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Mechanical transmission of *Potato spindle tuber viroid* between plants of *Brugmansia suaveoles, Solanum jasminoides* and potatoes and tomatoes

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Abstract *Potato spindle tuber viroid* (PSTVd) has been recently found in many solanaceous ornamental plant species. This study reports on the effectiveness of mechanical transmission between *Brugmansia suaveolens*, *Solanum jasminoides*, potato and tomato. Inoculation with 'infected' plant sap diluted in water, rubbing with contaminated finger tips and cutting with contaminated razor blades all resulted in transmission of PSTVd. Temperature, plant species and source of inoculum were found to be critical factors. An average temperature of 15°C only resulted in a few infections, whereas transmission at 20 and 25°C was more successful. Tomatoes were more susceptible to PSTVd than *B. suaveolens*, *S. jasminoides* and potatoes. Furthermore, *S. jasminoides* was a better source of inoculum than *B. suaveolens*. No transmission was obtained after repeated addition of inocula to tomato roots. These results indicate that PSTVd can be transmitted between plant species in practice by crop handling.

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Viroids are the smallest infectious agents known. They consist of circular single-stranded RNA molecules of approximately 250 to 400 nt (Diener 1999; Flores et al. 2005a) that do not encode for any protein. The currently characterized viroid species have been assigned to eight genera in two families, the *Avsunviroidae* and the *Pospiviroidae* (Flores et al. 2005b). *Potato spindle tuber viroid* (PSTVd) is the type member of the genus *Pospiviroid* from the latter family. It has been studied extensively because of both its scientific and economic importance (Owens 2007; Owens and Verhoeven 2009).

The fact that pospiviroid infections in tomatoes in the Netherlands could not be related to infected seed batches raised questions about the origins of these viroids (Verhoeven et al. 2004). As PSTVd had been found in plants of *Brugmansia spp.* and *Solanum jasminoides* (Di Serio 2007; Verhoeven et al. 2008, 2010), and on the basis of nucleotide sequence identity, evidence has been obtained that several PSTVd infections in tomato originated from these ornamental species (Navarro et al. 2009; Verhoeven et al. 2010). However, it was still not known how the PSTVd isolates had been transmitted to tomatoes. Transmission by vegetative propagation and true seed

could be excluded, as these only enable transmission within a plant species. The question therefore arose of whether mechanical transmission and/or insects could account for transmission from ornamentals to tomatoes. Here we are reporting on the efficiency of PSTVd transmission by crop handling within *B. suaveolens* and *S. jasminoides,* and from these plants to potatoes and tomatoes.

For inoculation, we used PSTVd genotypes B1 from *B. suaveolens* cv. Light Red and S1 from *S. jasminoides* (NCBI GenBank Accession Numbers EF192394 and EF192393; Verhoeven et al. 2008, 2010). Both inoculum sources were maintained in a quarantine greenhouse at common temperatures for these plants, i.e. 21/18°C during day/night, with at least a 13-hour photoperiod. Potato cv. Nicola was raised from eye-plugs, tomato cv. Sheyenne and cv. Moneymaker from seeds and *B. suaveolens* cv. Geel and *S. jasminoides* cv. Variegata from cuttings. Absence of viroid was assured by testing. In the first two experiments, inoculated potato and tomato cv. Sheyenne plants were grown in two groups at constant temperatures of 15 and 25°C for 6 weeks; 15°C simulating the average temperature per 24-hour period during the growing season for seed potatoes in the Netherlands, and 25°C being a good temperature for viroid replication/multiplication (Verhoeven and Roenhorst 2010).

For the first experiment, carborundum-dusted leaves of five potato and tomato cv. Sheyenne plants were inoculated with diluted plant sap when they had 4–6 and 3–4 true leaves, respectively. The inocula were prepared by grinding 1 g of leaves from the inoculum sources in water (milli-Q). For each inoculum, a 10-fold dilution series was prepared from 10^{-1} (1 g leaf tissue/10 ml) to 10^{-5}. Using real-time RT-PCR (Boonham et al. 2004), these diluted inocula produced Ct-values from 21.2 to 31.2 for *B. suaveolens* cv. Light Red, and from 22.0 to 30.7 for *S. jasminoides,* indicating similar viroid concentrations. The experiment was performed twice. At 15°C, only a few tomato plants became infected with PSTVd-S1 when the inoculum was not diluted more than 100-fold (Table 1). At 25°C, inoculum dilutions of up to 10^{-5} resulted in infections of tomatoes. In contrast, inocula from *B. suaveolens* only caused infections with up to 10-fold sap dilutions. Potatoes were less susceptible to PSTVd-S1 than tomatoes.

