Induction of Stem Pitting in Peaches by Mechanical Inoculation with Tomato Ringspot Virus

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ABSTRACT

Isolates of tomato and tobacco ringspot viruses were obtained from soil around stem-pitted peach and cherry trees. These isolates and recognized strains of tomato ringspot, tobacco ringspot, and Prunus necrotic ringspot viruses were used to mechanically inoculate peach seedlings. Inoculated seedlings were maintained in the greenhouse for 8-9 months before being planted outside in fumigated soil. Thirteen months later, seedlings inoculated with five of the six isolates of tomato ringspot virus exhibited symptoms characteristic of stem pitting. The percentage of inoculated seedlings showing stem pitting varied from 2.5-50.0. Stem pitting did not appear in the controls, in the peaches inoculated with tobacco ringspot virus isolates or the trees inoculated with prunus necrotic ringspot virus.

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The disease now designated as peach stem pitting (PSP), was described in detail on peach, *Prunus persica* (L.) Batsch, by Barrat et al. (3) in 1968. The association of pitting with the leaf symptoms was noted by Barrat in 1958 and reported by Christ (8) in 1960 and by Barrat in 1964 (1) and 1966 (2) as a nursery and young tree problem. Lewis et al. reported their observations on PSP in Pennsylvania in 1968 (10).

The characteristic symptoms of PSP were reproduced following graft inoculation (14). Bark patches taken from roots of PSP-affected trees were found to be more efficient than buds as graft inocula in reproducing the characteristic symptoms (12). Graft transmission of the PSP-agent by use of bark patches from roots of affected trees was confirmed by Soulen et al. (18, 19).

Several lines of evidence have indicated soil transmission of PSP: (i) natural infections tended to be clustered (13, 22); (ii) trees adjacent to source of infection had the highest probability of developing PSP (14); and (iii) soil fumigation prior to planting reduced the incidence of disease (20). Soil transmission was established when trees planted in soil from around stem-pitted trees developed PSP but remained apparently healthy if the soil was sterilized prior to planting (23). Strong evidence indicates that the PSP-agent is transmitted by the soil-borne nematode *Xiphinema americanum* Cobb (4).

Smith & Traylor (17) found stem pitting symptoms associated with the soil borne peach yellow bud mosaic virus (PYBMV), a strain of tomato ringspot virus (TomRSV) (6) which is transmitted by *X. americanum* (5). Smith & Stouffer surveyed viruses present in soils around trees showing symptoms of PSP and obtained numerous isolates of TomRSV and tobacco ringspot virus (TbRSV) (15). Although not consistently, TomRSV has been recovered from stem-pitted trees (11). Prunus necrotic ringspot (PNRSV) virus was also isolated from PSP-affected trees, but was shown not to be responsible for PSP (12, 22).

Young peaches mechanically inoculated with TomRSV and the PYBMV strain of TomRSV, exhibited characteristic PYBM symptoms but apparently were not examined for PSP (7). The present paper describes the effects of using the viruses recovered from soil around stem-pitted trees along with recognized strains of TomRSV, TbRSV, and PNRSV viruses to mechanically inoculate peach seedlings.

MATERIALS AND METHODS.—Trees were selected from within orchards showing natural infection of stem pitting. Soil samples were taken from around the following trees: peach (*Prunus persica* Batsch); nectarine (*P. persica* var. *nectarina* Maxim.); sour cherry (*P. cerasus* L.); and sweet cherry (*P. avium* L.). Samples were taken from around apparently healthy trees, around trees showing stem pitting, or from areas from which diseased trees had been removed. The top 10- to 15-cm of soil were removed from three selected sites under each tree within the drip line, usually 1-2 m from the base of the trunk. A sample of approximately 454 g (1 lb) was removed from the next 10- to 15-cm layer of soil and placed in 20.3-cm diam clay pots. Care was taken that the samples were not allowed to dry; they were maintained in a greenhouse at 21-27 C and watered daily.

Although several species of herbaceous trap crops were used, only cucumber, *Cucumis sativus* L. 'National Pickling' or 'Chicago Pickling', gave consistent results. At least 6-15 cucumber seeds were planted in each pot. If the seeds failed to germinate, or the developing seedlings were destroyed by fungi or bacteria, then cucumber seedlings in the first true leaf stage were transplanted in the soil samples. Severely virulent isolates induced symptoms within

10-14 days. Plants that showed no symptoms by the third-leaf stage were used to mechanically inoculate other cucumbers. Inoculations were accomplished by grinding leaf tissue in a mortar containing 0.05 M phosphate buffer at pH 7.1. All isolates were maintained in cucumbers and transferred weekly to fresh cucumbers to maintain a high level of virus infectivity for inoculation and purification studies.

Naturally infected 'Montmorency' sour cherry, a clone of *P. cerasus*, was used as a source of PNRSV. Buds were collected from at least three sides of trees showing PNRSV symptoms and ground in a mortar containing 0.05 M PO_4 buffer at pH 7.1. This homogenate was rubbed on Carborundum-dusted cucumber cotyledons. Yellow, chlorotic local lesions appeared in approximately 7-10 days followed by systemic infection. The cultures were maintained by weekly transfers to new cucumber seedlings.

