CMV can spread through plant to plant contact: implications for experimental practices

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ABSTRACT

Cucumber mosaic virus (CMV) is a major plant pathogen with a worldwide distribution and the widest host range among all known plant viruses. It affects numerous crop species and can cause symptoms that significantly reduce yield. CMV is primarily transmitted by aphids and more sporadically through seeds. It is frequently studied in laboratory settings with the aim of developing effective control strategies. In many experiments, infected plants are placed in direct contact with healthy ones assuming that CMV cannot be transmitted in this way. However, this has not been formally demonstrated. Therefore, this study aimed to assess whether CMV can be transmitted through plant-to-plant contact. Infected plants were first rubbed against healthy ones and then left in contact for 28 days. Target plants were subsequently tested using DAS-ELISA to detect potential transmission. We applied this protocol in two separate tests totalizing 15 combinations of plant species including pepper (*Capsicum annuum*) and five weed species (*Capsella bursa-pastoris*, *Cerastium glomeratum*, *Stellaria media*, *Stachys arvensis* and *Trifolium repens*). We found that CMV could be transmitted through contact between pepper and all tested weed species except *T.repens*. These findings highlight the importance of verifying whether a virus is capable of contact transmission before carrying out experiments in controlled conditions. In case of transmission, appropriate precautions will be crucial to avoid unintended transmissions.

Keywords: cucumber mosaic virus, contact transmission, pepper, weed, arena, propensity

Introduction

Plant-to-plant transmission of several plant viruses, such as potato virus Y or zucchini yellow mosaic virus, is known to be possible by simple contacts between plants, although their primary mode of transmission is aphid vection (Coutts, Kehoe, and Jones 2013; Coutts and Jones 2015). Strict measures must be implemented during experiments involving those viruses to prevent accidental contamination. Indeed, experiments in plant virology often involve a large number of plants. Whether conducted in fields, greenhouses, or trays, these plants may come into physical contact due to their close proximity in experimental facilities. Environmental perturbations such as wind or ventilation can further promote friction between neighbouring plants. Handling plants, such as touching infected plants before healthy ones, or using watering hoses that come into contact with both infected and healthy plants, can also represent a potential source of contact transmission. Experimental protocols involving these viruses are designed to avoid plant-to-plant contact. However, such precautions may not be taken with viruses for which no contact transmission has been reported. Since data on the topic are scarce, it is essential to test whether a virus can be transmitted through plant-to-plant contact before conducting experiments involving many plants in a confined space.

This is particularly important when assessing viral transmission by aphids, for which inadvertent contact transmission would compromise the results. There are two main approaches to measuring the aphid-mediated transmission of a virus. The first one is in completely controlled conditions, and consist in placing one or several aphids on an infected 'source' leaf and then transferring the aphids to the leaf of a healthy 'target' plant. After a certain period, target plants are diagnosed and the proportion of infected ones gives the efficiency of transmission (Doumayrou et al. 2013; Lehrer et al. 2007). The second approach comes closer to natural transmission and relies on arenas. In this setup, aphids are placed in a closed environment, containing both infected source plant and healthy targets. The proportion of infected target plants gives the propensity of transmission (Chatzivassiliou et al. 2016; Kanavaki et al. 2006). Aphids should be winged to better represent natural conditions of viral transmission, and effectively move between source and target plants within the arena experiments (Yuan 1996). However, managing winged aphids is challenging due to the difficulty of synchronizing generations in aphid breeding facilities, as well as the risk of escape beyond the experimental arenas. As a result, non-winged (apterous) aphids are often used instead (Katis et al. 2006; Claflin, Thaler, and Power 2015; Webb and Leng Kok-Yokomi 1993; Hobbs et al. 2000). To allow their movement between plants, the plants are positioned in direct contact with one another, which could inadvertently enable contact transmission of the virus, should this mechanism be possible. This would lead to inaccurately calculate transmission rates.

