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Short communication

Transmission by aphids of potato spindle tuber viroid encapsidated by potato leafroll luteovirus particles

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Abstract

Potato spindle tuber viroid (PSTVd) was transmitted by *Myzus persicae* to *Physalis floridana* from *P. floridana* plants that also were infected with potato leafroll luteovirus (PLRV), whereas it was not transmitted by aphids from plants infected with PSTVd alone. Dot-blot hybridisation was used to detect PSTVd. The results indicate that PLRV can assist PSTVd in its transmission by *M. persicae*. Doubly infected, aphid-inoculated *P. floridana* plants from the previous experiment were used as the source plants in aphid transmission tests to the tomato cv. Rutgers, *P. floridana* and *Datura stramonium*. PSTVd was detected in 17 of 30 plants of tomato. The viroid was not detected by dot-blotting in any plant of *P. floridana* and *D. stramonium* in this experiment, but it was recovered from some plants by sap inoculation of the Rutgers plants. Treatment with RNase A of PLRV preparations purified from doubly infected plants indicated that PSTVd was encapsidated by PLRV particles.

Potato spindle tuber viroid (PSTVd) can decrease tuber yield and quality in the potato (*Solanum tuberosum* L.) (Beemster and de Bokx, 1987). It also reduces pollen viability in infected tomato (*Lycopersicon esculentum* Mill.) (Hooker et al., 1978) and potato (Grasmick and Slack, 1986).

PSTVd, first described by Diener (1971]) is a coat protein-free, single-stranded circular RNA consisting of 359 nucleotides (Gross et al., 1978; Diener, 1979). The viroid is highly seed-transmissible in several host plants including potato and tomato (Fernow et al., 1970; Singh, 1970; Grasmick and Slack, 1986; Singh et al., 1988). It can also be spread by contact of healthy foliage with diseased foliage or contaminated planting and cultivating machinery (Merriam and Bonde, 1954; Singh, 1983). Because of the economic importance and ease of spread, PSTVd has been included in most quarantine regulations.

The role of insect vectors in transmission of PSTVd is unclear. According to Kennedy et al. (1962) and Smith (1972), transmission of PSTVd by the aphids *Myzus persicae* Sulz. and *Macrosiphum euphorbiae* Thomas, and several other insect pests of potato, has been reported since 1925. However, for the most part these findings have not been corroborated in later studies (Schumann et al., 1980; de Bokx and Piron, 1981). Among aphids, *M. euphorbiae* has been reported to transmit PSTVd with a very low efficiency (de Bokx and Piron, 1981), while *M. persicae* was found unable to transmit this viroid (Schumann et al., 1980; de Bokx and Piron, 1981).

Recently, Salazar et al. (1995) reported that the aphid *M. persicae* readily transmitted PSTVd to potato, *Physalis floridana* and *Datura stramonium* plants from the source plants doubly infected with the viroid and potato leafroll luteovirus (PLRV) but not from plants infected with PSTVd alone. Since PLRV occurs wherever potato plants are grown and is transmitted by *M. persicae* with a high efficiency, its assistance in transmission of PSTVd by aphids under natural conditions would be of great importance for the epidemiology of this viroid in potato crops.

This paper presents the results of studies on PLRV-assisted transmission of the PSTVd by *M. persicae*.

In vivo encapsidation of PSTVd by PLRV particles as an explanation for transmission of the viroid by *M. persicae* from doubly infected plants has been examined.

Two PSTVd isolates, severe (s-PSTVd) and mild (m-PSTVd), originating respectively from Canada and USA (Kryczyński et al., 1980), were maintained in the tomato cv. Rutgers. The isolate L13D of PLRV, causing relatively mild symptoms in *Physalis floridana* Rydb. (Syller, 1985), was maintained in the potato cv. Osa.

A clone of *Myzus persicae* Sulz. was reared on healthy plants of Chinese cabbage (*Brassica pekinensis* Rupr.) in cages in a greenhouse. Apterous individuals were used in transmission experiments.

