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SHORT COMMUNICATION

Transmissibility of citrus yellow vein clearing virus by contaminated tools

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Abstract

In 2009, a new citrus viral disease caused by citrus yellow vein clearing virus (CYVCV) was first discovered in China, and now CYVCV is widely distributed in the field. CYVCV is transmissible by grafting and is spread by aphids from lemon to bean, and from bean to bean. However, whether CYVCV is transmitted by contaminated tools is unknown. In this study, the transmissibility of CYVCV with contaminated knife blades was investigated in sour orange and rough lemon. Three months after knife blade inoculation, CYVCV was detected in 16.5% of sour orange and 20.0% of rough lemon, respectively. Furthermore, six months post inoculation, the presence of CYVCV in sour orange and rough lemon was 23.3 and 20.0%, respectively. The results indicated that CYVCV is transmitted from citrus to citrus by contaminated knife blades.

Keywords Citrus · *Mandarivirus* · Virus disease · Cutting transmission

Yellow vein clearing disease (YVCD), an emerging viral disease, was first reported from Pakistan in 1988 in lemon [*Citrus limon* (L.) Burm.f.] and sour orange [*C. aurantium* (L.) Amara] (Catara et al. 1993). Symptoms in affected lemon consist of strong yellow vein clearing, leaf distortion and water soaking of the veins on the abaxial side of the leaf. The same symptoms were also observed on 'Etrog' citron [*C. medica* (L.) Ethrog], 'Rangpur' lime [*C. limonia* (L.) Osbeck], sour orange, and some lemon cultivars in Turkey and India (Önelge 2002; Alshami et al. 2003). Recently, YVCD has been observed in Yunnan and Sichuan Provinces of China where more than 80% of Chinese lemon production is concentrated (Zhou et al. 2013; Chen et al. 2014).

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Citrus yellow vein clearing virus (CYVCV), a member of the genus *Mandarivirus*, family *Alphaflexiviridae*, is considered the causal agent of YVCD (Loconsole et al. 2012). A positive sense flexuous RNA virus with a genome of 7.5 kb and six predicted open reading frames (ORFs) (Loconsole et al. 2012), CYVCV can be transmitted to most citrus species, cultivars and hybrids by grafting and mechanically to some herbaceous hosts (Ahlawat and Pant 2003; Önelge et al. 2011, 2016). CYVCV was also transmitted by *Aphis craccivora* and *A. spiraecola* from lemon to bean, and from bean to bean (Önelge et al. 2011). However, until now no vector has been shown to transmit CYVCV from citrus to citrus (Loconsole et al. 2012).

Since CYVCV was first detected in China in 2008, the virus has become widespread in all of Chinese citrus growing provinces (Zhou et al. 2017). The questions that arise from the present study are: why is CYVCV spreading so quickly? And how did CYVCV spread in China? In addition to vector transmission and virus-infected propagative materials, contamination of tools is another important route for citrus virus transmission. Previous studies showed that citrus tristeza virus (CTV) (Garnsey et al. 1977), citrus tatter leaf virus (CTLV) (Sun et al. 2009) and citrus leaf blotch virus (CLBV) (Chavan et al. 2013) could be transmitted by contaminated tools with high transmission rates. The high incidence of CYVCV in China may result from the use of contaminated knife blades. This prompted us to investigate the transmissibility of CYVCV by contaminated knife blades.

Table 1 Mechanical transmission of citrus yellow vein clearing virus by knife-blade inoculation under experimental greenhouse conditions

Citrus species	Three months post inoculation		Six months post inoculation	
	Transmissibility	Average transmission rate (%)	Transmissibility	Average transmission rate (%)
Sour orange	4/30[a]	16.5	7/30	23.3
	6/30		10/30	
	7/43		7/43	
Rough lemon	6/30	20.0	6/30	20.0

[a] Numerator = number of plants infected; denominator = number of test plants used. Three groups of sour orange plants were inoculated separately

CYVCV isolate CY-T1 was collected from Chongqing province of China and was graft-inoculated onto Pineapple sweet orange [*C. sinensis* (L.) Osbeck] seedlings to create virus donor plants. Virus-free sour orange and rough lemon [*C. jambhiri* (L.) Lush] seedlings were used as receptor plants. The presence of CYVCV in the donor plants and receptor plants was confirmed by RT-PCR as described previously (Chen et al. 2015). All of the plants were planted individually in 12 cm pots with sterilized potting soil (1/3 sand, 1/3 chaff, 1/3 peat).

One hundred and three sour orange and thirty virus-free rough lemon seedlings were used to inoculate CYVCV by the knife-cut procedure, described by Garnsey et al. (1977). CYVCV was inoculated in stem tissue 25 cm above the soil level using a sterile surgical blade. The knife blade was twice sliced slowly through the stem of the infected donor plant, and then sliced diagonally about 3 mm into the stem of the recipient plant, 50 cuts per receptor plant were made. The incision wound made in the receptor stem was wrapped with Parafilm (Beacon and Janis, Ltd., London, England). An additional five virus-free sour orange and five virus-free rough lemon seedlings were cut by sterile surgical blades after cutting into stems of healthy Pineapple sweet orange, and used as negative controls. CYVCV isolate CY-T1 was graft-inoculated onto virus-free sour orange and virus-free rough lemon as positive controls. After inoculation, all of the plants were decapitated and maintained in the greenhouse at 18 °C to 25 °C.

Three months after inoculation, the young shoots of the receptor plants were analyzed by RT-PCR analysis (Chen et al. 2015). The results showed that CYVCV was detected in 16.5% of sour orange and 20.0% of rough lemon, respectively. CYVCV was detected in all of the positive control plants at three months post inoculation. Six months post inoculation, more sour orange receptor plants tested positive for CYVCV, and about 23.3% of sour orange and 20.0% of rough lemon were CYVCV-positive (Table 1); the CYVCV-infected sour orange showed strong yellow vein clearing and leaf distortion like the positive controls. Six months post inoculation, no CYVCV was detected in negative control.

In this preliminary greenhouse transmission study, the results showed that the transmissibility of CYVCV by knife-blade inoculation is lower than grafting (Alshami et al. 2003), but CYVCV was easily transmissible from citrus to citrus with a contaminated knife blade. Therefore, contaminated cutting tools are probably one important route of CYVCV transmission in the field, and local spread. Previous studies showed that decontamination procedures are necessary when clean and virus-infected citrus plants are grown in the same orchard (Garnsey et al. 1977; Kyriakou 1992; Nishiio et al. 1982; Roistacher et al. 1980). Further studies are required to identify the effect of knife blade decontamination to inhibit the CYVCV transmission. Furthermore, even though a citrus virus-testing scheme has been implemented in China for decades, the evaluation of the virus status of budwood source trees in some nurseries, especially in small nurseries, is primarily based on visual inspection and the identification of characteristic symptoms (Zhou et al. 2013). However, CYVCV is frequently latent in most sweet orange, pummelo [*C. grandis* (L.) Osbeck] and mandarin [*C. reticulata* (L.) Blanco] varieties (Chen et al. 2015), and therefore this visual "virus testing" approach might also exacerbate the dissemination of CYVCV in China.

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