As mentioned before, for viruses that do not primarily rely on plant-to-plant contact for transmission, their ability to spread in this way is not always tested, and experimental setups are not necessarily designed with this eventuality in mind. This is the case of cucumber mosaic virus (CMV), a virus belonging to the genus *Cucumovirus* within the *Bromoviridae* family. CMV is present worldwide and has the largest host range among plant viruses (Hirsch and Moury 2021). It is primarily transmitted by aphids, with more than 80 species known to act as vectors. It is also transmitted by the seed of some of its host plants. It induces various symptoms in plants, including mosaic patterns, yellowing, and leaf and fruit distortions, among others (Hirsch and Moury 2021). It is the most economically important virus for six different crop varieties and the first virus affecting annual crops in 14 countries (Gallitelli 2000). It induces crop losses not only in quantity (yield) but also in quality (Lepage et al. 2025). For these reasons, CMV is well studied in laboratory, and even serves as a model for studying plant viruses (Scholthof et al. 2011). Numerous experiments are therefore carried out with CMV. And yet, to the best of our knowledge, the possibility of plant-to-plant contact transmission of CMV is not documented. This work thus aims at addressing this question.

Material and methods

The experiment has been carried out in two independent tests.

Virus and plant material, virus detection, and greenhouse conditions

Virus isolate:

The CMV isolate used in this study was collected on an infected pepper (*Capsicum annuum*, cultivar 'Gorria') in the area of Espelette in 2022 (Gaudin et al. 2025). It belongs to the taxonomic subgroup IB of CMV and was stored in dried leaves (Bos 1983) from tobacco plants and conserved at 4°C.

Mechanical inoculation and virus detection:

Mechanical inoculations and viral detection both require the preparation of plant homogenates. For this, one gram of plant material (dried or fresh leaves) was ground in 4 mL of extraction buffer containing 0.03M sodium phosphate (Na_2HPO_4) and 0.2% diethyldithiocarbamate (DIECA).

For mechanical inoculation, the homogenate was mixed with 90 mg of carborundum and 90 mg of activated charcoal before being applied to 2 leaves per plant using two strokes. After five minutes, the leaves were rinsed with tap water.

For virus detection, a DAS-ELISA test was performed on the homogenate following the method described by (Hirsch et al. 2023). Plants were considered negative when their optical density (OD) at 405 nm was under three times the OD of a healthy control, and positive when higher.

Greenhouse conditions:

All plants were grown in an insect-proof greenhouse from October to December (first test) and from April to June (second test) in Avignon, France. Greenhouse temperatures ranged from 11.9°C to 25.1°C during the first test and 11.4°C to 37.9°C during the second test.

Mechanical inoculations to prepare source plants

To obtain CMV infected plants serving as sources for contact inoculation, tobacco plants were first mechanically inoculated with CMV from dried material, and maintained in a greenhouse for 14 days post-inoculation (dpi). Then, a homogenate of these infected tobacco plants was used to mechanically inoculate 5-week old plants from the following species: Espelette pepper (*Capsicum annuum* cultivar 'Gorria'), shepherd's purse (*Capsella bursa-pastoris*), clammy chickweed (*Cerastium glomeratum*), chickweed (*Stellaria media*), staggerweed (*Stachys arvensis*), and white clover (*Trifolium repens*). Ten (first test) or twelve (second test) plants per species were inoculated, except *T. repens* for which 100 (first test) or 105 plants (second test) were inoculated as infection was difficult to achieve in preliminary experiments (data not shown). All these plants were diagnosed by ELISA at 28 dpi. Source plants in the contact transmission tests were chosen among the infected plants.
All plants were approximately five weeks old at the time of inoculation (mechanical or by contact).

Contact transmission test

Contact transmission was tested in two independent tests, from *C. annuum* to *C. annuum* (1 replicate in the first test and 4 replicates in the second test), from *C. annuum* to

the five weed species and from the weed species to *C. annuum* (1 replicate per test). In the second test, we also tested the transmission from the weed species to themselves, except *T. repens* for which not enough infected plants had been obtained. This resulted in a total of 15 different modalities. For each modality, one infectious plant was used as a source for 8 healthy target plants. In every test, 12 healthy plants were used as negative controls (two per plant species).