For the second experiment, finger tips were contaminated by rubbing young leaves of the inoculum source between thumb, index and middle finger. One minute, 10 min and 2 h after acquiring the viroid, a group of five plants of potato (4–6 true leaves) or tomato (3–4 true leaves) was inoculated by smoothly rubbing non-carborundum-dusted leaves. The experiment was performed twice. Results were comparable to those of inoculations with diluted plant sap. At 15°C, only a single tomato plant became infected by PSTVd-S1 (Table 2). At 25°C, all tomato plants became infected by this isolate and 25 out of 30 plants by PSTVd-B1. Where all five plants in a series did not become infected, the infections were randomly spread. Potatoes also appeared to be less susceptible than tomatoes in this experiment, but PSTVd-S1 still evoked infections up to 10 min after acquiring the viroid.

For the third experiment, plants of *B. suaveolens* cv. Geel, *S. jasminoides* cv. Variegata and tomato cv. Moneymaker were inoculated with PSTVd-contaminated razor blades at plant heights of between 10 and 20 cm. Plants were grown under conditions as described for the inoculum sources. To obtain the viroid, leaves and stems of each inoculum source were cut with a razor blade 8 to 10 times. For inoculation, the tops of a group of five healthy plants were cut. Groups of five plants of each plant species were inoculated once, twice or three times at intervals of 2 weeks. For each group, the viroid was only obtained before inoculation of the first plant. The order of inoculation within the groups was always the same. The experiment was performed in three replications. With the inoculum PSTVd-S1, 12 out of 15 tomato plants became infected after a single cut with a contaminated razor blade; two or three cuts resulted in infection of all inoculated plants (Table 3). With PSTVd-B1, the majority of the inoculated tomato plants became infected after two or three cuts. Where all five plants of a series did not become infected, the infections were randomly spread. In contrast to tomatoes, viroid transmission within the ornamentals was very low: no infections were detected in plants of *S. jasminoides* cv. Variegata, and only a single plant of *B. suaveolens* cv. Geel became infected after three razor inoculations with PSTVd-S1. Remarkably, this was the last of the five *Brugmansia* plants of a series inoculated with the same razor blade.

Table 1 Transmission of PSTVd by mechanical inoculation of diluted plant sap

Inoculum from *Brugmansia suaveolens* (B1)					Inoculum from *Solanum jasminoides* (S1)			
Dilution[a]	Potato		Tomato		Potato		Tomato	
15°C	I[b]	II	I	II	I	II	I	II
10^{-1}	0[c]	0	0	0	0	0	5	0
10^{-2}	0	0	0	0	0	0	4	0
10^{-3}	0	0	0	0	0	0	0	0
10^{-4}	0	0	0	0	0	0	0	0
10^{-5}	0	0	0	0	0	0	0	0
25°C	I	II	I	II	I	II	I	II
10^{-1}	4	4	4	4	4	5	5	5
10^{-2}	0	0	0	0	0	1	5	5
10^{-3}	0	0	0	0	0	2	5	5
10^{-4}	0	0	0	0	0	0	5	3
10^{-5}	0	0	0	0	0	0	3	4

[a] Leaf sap was diluted in 10-fold steps; [b] I = first replication; II = second replication; [c] number of infected plants from five plants of potato cv. Nicola and tomato cv. Sheyenne; all inoculated plants were individually tested for PSTVd by real-time RT-PCR (Boonham et al. 2004) 6 weeks after inoculation

For the last experiment, both inoculum sources and plants of tomato cv. Sheyenne were grown in a quarantined growing chamber at 25°C, 70% humidity and 16/8 h day/night photoperiod. The inocula for PSTVd-B1 and S1 were prepared daily by grinding 0.5 g leaf tissue in 10 ml RNAse-free water (Milli-Q). At the 2–3 leaves stage, the tomato plants, which were being grown in pots with 40 ml of silicate sand (0.1–1.3 mm), were inoculated by adding 2 ml of inoculum to the soil via a pipette, followed by 3–4 ml nutrient solution after 2 h. For both PSTVd-B1 and S1, eight plants were inoculated, four plants for five consecutive days and four plants for 10 days. After the final inoculation, the plants were transplanted individually to 1.5 l pots with a Klasman Surfinia/Carilon substrate. After 6 weeks, none of the plants was found to be infected by testing with real-time RT-PCR (Boonham et al. 2004), irrespective of the viroid isolate or frequency of inoculation.

