



Transmission of *Physalis rugose* mosaic virus via pruning, leaves contact and soil

Heron Delgado Kraide¹ · Eike Yudi Nishimura Carmo¹ · Arnaldo Esquivel Fariña² · Camila Geovana Ferro¹ · Jorge Alberto Marques Rezende¹

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Abstract

Physalis peruviana (Solanaceae) is an exotic plant in Brazil that has been increasingly known for its fruits' vitamins, nutrients, and high-added value. Like other solanaceous plants, physalis is also the target of some plant viruses. Until now, five viruses have been reported to naturally infect *P. peruviana* in Brazil. Among them, the physalis rugose mosaic virus (PhyRMV) has attracted attention due to the severity of the symptoms, characterized by leaf mosaic, malformation, leaf abscission, and a significant reduction in plant development and yield. This work evaluated the efficiency of different forms of PhyRMV transmission. The virus was efficiently transmitted to healthy *P. peruviana* plants through contact between the leaves, pruning, and contaminated soil with remains of infected *P. peruviana* plants. PhyRMV remained active and infectious in contaminated soil for up to ninety days.

Keywords *Sobemovirus* · *Solanaceae* · *Transmission* · *Pruning* · *Leaves Contact* · *Soil*

Physalis peruviana, from the Andean region, is an exotic plant in Brazil. It has been increasingly known for its fruits' vitamins, nutrients, and high-added value, as the cosmetics, pharmaceuticals, and food industries use them (Chaves et al. 2005; Patro 2010). Like other solanaceous plants, physalis is also affected by virus diseases. To date, five viruses have been reported to naturally infect *P. peruviana* in Brazil: the sobemovirus physalis rugose mosaic virus (PhyRMV) (Fariña et al. 2019), the potyviruses potato virus Y, and pepper yellow mosaic virus (Esquivel-Fariña et al. 2022) and the orthospoviruses tomato chlorotic spot virus and groundnut ringspot virus (Eiras et al. 2012; Fariña et al. 2018).

PhyRMV causes severe leaf mosaic symptoms, malformation, leaf abscission, and reduced plant development (Kraide et al. 2023). Additionally, infected plants have yield reduction varying from 66% to 70%, and some postharvest

fruit characteristics are negatively affected (Gorayebe et al. 2020; Kraide et al. 2023). This sobemovirus is readily transmitted mechanically but not by seeds (Savi et al. 2021). Its vector remains unknown. Knowledge of PhyRMV transmission is essential for establishing management strategies to prevent its spread and minimize disease damage. This work evaluated the transmission of PhyRMV by pruning, leaf contact, and soil.

The experiments were performed with PhyRMV isolate (GenBank accession number MK681145) collected from a field-infected physalis plant in the municipality of Piracicaba, State of São Paulo (SP; Brazil), in 2019 (Fariña et al. 2019). The viral isolate was maintained in physalis plants in insect-proof cages in a greenhouse. It was regularly renewed via mechanical transmission to younger plants. Inoculum was prepared by grinding symptomatic leaves in 0.02 M phosphate buffer (pH 7.0) containing 0.02 M sodium sulphite in a 1:10 dilution (w/v). Inoculum was applied to leaves previously sprinkled with carborundum. Virus identification was confirmed by RT-PCR, using the primer set *Sobemo1F* (5'-TAGCCAAGCTCAATCCATTT-3') and *Sobemo1R* (5'-GTCTTAGGCCAAGAAGTCAA-3') following the thermocycler regime described by Fariña et al. (2019), generating an amplicon of 937 bp. Amplified amplicons were analyzed on 1% agarose gel electrophoresis

✉ Heron Delgado Kraide
heron.kraide@usp.br

¹ Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (USP), Piracicaba, SP, Brazil

² Facultad de Ciencias Agrarias, Universidad Nacional de Asunción (FCA/UNA), San Lorenzo, Paraguay

stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific) and visualized using a UV light transilluminator. According to the manufacturer's recommendations, they were purified with the Wizard SV Gel and PCR Clean-Up System purification kit (Promega), and directly sequenced at MacroGen Inc. (Seoul, South Korea).

Transmission assays of PhyRMV via pruning were performed as follows. *P. peruviana* seeds were sown on a Styrofoam tray containing autoclaved substrate. After germination, the seedlings were transplanted into 5-L pots. Thirty days after transplanting (DAT), five healthy physalis plants were used for transmission by pruning, using PhyRMV-infected plants as a source of inoculum. Virus-free pruning shears were used to cut two branches of an infected plant. After that, they cut the main branch of one healthy plant. The pruning shears were then disinfected in 99° ethanol and used to prune PhyRMV-infected plants again, followed by the second healthy plant. The procedure was repeated until the fifth healthy plants were used. The experiment was repeated ten times. The pruned healthy plants were then individualized to avoid contamination. Thirty days later, they were analyzed for symptom expression, and virus detection by RT-PCR.

