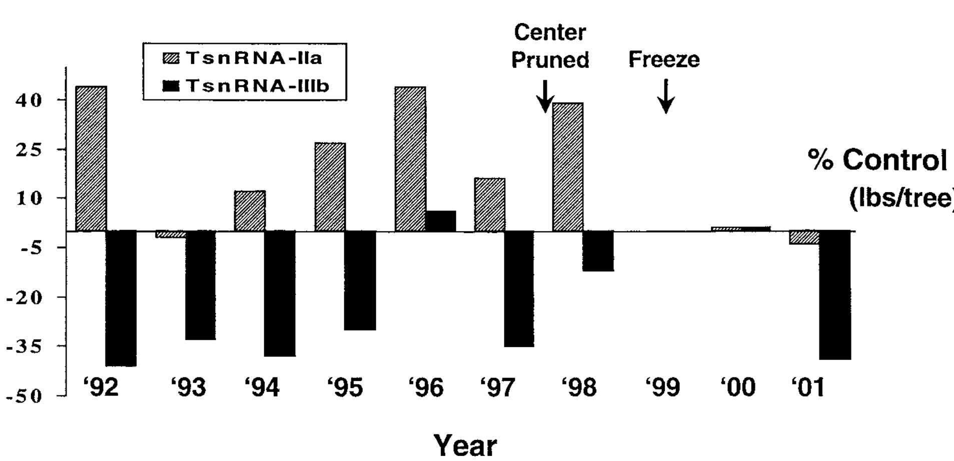

Fig. 1. Effects of TsnRNAs on the yield of Valencia sweet orange on trifoliate orange rootstock.

