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International Organization of Citrus Virologists Conference Proceedings (1957-2010)

Title
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Permalink
https://escholarship.org/uc/item/50k9h224

Journal
International Organization of Citrus Virologists Conference Proceedings (1957-2010), 12(12)

ISSN
2313-5123

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Publication Date
1993

DOI
10.5070/C550k9h224

Peer reviewed

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Graft Transmission of Citrus Measles

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ABSTRACT. Measles is a disorder that occasionally affects a few trees in a grove. Symptoms produced are numerous pale yellow spots that appear on leaves as they mature. Once a tree is affected with measles, the symptoms reappear year after year. The cause of measles is unknown, and it was thought to possibly be a genetic variant. We have succeeded in graft transmitting measles to indicator plants using blind buds from symptomatic field trees. The indexing was done with a temperature regime of 18 C night and 38 C day temperatures. Faint translucent spots occurred on young leaves which then developed into typical measles spots as the leaves matured. The graft transmission of measles symptoms suggests this disorder is caused by an infectious agent, although no double stranded RNAs or virus-like particles have been associated with it.

In Florida a condition, commonly referred to as citrus measles, occurs on rare occasions. When found, it usually occurs on only a few trees in a grove. On affected trees, pale yellow spots appear on the leaves, which can be seen from both the adaxial and abaxial surfaces. Sometimes only one branch or limb is affected, but the symptoms can appear throughout the tree canopy. Once a tree develops measles, the symptoms reappear year after year. Vigorous sweet orange varieties, such as Hamlin and Pineapple, are most often affected by this disorder, although the symptoms also have been observed on Valencia and Navel orange trees, as well as other citrus species. It has been reported that measles may be a genetic variant (3).

The purpose of this study was to determine if citrus measles is caused by an infectious agent that could be graft transmitted to indicator plants.

METHODS AND MATERIALS

Hamlin sweet orange grafted onto rough lemon rootstock, Valencia grafted onto sour orange rootstock, Mexican lime seedlings, citron clone '861', and Madam Vinous sweet orange seedling were used as indicator plants. Indicator plants were kept to a single shoot and were maintained in an insect proof greenhouse with median temperatures of 38 C day and 18 C night. Metro-Mix 500 potting mixture was used, and standard greenhouse fertilization and pesticide procedures were followed.

Budwood was collected from a measles affected Hamlin tree in a field location in October, 1991. Inoculation to indicator plants was done by cutting blind bud chips from the budwood which had been collected from branches showing measles symptoms. Three bud chips were grafted onto each of the indicator plants using a T cut and tightly wrapped with grafting tape. Three plants of each indicator species were used. Bud survival was checked after three weeks. Indicator plants having no bud chips alive were regrafted from the original source material that had been stored at 4 C.

Double stranded RNA (dsRNA) analysis was performed as described by Lee (2) using 4 g of bark or symptomatic leaf tissue and CF-11 cellulose chromatography. The dsRNAs were analyzed on a 5% polyacrylamide gel after staining with ethidium bromide (2). Positive controls of citrus tristeza virus (CTV) infected tissue and healthy tissue were included in each dsRNA analysis.

Quick dip extracts from symptomatic leaves at various stages of measles development were examined on formvar coated grids using a Phillips 301 transmission electron microscope after either positive straining with 2% uranyl acetate (1) or negative straining in 1% potassium phosphotungstate, pH 7.5.

RESULTS AND DISCUSSION

When the second growth flush developed about 60 days after graft inoculation, faint translucent spots were observed on young leaves as they appeared (Fig. 1). As the leaves enlarged and matured, spots became more apparent and started to develop the typical pale yellow color (Fig. 2), and as the leaves hardened, typical measles symptoms developed (Fig. 3). While symptoms developed on all the indicator plants used, Mexican lime and Madam Vinous were the first to produce symptoms. These indicators have been the most consistent in displaying measles symptoms as compared to the other indicator plants, although all indicators developed measles symptoms. Moving the indicator plants into greenhouses with warmer night temperatures resulted in loss of the measles symptoms on the new flush tissue; returning the plants to the original greenhouse resulted in symptoms again appearing on the newly emerging flush. This indicates that the temperature at which the indicator plants are maintained may be critical for expression of measles symptoms.