For aphid transmission tests, plants of *Nicotiana glutinosa* L. and *P. floridana*, both reported to be infectible by PSTVd (O'Brien and Raymer, 1964) and PLRV (Natti et al., 1953), were chosen as sources. To obtain plants infected with PSTVd and PLRV separately and together, 230 seedlings of each species were inoculated as follows: 40 seedlings were inoculated with PLRV followed by s-PSTVd, 40 with PLRV followed by m-PSTVd, 20 with PLRV followed by buffer alone, 40 with s-PSTVd followed by PLRV, 40 with m-PSTVd followed by PLRV, 20 with s-PSTVd alone, 20 with m-PSTVd alone, and 10 with buffer alone to serve as a healthy control. Inoculations with viroid and virus were one week apart. Inoculation with PLRV by aphids was performed essentially as described earlier (Syller, 1985). PSTVd was used as a viroid nucleic acid extract. Total nucleic acids from PSTVd-infected tomato leaves were phenol extracted and PEG fractionated, as described by Wełnicki et al. (1989). PSTVd inoculum in 0.1 mol Na_2HPO_4 contained 0.7–0.8 mg ml^{-1} of nucleic acid, of which 20–25 μg ml^{-1} was PSTVd. After inoculation plants were grown in a growth chamber with a constant temperature of 27 °C and 16h artificial illumination of 6000 lux.

All source plants and test plants used in the studies were monitored for symptom appearance and assayed for the presence of viroid by dot-blot hybridisation with a PSTVd-specific RNA probe, as described by Wełnicki and Hiruki (1993) except that ^{32}P-labeling, according to Salazar et al. (1988), was applied. Leaf extracts and purified PLRV and PSTVd preparations were denaturated with formaldehyde before blotting on nitrocellulose, as described by Wełnicki et al. (1990). Infection with PLRV was verified by ELISA, done essentially as described previously (Syller, 1994). Unless otherwise stated, the plants were assayed for the presence of PSTVd and/or PLRV 3–4 wk after inoculation. In case of doubtful results, recovery tests on tomato cv. Rutgers and *P. floridana* for PSTVd and PLRV, respectively, were done.

Infection with PSTVd and/or PLRV was detected in *P. floridana* but not in *N. glutinosa* plants. The isolate s-PSTVd was detected in 66 out of 80 *P. floridana* plants infected with PLRV, and in all the 20 plants inoculated with s-PSTVd alone. The isolate m-PSTVd was detected in 56 out of 78 plants infected with PLRV, and in all the 20 plants inoculated with m-PSTVd alone. Infection with either strain of PSTVd in *P. floridana* was symptomless, as reported by O'Brien and Raymer (1964), while plants infected with PLRV developed symptoms typical for infection with this virus (Syller, 1985).

There was no essential difference in the infectability of *P. floridana* with mechanically inoculated PSTVd, whether the plants were inoculated with the viroid before or after aphid inoculation with PLRV. This finding is unlike that reported by Francki et al. (1986), who found that PSTVd could not be detected in *Nicotiana clevelandii* Gray, a host of the viroid, if plants had been first or simultaneously inoculated with velvet tobacco mottle virus (VTMoV). Signals in the dot-blot were stronger with the sap samples from plants infected with either strain of PSTVd alone than with samples from plants doubly infected with PSTVd and PLRV (not shown). Since all sap samples were prepared in the same way, weaker hybridisation signals in PSTVd detection from doubly infected plants might have reflected an inhibiting effect of PLRV on PSTVd accumulation in *P. floridana* plants.

P. floridana plants infected with PSTVd and PLRV separately and together were used as sources of inoculum for aphids 5–6 wk after inoculation with PSTVd. During a 3-day acquisition feeding period in a greenhouse, the aphids were confined to source leaves in groups of 10–15 individuals with small clip cages. The aphids were transferred from the cages onto test plants (5–7 aphids per seedling) with a fine brush. This method of aphid transfer protected the test plants from accidental mechanical contamination with the PSTVd. During a 3-day inoculation feeding period in a greenhouse, the seedlings were covered with small cages to prevent aphid escaping. After killing the aphids with an insecticide, test plants were grown in the growth chamber.