To perform the transmission test mimicking situations where plants might come into contact and potentially transmit viruses, each CMV-infected source plant was gently rubbed against the 8 target plants for 10 seconds, then placed at the centre of the group of target plants, allowing them grow while remaining in direct contact, a process known as "intertwining". The experimental setup is illustrated in Figure 1. Plants were then kept in an aphid free greenhouse for 28 days. At the end of this period, each target plant was tested using ELISA to determine whether contact transmission had occurred.

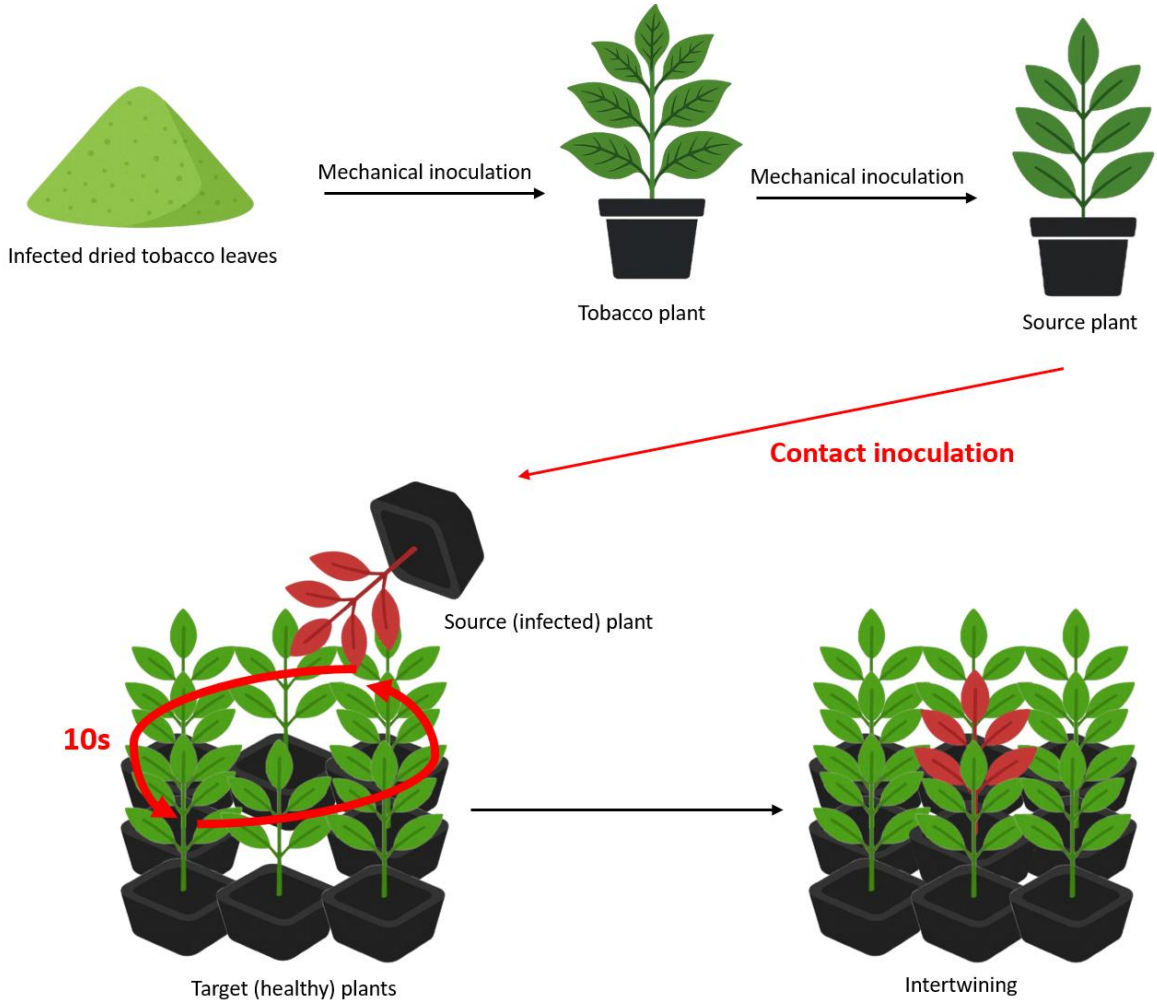

Figure 1. Scheme of the experimental setup to test for viral contact transmission. Infected source plants are obtained after successive mechanical inoculations. Then, a single source plant is rubbed against 8 healthy target plants during 10 seconds and next placed in the middle for 28 days of intertwining.