Two PhyRMV-infected plant was placed between four healthy plants of the same age and height (30 days old, 0.3 m tall) to assess virus transmission by contact among leaves. These plants were placed one meter away from an Arno fan running for 2 h day⁻¹, at a speed of 3 m s⁻¹, for five days

(Fig. 1A). The assay was repeated six times. Symptoms were evaluated 30 days later. Total RNA was extracted from the leaves of all plants to confirm virus infection by RT-PCR.

To evaluate the transmission of PhyRMV from plant residues in the soil, 30 pots containing systemically PhyRMV-infected physalis plants and five pots containing healthy plants were used. The aerial part of all plants was cut at the soil level and discarded. The soil in each pot was turned over. Samples of roots in the pots were collected separately and analyzed by RT-PCR for PhyRMV detection. Fifteen days after aerial plant removal, healthy physalis seedlings, produced in autoclaved substrate, were transplanted (one plant/pot) into these pots containing only remnants of roots. Root and leaf samples were collected 30 days after plant development and analyzed by RT-PCR for virus detection. Symptoms expression on the leaves were also evaluated.

Additionally, the persistence of PhyRMV in the soil was evaluated. Sterilized soil in 20 L pots were irrigated with an extract of symptomatic physalis leaves, diluted 1:10 (w/v) in phosphate buffer: 1 g of infected leaf in 10 ml of phosphate buffer. One hundred mL of extract was applied in each pot one time. The pots were kept in the greenhouse to avoid contamination. Thirty days after irrigation with the leaf extract, healthy physalis seedlings were transplanted into five pots (one plant per pot). This procedure was repeated at 60, 90, 120, and 150 days after application of the leaf extract, always using five new pots. The infection was evaluated by detecting the virus by RT-PCR 30 days after each transplant.

Fig. 1 Transmission of Physalis rugose mosaic virus (PhyRMV) through contact between leaves caused by a fan (A), symptomatic new leaf after pruning a healthy plant with infected scissors (B); symptomatic plant after transplantation and development in PhyRMV infested soil (C); symptomatic plants after mechanical inoculation with extract from infested soil (D) and partially purified virus from infested soil (E)

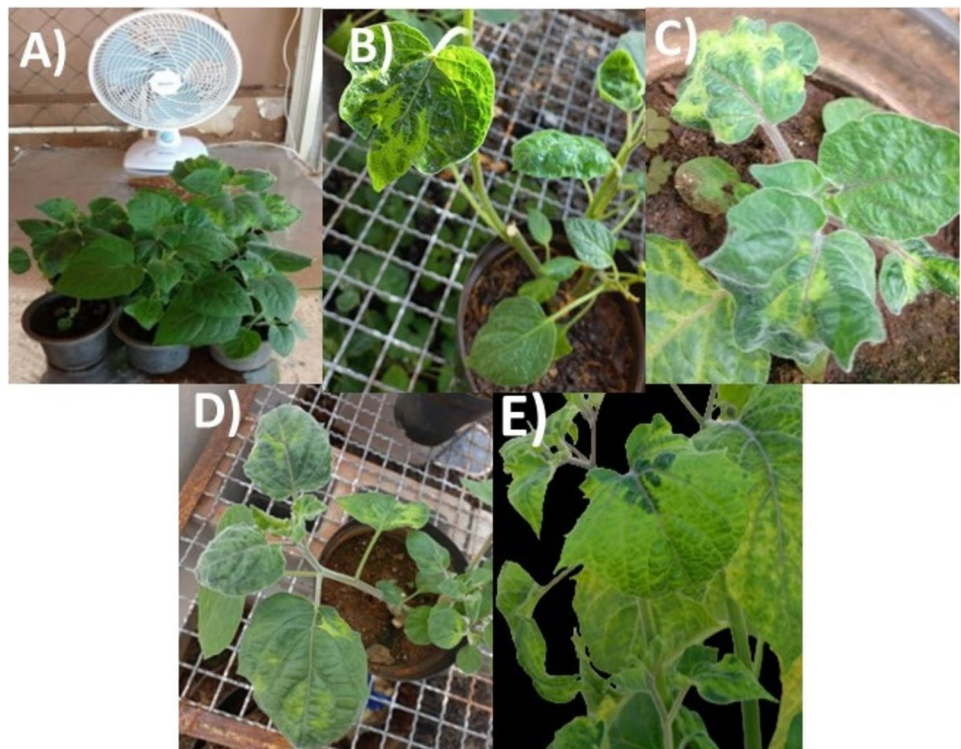


Table 1 Transmission efficiency of *Physalis rugose* mosaic virus (PhyRMV) via pruning, wind-mediated leaf contact, contaminated soil, and mechanical inoculation with extracted virus from the soil, evaluated by detecting the virus by RT-PCR

Transmission Methods	No. infected plants / No. tested plants
Pruning	19 / 50 (38%)
Leaf Contact	14 / 24 (58.34%)
Soil	16 / 30 (53.34%)
Soil Extract	10 / 10 (100%)
Soil Extract purified	10 / 10 (100%)