In the first experiment, transmission of PSTVd by *M. persicae* to healthy *P. floridana* from *P. floridana* plants infected with the viroid alone and from

Table 1. Detection of PSTVd in *Physalis floridana* test plants inoculated by aphids *Myzus persicae* transferred from *P. floridana* plants infected with PSTVd and/or PLRV

Pathogens present in sources of inoculum	Number of plants inoculated by aphids	Infection of aphid-inoculated *Physalis floridana* plants[1]		
		PSTVd+PLRV	PLRV	PSTVd
s-PSTVd[2]+PLRV	90	13	70	0
s-PSTVd[2]	59	0	0	0
m-PSTVd[2]+PLRV	90	3	73	0
m-PSTVd[2]	60	0	0	0
PLRV	30	0	28	0

[1] Dot-blot hybridisation and ELISA were used to detect PSTVd and PLRV, respectively.
[2] s-PSTVd denotes a severe isolate of PSTVd; m-PSTVd denotes a mild isolate of PSTVd.

plants infected with the complex PSTVd+PLRV was compared for each PSTVd isolate used (Table 1). *P. floridana* plants singly infected with PLRV were included as viroid-free controls. The following numbers of plants were used as sources of inoculum for aphids in particular experimental variants: s-PSTVd+PLRV and m-PSTVd+PLRV (30 plants each), s-PSTVd alone and m-PSTVd alone (20 plants each), and PLRV alone (10 plants). Three *P. floridana* seedlings were inoculated by the aphids from each source plant. A batch of 10 noninoculated seedlings was included as a control. The plants were assayed for the presence of PSTVd and PLRV 6–7 wk after inoculation. As shown in Table 1, PSTVd was only detected in plants that were inoculated by aphids transferred from the sources infected both with PSTVd and PLRV. Moreover, PSTVd was only detected in plants that also became infected with PLRV. These results indicate that transmission of PSTVd by *M. persicae* from and to *P. floridana* was assisted by PLRV.

In the next experiment, 10 of 16 *P. floridana* plants that became infected both with PSTVd and PLRV after aphid inoculation in the previous experiment (Table 1) were tested as sources of inoculum for aphid transmissions. As test plants, the tomato cv. Rutgers, *P. floridana* and *Datura stramonium* L. were used. Transmission trials were done as described above. Three healthy seedlings of each species were inoculated by aphids from each source plant. In the tomato cv. Rutgers, PSTVd was detected by dot-blotting in 17 of 30 inoculated plants. The viroid was transmitted to at least one tomato plant from each source plant used. Infected plants developed symptoms characteristic of PSTVd 3–5 wk after inoculation. The results indicate that also in this trial PSTVd was transmitted by *M. persicae* from the source plants coinfected with PLRV. No presence of PLRV in the Rutgers plants was detected by ELISA, although many plants developed weak symptoms of virus infection. Apparently PLRV reached a very low concentration in the tomato that is a poor host for this virus. PLRV was subsequently detected in all the 30 Rutgers plants in the recovery test on *P. floridana*.

PSTVd was not detected by dot-blotting *D. stramonium* and *P. floridana*, although all plants became infected with PLRV. However, the viroid was recovered from several plants of each species by back testing on the Rutgers plants inoculated by sap. Evidently low concentration of aphid-inoculated PSTVd in *D. stramonium* and *P. floridana* that are the excellent hosts for PLRV, might have been the result of an inhibiting effect of PLRV on the viroid multiplication. Unlike that in *D. stramonium* and *P. floridana*, PSTVd multiplication in tomato plants was apparently not impeded by PLRV and the viroid was easily detected by dot-blotting.