Results

Mechanical inoculations to prepare source plants:

To obtain infected source plants, mechanical inoculations were performed using dried leaves from infected tobacco plants as inoculum. The success rate of mechanical inoculation

(based on ELISA) varied by species (Figure 2), reaching 100% for *C. annuum* and *C. bursa-pastoris*, 90–100% for *S. arvensis*, 80–100% for *S. media*, 70–75% for *C. glomeratum*, and only 0.9–4% for *T. repens*. Symptom assessment showed consistent expression in *C. annuum* (90–94%), while results fluctuated between tests for the weeds: *C. bursa-pastoris* (0–50%), *C. glomeratum* (0–75%), *S. media* (70–92%), and *S. arvensis* (40–100%). No symptom assessment was conducted for *T. repens* due to the difficulty of identifying CMV symptoms on this species.

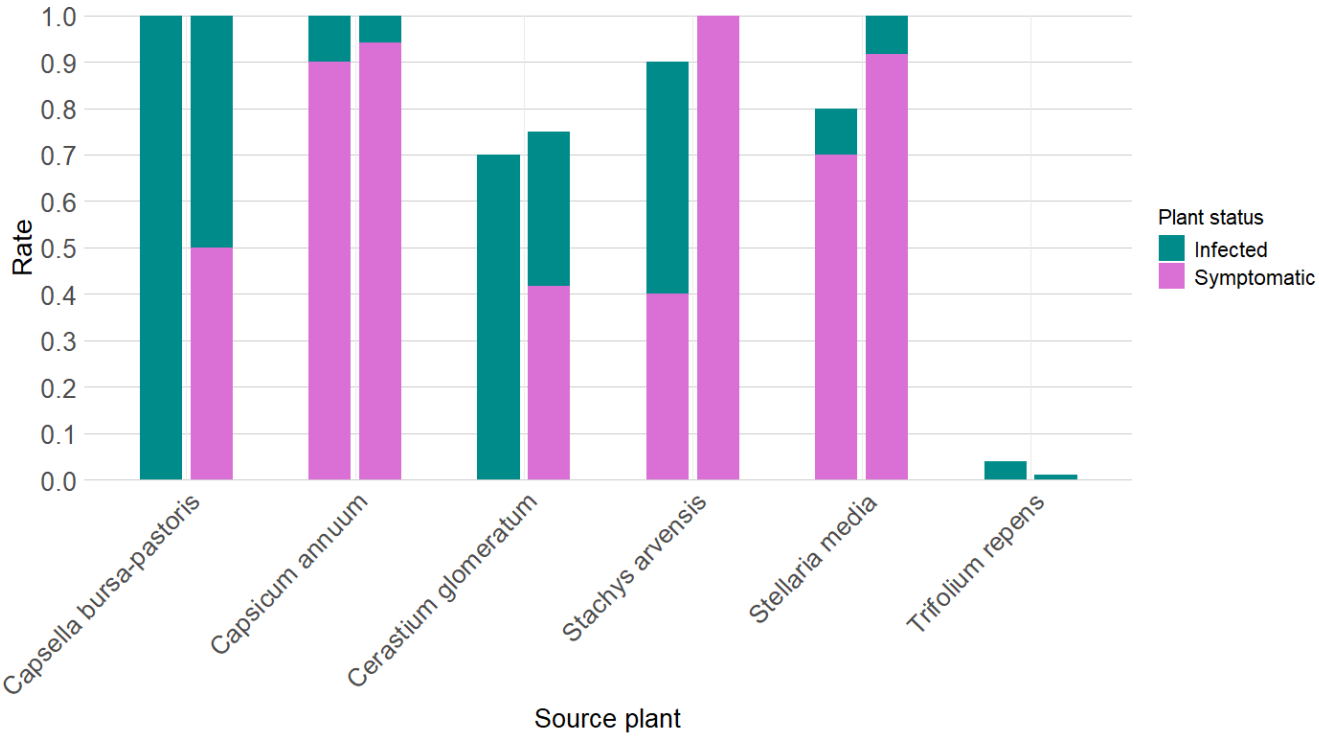

Figure 2 – Rates of mechanical transmission (cyan) and symptom expression (purple) for each plant species. The first bar corresponds to Test 1 (n=10 plants except for *T. repens* (n=100 plants)) , the second to Test 2 (n=12 plants except for *T. repens* (n=105 plants))

Contact transmission test

Contact transmission test involved rubbing infected and healthy plants and allowing them to grow in direct contact. Each target plant was then monitored for symptom expression and its sanitary status (healthy or infected) checked by ELISA (Table 1).

Transmission through contact was detected in 11 out of the 15 plant combinations tested. Transmission was observed from *C. bursa-pastoris* to *C. annuum* (75%), from *C. glomeratum* to *C. annuum* (13%), from *S. media* to *C. annuum* (13%), and from *S. arvensis* to *C. annuum* (44%). Transmission also occurred between *C. annuum* plants, although at a very low rate (8%). In the opposite direction, *C. annuum* transmitted CMV to *C. bursa-pastoris* (56%) and to *S. arvensis* (13%).

Transmission between individuals of the same species was also observed: 100% for *C. bursa-pastoris*, 25% for *C. glomeratum*, 25% for *S. media*, and 88% for *S. arvensis*. No transmission was detected from or to *T. repens*. Likewise, no infection was recorded from *C. annuum* to *C. glomeratum* or to *S. media*. Self-transmission in *T. repens* could not be tested due to the limited number of infected source plants.