The results of the inoculation experiments show that PSTVd is transmitted successfully by traditional mechanical inoculation and by contaminated finger tips and razor blades. This supports the hypothesis that ornamentals formed the origin of viroid infections in tomatoes in the past (Verhoeven et al. 2004, 2010). The efficiency of transmission varied and appeared to be clearly influenced by temperature. Only a few tomato plants became infected at 15°C, whereas many plants became infected at 25°C. For tomatoes, these results confirm

Table 2 Transmission of PSTVd by contaminated finger tips

Inoculum from *Brugmansia suaveolens* (B1)					Inoculum from *Solanum jasminoides* (S1)			
Latency[a]	Potato		Tomato		Potato		Tomato	
15°C	I[b]	II	I	II	I	II	I	II
1 min	0[c]	0	0	0	0	0	0	0
10 min	0	0	0	0	0	0	1	0
2 h	0	0	0	0	0	0	0	0
25°C	I	II	I	II	I	II	I	II
1 min	0	0	5	5	0	3	5	5
10 min	0	0	1	5	0	5	5	5
2 h	0	0	5	4	0	0	5	5

[a] Period between viroid acquisition and inoculation; [b] I = first replication; II = second replication; [c] number of infected plants from 5 plants of potato cv. Nicola and tomato cv. Sheyenne; all inoculated plants were individually tested for PSTVd by real-time RT-PCR (Boonham et al. 2004) 6 weeks after inoculation

Table 3 Transmission of PSTVD by contaminated razor blades

Inoculum from *Brugmansia suaveolens* (B1)				Inoculum from *Solanum jasminoides* (S1)		
Slashes	*B. suaveolens*	*S. jasminoides*	*S. lycopersicum*	*B. suaveolens*	*S. jasminoides*	*S. lycopersicum*
1	0[a]	0	4 (0–3)	0	0	12 (3–5)
2	0	0	11 (3–4)	0	0	15
3	0	0	9 (1–5)	1 (0–1)	0	15

[a] total number of infected plants of three replications (maximum 15) of *B. suaveolens* cv. Geel, *S. jasminoides* cv. Variegata and tomato cv. Moneymaker, range within numbers of infected plants per replication between parentheses; all inoculated plants were individually tested for PSTVd by real-time RT-PCR (Boonham et al. 2004) 8 weeks after the last inoculation

those of Grasmick and Slack (1985) and Sänger and Ramm (1975). Inoculation at 15°C was unsuccessful for potatoes. Although Singh and Dilworth (2009) had shown that TCDVd survived temperatures of −12°C in *Vinca minor*, our results indicate that 15°C may be too low for adequate replication of the viroid, thereby substantially reducing the chance of successful inoculation. This might explain both the lack of transmission during previous field experiments (Verhoeven et al. 2004) and the fact that outbreaks of PSTVd have not been observed in potatoes in the Netherlands. The average temperature per 24-hour period has been recorded as 15.1°C during the growing season of seed potatoes between 1901 and 2008 (KNMI daily meteorological data, undated).

With regard to the inoculum source, PSTVd-S1 from *S. jasminoides* was transmitted more successfully than PSTVd-B1 from *B. suaveolens*. This is rather due to the host plant than to the PSTVd genotype. Poor transmission rates were also obtained in previous experiments when using leaves of *Brugmansia spp.* as the source of PSTVd inoculum (Verhoeven et al. 2010). In these experiments, five out of seven mechanical inoculations with PSTVd-S1 from *S. jasminoides* were successful, whereas all five inoculations with the same genotype in similar concentrations from *Brugmansia* spp. were unsuccessful. Since three genotypes of PSTVd and one of *Tomato chlorotic dwarf viroid* (TCDVd) were transmitted successfully from roots of *Brugmansia* spp. (Verhoeven and Roenhorst 2010), an unknown leaf component is presumed to inhibit viroid transmission. In the case of finger tip inoculation, this negative effect on viroid transmission is less pronounced, probably because the leaves are less damaged in this case.

The finger tip inoculation method appeared very successful in transmitting PSTVd from ornamentals to tomatoes. Seigner et al. (2008) already demonstrated transmission after a short latency period with a PSTVd isolate from *S. jasminoides*. Our experiments show that infectivity may even be maintained over latency periods of 10 min and 2 h. This would enable transmission from ornamentals to potatoes and tomatoes in practice, even if these plant species are not grown in the vicinity of each other.

Furthermore, transmission via contaminated razor blades shows that knives and scissors may transmit viroids in practice. Several viroids have been reported to be transmitted by contaminated knives at low frequencies for *Citrus* too (Barbosa et al. 2005). Although transmission within ornamentals hardly occurred in our experiments, the role of viroid transmission via contaminated knives and scissors should not be neglected because plants such as *S. jasminoides* may be cut very frequently for pruning and taking cuttings.

Finally, Seigner et al. (2008) did not observe transmission after a single addition of PSTVd inoculum to pots with healthy tomato plants. We showed that even repeated additions of inoculum to the rooting medium of tomato plants did not result in viroid transmission. These results indicate that the spread of pospiviroids in tomato crops along the rows (Verhoeven et al. 2004) does not go via soil or hydroponic systems, but most likely results from crop handling.

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