No dsRNAs have been associated with the measles symptoms being expressed in the symptomatic indicator plants. No virus or virus-like particles

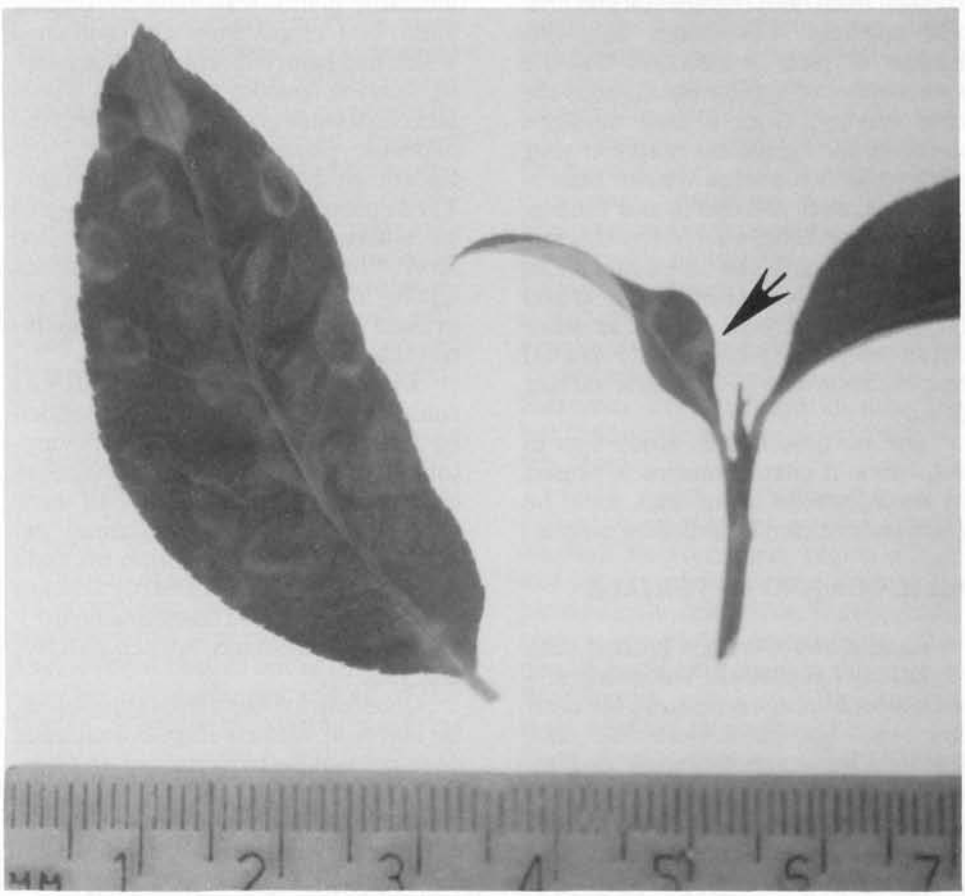

Fig. 1. Translucent spots on citron occurring on the second growth flush and subsequent growth flushes after inoculation with bud chips from measles affected field source as viewed over transmitted light. The arrow points to a newly developing spot on the second leaf from the terminal. The detached leaf with several translucent spots was the third leaf from the shoot terminal.

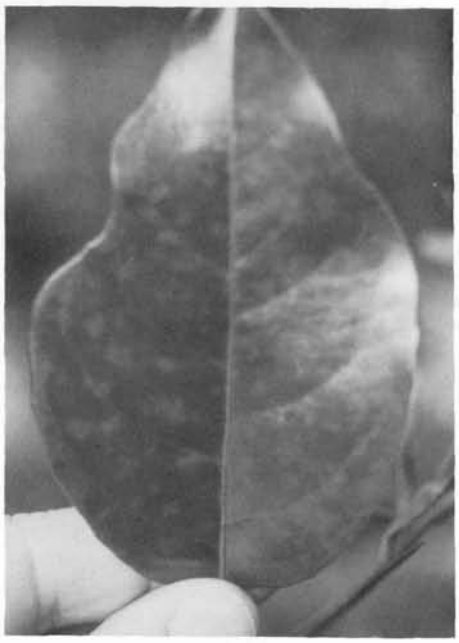

Fig. 2. A leaf from Madam Vinous showing the stage when the translucent spots are beginning to develop the characteristic pale yellow color typical of citrus measles.

have been identified by electron microscopy.

In Florida, measles occurs at a very low rate. In a recent field experiment involving 8,000 Hamlin on various rootstock sources, one plant has developed measles symptoms after two years. However, in Brazil there have been reports of up to 40% incidence of measles and with measles spots occurring on fruit as well as leaves (M. J. G. Beretta, personal communication).

Graft transmission of measles symptoms through a bud chip indicates this disorder is caused by an infectious agent and not as previously thought, a genetic disorder (3). Growers should be aware that measles can be graft transmitted and take care not to propagate from source material that has measles symptoms.

ACKNOWLEDGMENTS

We thank Neil Berger and Beverly Hewitt for technical assistance. This

Fig. 3. Back (left) and front (right) sides of leaves of a Hamlin indicator plant showing typical measles symptoms. The measles spots, as seen in field trees, are also present on both sides of the leaf. Note that it is not unusual for the measles spots to be limited to one side of the leaf.

study would not have been possible without the temperature controlled greenhouses donated by the Florida citrus growers.

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