Symptom assessment on pepper had a specificity (number of infected plants showing symptoms/number of symptomatic plants) of 85%. Indeed, among the 20 peppers displaying symptoms, only 17 tested positive by ELISA. In contrast, 8 peppers tested positive for CMV without exhibiting any symptom. Symptoms on weed plants were observed only during the second test on *S. media* and *C. glomeratum* with a frequency of 25%.

None of the negative control plants presented symptoms nor was tested positive in ELISA (data not shown)

Table 1. Number of infected and symptomatic plants in the contact transmission tests. Symptomatic plants were identified based on the presence of typical CMV symptoms, including mosaic, deformation, and necrosis. Infected plants were diagnosed based on ELISA tests. Modalities resulting in contact transmission are highlighted in bold.

Source plant	Target plants	Number of symptomatic plants		Number of infected plants		Transmission rate
		Test 1	Test 2	Test 1	Test 2	
Capsella bursa-pastoris	***Capsicum annuum***	4/8[a]	3/8	**5/8**	**7/8**	**0.75**
Cerastium glomeratum	***Capsicum annuum***	1/8	1/8	**1/8**	**1/8**	**0.13**
Stellaria media	***Capsicum annuum***	1/8	2/8[a]	**1/8**	**1/8**	**0.13**
Trifolium repens	*Capsicum annuum*	0/8	0/8	0/8	0/8	0.00
Stachys arvensis	***Capsicum annuum***	5/8[a]	1/8	**6/8**	**1/8**	**0.44**
Capsicum annuum	***Capsicum annuum***	0/8	1/8	0/8	**1/8**	**0.08**
		NA	1/8	NA	**1/8**	
		NA	0/8	NA	0/8	
		NA	0/8	NA	0/8	
Capsicum annuum	***Capsella bursa-pastoris***	0/8	0/8	**5/8**	**4/8**	**0.56**
Capsicum annuum	*Cerastium glomeratum*	0/8	0/8	0/8	0/8	0.00
Capsicum annuum	*Stellaria media*	0/8	2/8[b]	0/8	0/8	0.00
Capsicum annuum	*Trifolium repens*	0/8	0/8	0/8	0/8	0.00
Capsicum annuum	***Stachys arvensis***	0/8	0/8	**1/8**	**1/8**	**0.13**
Capsella bursa-pastoris	***Capsella bursa-pastoris***	NA	0/8	NA	**8/8**	**1.00**
Cerastium glomeratum	***Cerastium glomeratum***	NA	2/8	NA	**2/8**	**0.25**
Stellaria media	***Stellaria media***	NA	0/8	NA	**2/8**	**0.25**
Stachys arvensis	***Stachys arvensis***	NA	0/8	NA	**7/8**	**0.88**