The soil-borne viruses, and the virus isolated from sour cherry used in this work, were transferred every week for 4-5 weeks using single lesions obtained on cucumber cotyledons. The single lesion transfers were used in an attempt to assure that only a single virus was present in each isolate. Virus purification was as previously described: TomRSV (25), TbRSV (21), and PNRSV (9). Each virus reacted with antisera prepared against recognized strains of the same virus using gel diffusion as previously described (24).

TABLE 1. Examination for stem pitting following mechanical inoculation of peach seedlings with TomRSV, TbRSV, and PNRSV (*P.* = *Prunus*)

Inoculum Source	No. stem pitted/ No. inoculated
TomRSV	
Soil isolate - *P. persica* var. *nectarina* 'Nectarose'	0/40[d], 0/8
Soil isolate - *P. persica* 'Kolhaven'	4/40, 0/10
Soil isolate - *P. persica* 'Red Globe'	0/10, 1/13
Soil isolate - *P. cerasus* 'Montmorency'	18/36, 1/10
Soil isolate - *P. cerasus* 'Montmorency'	1/40, 0/10
Cornell strain[a]	4/40, 0/10
TbRSV	
Soil isolate - *P. persica* var. *nectarina* 'Nectarest'	0/40
Soil isolate - *P. persica* var. *nectarina* 'Cavalier'	0/10, 0/14
Soil isolate - *P. persica* 'USDA 572115'	0/10, 0/10
Soil isolate - *P. persica* 'Halford'	0/20
Bud isolate - *P. persica* 'Jerseyland'	0/10, 0/10
Cornell strain[a]	0/40, 0/10
PNRSV	
Bud isolate - *P. cerasus* 'Montmorency'	0/20, 0/8
Bud isolate - *P. persica*[b]	0/40, 0/10, 0/9
Control	
Inoculated with healthy *Cucumis sativus* 'National Pickling'	0/20, 0/60, 0/18
Noninoculated *P. persica* 'Kent Redleaf[c]	0/78

[a] Supplied by A. F. Ross, Cornell University.
[b] Supplied by E. L. Civerolo and S. M. Mircetich; USDA, Beltsville.
[c] Used as markers to separate treatments in the nursery.
[d] No. stem pitted/No. inoculated for individual experiments.

Fig. 1. Stem pitting symptoms in the wood of 'Halford' peach seedlings mechanically inoculated with TomRSV.

The virus isolates described above and known strains of TomRSV, TbRSV, and PNRSV were used to mechanically inoculate 'Halford' and 'Tennessee Natural' peach seedlings. Cucumbers infected with these viruses were ground in 0.05 M phosphate buffer at pH 7.1 and rubbed on Carborundum-dusted leaves of peach seedlings 13- to 16-cm in height. The peach seedlings were maintained in the greenhouse for 8-9 months, before being planted outside in soil previously fumigated with Nemagon (12.1 EC) 18.7 liters/hectare (active material) [2 gal (active)/acre]. The trees were examined for stem pitting symptoms 13 months later.

RESULTS.—Examination of the peach seedlings revealed that only those inoculated with the TomRSV isolates developed stem pitting (Table 1). No pitting was observed on any of the seedlings inoculated with TbRSV, PNRSV, or juice prepared from healthy cucumbers. The noninoculated controls also remained symptomless.

Peach seedlings inoculated with the TomRSV isolates developed pitting indistinguishable from that observed on naturally infected young trees (Fig. 1). The bark in the area of the lower trunk was thickened and spongy. When the bark was removed elongated

pits and grooves were seen in the wood. The TomRSV-infected trees showed a reduction in terminal growth, and the leaves had become chlorotic or reddish to purple.

TomRSV and TbRSV could be recovered from the mechanically inoculated peach seedlings up to 3 weeks after inoculation. PNRSV could be recovered from the inoculated peach seedlings throughout the entire 13-month period.

DISCUSSION.—This report establishes TomRSV as being capable of inducing stem pitting in peaches. The variation between isolates as to the percentage of mechanically inoculated trees developing PSP symptoms would indicate that isolates vary in their ability to induce stem pitting.This is not surprising as the peach yellow bud mosaic strain of TomRSV is associated with a stem pitting in the western U.S. (17); but TomRSV-infected peach trees have never displayed the yellow bud symptom in any of our experiments (16).

The reported isolation of TomRSV from stem-pitted trees (11), although these isolations were not consistent enough to indicate causality, provides further evidence that TomRSV may be involved in the disease called Prunus Stem Pitting.

The consistent association of soil-borne viruses of TomRSV and TbRSV types from soils around stem-pitted trees (15), the ability of *X. americanum* to transmit the PSP-agent (4), and the effects of soil fumigation on the spread of this disease (23) lend substantial support to the concept that TomRSV is capable of causing stem pitting in peaches.

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