To examine the ability of PLRV particles to encapsidate PSTVd in mixed infections of *P. floridana*, PLRV was purified from the following leaf samples: s-PSTVd+PLRV-infected *P. floridana*, PLRV-infected *P. floridana*, s-PSTVd-infected tomato Rutgers, and the mixture (1:1, w/w) of PLRV-infected *P. floridana* and s-PSTVd-infected tomato. Infected leaves were homogenized with 2 volumes (w/v) 0.1 mol citrate buffer at pH 6.0, containing 0.5% 2-mercaptoethanol, 0.5% cellulase from *Penicillium funiculosum* (Sigma) and 1% pectinase from *Aspergillus niger* (Serva) and incubated for 12 h at room temperature. The homogenate was emulsified with 0.2 vol. chloroform/butanol (1:1, v/v), precipitated by PEG (8%) and NaCl (0.2 mol), concentrated by two cycles of high-speed centrifugation (100 000g, 3 h) and purified on 10–40%

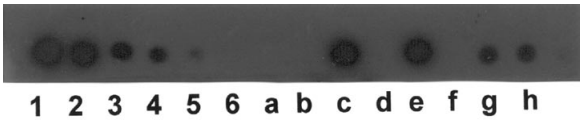

Figure 1. Detection of PSTVd in the RNase A treated (b,d,f,h) and untreated (a,c,e,g) preparations of: PLRV (a,b), s-PSTVd (c,d), a mixture of PLRV and s-PSTVd (e,f), and PLRV purified from s-PSTVd+PLRV-infected plants (g,h). Standards s-PSTVd per blot: 1, 0.5, 0.1, 0.05, 0.01, 0 ng (1–6, respectively). Amount of PSTVd per blot: 0.5 ng (c,d,e,f). Amount of PLRV per blot: 100 ng (a,b,e,f), c. 5 μg (g,h).

sucrose density gradient (90 000g, 2 h). The final PLRV preparation was concentrated 10× with Microcon 30 (Amicon, Inc. USA).

Copurification of s-PSTVd did not occur using this procedure of PLRV purification. The viroid was not detected in the homogenates after incubation with the enzymes. However, it was detected in preparations of PLRV obtained from s-PSTVd+PLRV-infected *P. floridana* after the second high-speed and sucrose density gradient centrifugations, whether the plants became infected with PSTVd after mechanical or aphid inoculation.

To determine that s-PSTVd associated with the PLRV preparations was encapsidated and not adsorbed to the outsides of PLRV particles, the samples were digested with RNase A (0.2 μg/ml) at 37 °C for 30 min (Figure 1). No PSTVd hybridisation signals were detected in the preparation of s-PSTVd and in a mixture of PLRV and s-PSTVd preparations treated with the enzyme. Positive signals of the same intensity were observed with the RNase A treated and untreated samples of the purified preparations from *P. floridana* plants doubly infected with s-PSTVd and PLRV. This indicates that RNA of PSTVd was protected against digestion with the enzyme and that it was most likely encapsidated *in vivo* by PLRV particles.

Our results provide further evidence for a key role of PLRV in transmission of PSTVd by *M. persicae*. However, a rate of transmission of PSTVd by aphids in our study was not as high as that obtained by Salazar et al. (1995) who reported 100% transmission of the viroid in some experiments. This could be due to differences between the isolates of PSTVd used or to the experimental conditions applied in both studies.

Several plant viruses, including luteoviruses, have been reported to assist transmission by aphids of other viruses (Hull and Adams, 1968; Rochow, 1970, 1982; Waterhouse and Murant, 1983). Transcapsidation of virions, occurring in mixed infections, can be an acceptable explanation for this phenomenon (Mayo and Ziegler-Graff, 1996). Earlier evidence suggested that PSTVd can be encapsidated *in vivo* by VTMoV particles (Francki et al., 1986).

We also found evidence suggesting that aphid-inoculated PSTVd is barely detectable in plants which are highly susceptible to the assisting virus. On the contrary, plants that are non-susceptible to this virus can easily be invaded by aphid-transmitted PSTVd, as it was found in tomato plants.

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