[a] 1 plant exhibited symptoms but was tested negative by ELISA.
[b] 2 plants exhibited symptoms but were tested negative by ELISA.

Discussion

The objective of this study was to determine whether CMV could be transmitted through plant-to-plant contact. Active contact (plant rubbing) and passive contact (intertwining during growth) were tested jointly. The results showed that contact transmission was possible between certain plant combinations.

Mechanical inoculation does not exhibit equal efficiency across all plant species. Transmission rates ranged from 100% in *C. annuum* and *C. bursa-pastoris* to only 4% maximum, in *T. repens*. Although mechanical inoculation of CMV is generally very effective (Jacquemond 2012), the low success rate in *T. repens* required inoculating up to 105 plants, compared to just 12 for the other species. This may explain the absence of contact transmission involving *T. repens* in our experiment.

During symptom assessment, some asymptomatic plants tested positive by ELISA, suggesting they were still in the viral incubation period. Although the plants had been in contact for a month at the time of symptom evaluation, the exact moment of transmission remains unknown. It could have occurred early on during the leaf rubbing process or later during the

course of the experiment through plant intertwining. If the transmission happened only a few days before the experiment ended, the virus may have started replicating but not yet progressed enough to cause visible symptoms. At the opposite, some plants recorded as symptomatic were actually negative in ELISA. This could be due to the lack of specificity in our visual assessment, meaning that some symptoms may result from factors other than CMV infection. Another possibility is that the ELISA test lacked sufficient sensitivity to detect very low viral loads in the plant or that the virus had a heterogeneous accumulation within the plant.

These findings highlight the need to minimize plants contacts during experiments to avoid biases in the results. Slight friction between plants can occur spontaneously, even without direct intervention. Wind, air circulation, and even airborne sand particles can cause micro-wounds on plants. One potential solution is to increase the distance between plants in greenhouses whenever possible. To avoid plant contact in aphid arena experiments with apterous aphids, artificial bridges between plants could allow aphid movement between plants.

Additionally, this study emphasizes the importance of verifying whether a virus can be transmitted through contact. The absence of prior reports does not necessarily mean that such transmission is impossible. In aphid arena experiments, including a control modality without aphids is crucial to confirm that contact transmission does not occur and bias the results.

The transmission test used in this study combined two techniques: leaf rubbing, where plants were physically rubbed against each other, and intertwining, where plants were left to grow in contact. The exact mechanism underlying contact transmission of CMV remains unclear. Additional experiments could provide more detailed insights into these mechanisms, such as testing transmission with only one of the two techniques done here. Another approach could involve applying drops of viral homogenate directly onto leaves to assess whether this is sufficient to cause infection. Nevertheless, these investigations lie beyond the scope of the present study.

Our experimental setup does not necessarily reflect the real contact between plants in the field, leaving the impact of contact transmission unclear with respect to CMV epidemiology. Nevertheless, transmission between pepper plants appears rare: no transmission was observed in the first test, and only one plant in two arenas among four arenas became infected during the second. This suggests that contact transmission in peppers is infrequent, which is reassuring for both past and future experiments involving peppers, as well as for farmers growing peppers in close proximity in the field.

Authors contributions

LC and LR conceived and designed the experiments, and analyzed the data. The experiments were carried out by LR, LC, MS, and JT. The manuscript was written by LC, MS, and LR. LR supervised the project and secured funding.

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Data Availability

All data relevant to this work are available within the article.

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Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

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