Sixty grams of soil from pots containing infected *physalis* plants were collected for virus detection and inoculation, sixty grams of soil from pots containing healthy *physalis* plants was also collected as a control. Two hundred and fifty ml of phosphate buffer was added, and the solution was stirred overnight at room temperature. The solution was transferred to 50 mL Falcon tubes and centrifuged at 5,000 rpm for 30 minutes in a Sorvall centrifuge (SS-34 rotor). The supernatant was collected, placed on top of 8 ml of 30% sucrose cushion, prepared in the same buffer, and centrifuged at 29,000 rpm, at 4°C, for 90 minutes in a Beckman L8-60M ultracentrifuge (type 30 rotor). The supernatant was reserved. The pellets were resuspended in the same buffer under stirring for two hours at 8°C. It was then centrifuged at 12,000 rpm at 4°C for 30 minutes in a Nova Tecnica NT-805 centrifuge (fixed rotor). The supernatant was mechanically inoculated in 10 healthy *physalis* plants, while the reserved supernatant was inoculated into ten other healthy plants. Evaluations were as described before.

The results obtained on the different transmission methods of PhyRMV are in Table 1. Transmission rates *via* pruning, wind-mediated leaf contact, and contaminated soil were 38%, 58.3%, and 53.3%, respectively. All 20 *physalis* plants mechanically inoculated with the virus extracted from the contaminated soil were infected. PhyRMV persisted infective in the soil for up to 90 days with a decline in infection rate over time until it lost its infective capacity (Table and Fig. 1). Attempts to transmit PhyRMV with *Diabrotica speciosa*, *Bemisia tabaci* MEAM1, and *Myzus persicae*, by confinement of the insects in *P. peruviana* plants infected with PhyRMV for different periods of access to acquisition and different periods of access to inoculation on healthy *P. peruviana* plants failed. *Bemisia tabaci* MEAM1 and *Myzus persicae* fed on infected plants, but were not able to transmit the virus, while *Diabrotica speciosa* did not even feed on the plants (unpublished data).

Compared to transmission by vectors (insects, mites, nematodes, and fungi) or vegetative propagation, field spread by infested soil and mechanical means is uncommon

and of minor importance. However, with some viruses, it might be of considerable practical significance. The tobamovirus cucumber green mottle mosaic virus (CGMMV), which has no vector, is one of the most economically important viruses of cucurbit crops worldwide, particularly in Asia where water, soil, root, or pruning could be involved in facilitating its spread, besides seed transmission (Li et al. 2016). The soil transmission rate of CGMMV varied from 0.2% to 10.3% (Choi et al. 2004; Li et al. 2016; Lovelock et al. 2022). The infectivity of the virus in the soil was maintained from 17 months without host plants (Park et al. 2010). Li et al. (2016) found that CGMMV could be transmitted from infected watermelon plants to the ninth healthy plant by pruning. Tomato brown rugose fruit virus (ToBRFV) is another tobamovirus recently identified affecting tomato crops. It is soil-borne at a low percentage of ca. 3% when the soil contains root debris from a previous 30–50-day growth cycle of ToBRFV-infected tomato plants (Klein et al. 2023). Infested soil is also an essential medium for the survival and spread of maize chlorotic mottle virus (genus *Machlomovirus*; family *Tombusviridae*). Under natural conditions, this virus is also transmitted by chrysomelid beetle species and thrips (Regassa et al. 2022).

Transmission by leaf contact between infected and healthy plants can occur with highly stable viruses that reach high titers within their host plants, such as those in the genera *Potexvirus*, *Carlavirus*, *Sobemovirus*, and *Tobamovirus* (Hull 2014). Some less stable viruses, such as potyviruses, can also be transmitted this way. Pea seed-borne mosaic virus was transmitted when intertwining healthy and infected plants were blown by a fan to simulate wind (Congdon et al. 2016).

The primary transmission source of sobemoviruses is the mechanical wounding of host plants associated with different species of insect vectors. Sobemoviruses are easily transmitted by sap-inoculation under experimental conditions. Their particles are very stable with a thermal inactivation point near 80–90°C (Hull 1977). Some are seed-transmitted but not PhyRMV (Savi et al. 2021). The efficient transmission of PhyRMV from infected to healthy plants through contact between leaves, pruning, and contaminated soil, besides remaining infectious for up to three months in the soil, may have significant epidemiological consequences for this disease. This knowledge allows us to develop some management recommendations to minimize disease damage, while there is no efficient and lasting measure to control it in the field. The following measures are recommended: use of healthy and certified seedlings to start a new crop, use of spacing between plants that avoid contact between them, disinfection of pruning shears used to grow the plants, and avoid planting new crops in areas previously cultivated with infected *physalis* plants for at least four months.

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Declarations

Conflict of interest The authors declare no competing interests.

Consent to participate The authors declare their consent to publish the data presented in this research.

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