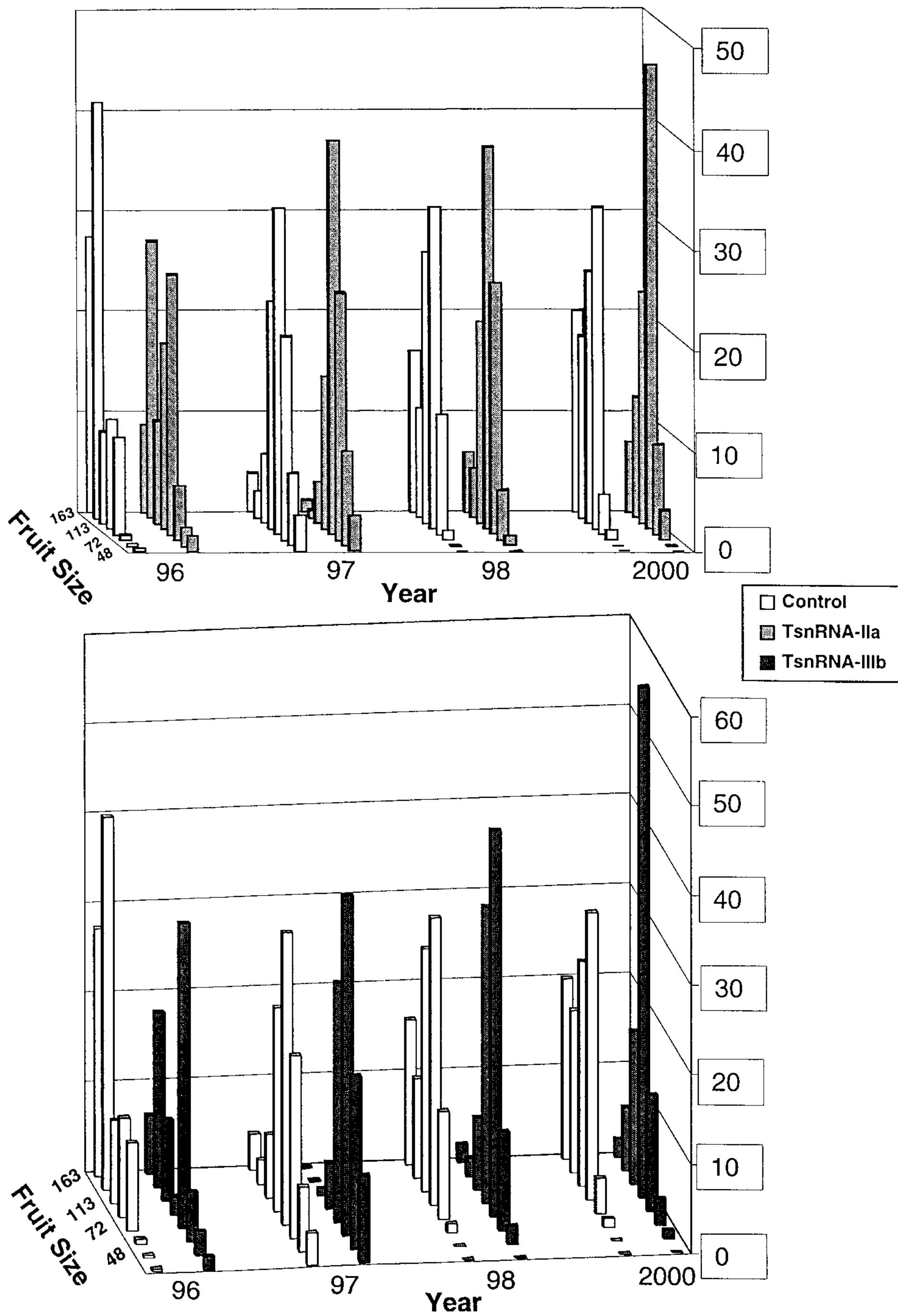

Fig. 2. Effect of TsnRNA-IIa and IIIb on fruit size of Valencia sweet orange on trifoliate orange rootstock.

When 8-yr old field trees of four cultivars of sweet orange on trifoliate rootstocks containing TsnRNA-IIa were cut and the bark removed, well defined green streaks were observed in the underlying wood

TABLE 2
REACTIONS OF SWEET ORANGE* AND *P. TRIFOLIATA*/CARRIZO CITRANGE ROOTSTOCKS WITH TsnRNAs

Treatment	Rootstock		Scion canopy
TsnRNA-Ia	Trifoliate:	Variable pitting	90%
	Carrizo:	NR	NR
TsnRNA-IIa	Trifoliate:	Bark cracking Wood streaks	80%
	Carrizo:	NR	NR
TsnRNA-IIIb	Trifoliate:	NR	50%
	Carrizo:	NR	NR
TsnRNA-Ia -IIa -IIIb	Trifoliate:	Bark cracking Wood streaks Mild pitting	50%
	Carrizo:	NR	60%

*Sweet orange: Atwood Navel, Lane Late Navel, Cutter Valencia, Olinda Valencia.

that coincided with areas of deepest fissures in the bark. It can only be conjectured whether this evidence of unusual photosynthetic activity might have some relationship with the enhanced performance of the trees so affected. Trees with TsnRNA-IIIb removed from the same trial displayed no evidence of gum pocket symptoms or any other disorder. Therefore, if it can be demonstrated that the gum pocket disorder is associated with CVd-III-like molecular species, it appears to be highly specific to the variants occurring in South Africa (5). However, the identification of the RNA band with the mobility of a possible CVd-III as a specific variant of CVd-III must first be demonstrated.

Included in these studies has been a preliminary investigation of the possibility of extending the dwarfing response of *P. trifoliata* to trifoliate hybrids such as citrange rootstocks. No reduced vegetative growth or symptoms were observed when a number of sweet orange scions were grafted to Carrizo citrange rootstocks containing single TsnRNA species. However, when a mixture of TsnRNA-Ia, -IIa and -IIIb was present, canopy growth was reduced by about 40% in the absence of any other symptoms on either the scions or rootstock (Table 2). This observation suggests that some combination of TsnRNA species may be effective in the induction of a dwarfing reaction in hybrids of *P. trifoliata*. These results may also offer an explanation for some of the erratic data reported (3, 4, 8) when *P. trifoliata* and citrange were employed as rootstocks in the presence of poorly characterized graft transmissible dwarfing factors that probably included complex mixtures of citrus viroids.

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