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SHORT COMMUNICATION

Further investigation on citrus phantom disorders of unconfirmed viral etiology

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Abstract

This brief report expands upon the original review article published in Journal of Citrus Pathology in 2023 on citrus "phantom" disorders of presumed virus and virus-like etiology and addresses five additional disorders: citrus seed-borne virus disorder in New Zealand, bergamot vein yellowing in Greece, bergamot gummosis in Italy, bud knot in Italy, and a disorder resembling citrus crinkly leaf in Cuba. Each disorder is characterized by distinct symptoms and transmission patterns yet remains unresolved in terms of causative agents or conditions. By providing comprehensive information on these phantom citrus disorders, this report aims to serve as an additional reference for the citrus research community, industry stakeholders, and regulatory offices.

Keywords: citrus disorders, citrus pathology, citrus virus, graft-transmissible, causal agent, disease etiology

Introduction

Recently, we made an effort to clarify and update the status of citrus disorders, which were at some point suspected to have viral causal agents (Aknadibossian et al., 2023). Some of these disorders have since been attributed to either known pathogens or abiotic conditions, however, others remain without any established connection to a causative agent or condition, and as such, further clarification on the etiology of those disorders was needed. On the other hand, some disorders seemed to disappear from the literature soon after they were reported, and no isolates from the affected trees were preserved. Those were termed "phantoms". Since the publication of our initial review article in 2023, which aimed to sort out the information on such disorders, it was brought to our attention that five additional phantom disorders should be discussed in continuation of this work. Therefore, the purpose of this communication is to address the unresolved cases of 'citrus seed-borne virus disorder', three bergamot disorders – 'bergamot vein yellowing', 'bergamot gummosis' and 'bud knot' - and a disorder with the symptoms resembling those of citrus crinkly leaf virus. Along with the original review article (Aknadibossian et al., 2023), this study provides the citrus research community as well as the citrus industry and regulatory offices with a comprehensive reference on the phantom citrus disorders of the past.

Citrus seed-borne virus disorder, New Zealand

A disorder associated with an agent named citrus seed-borne virus was reported in New Zealand in 1998 (Pearson et al., 1998). Virus-like symptoms, which included "boat and spoon-shaped" leaves, dwarfing, and small fruits, were observed in the mid-to-late 1980s in satsuma mandarin (*Citrus* × *aurantium* L. var. *chrysocarpa* (Hassk.) ined.) orchards. Transmission electron microscopy and enzyme-linked immunosorbent assays revealed the presence of citrus tristeza virus (CTV) in these trees along with another type of filamentous particles with a length ranging from 150 to 500 nm. The virus was later detected in other citrus species co-infected with CTV and expressing variable symptoms. It was also found in asymptomatic CTV-free 'Madame Vinous' sweet orange (Citrus × aurantium L. var. sinensis L.) and Citrus x excelsa Wester seedlings growing

in an insect-free glasshouse. Seed transmission tests were not reported, and the presence of the virus in the insect-free glasshouse is the only evidence supporting the seed-borne nature of the virus. The sap obtained from the diseased satsuma mandarins was mechanically inoculated into 20 different herbaceous indicator hosts, among which only common beans (Phaseolus vulgaris L.) occasionally exhibited systemic pinpoint chlorotic spots. The virus was reported to react with the antiserum raised against the Indian citrus ringspot virus (ICRSV). The European and Mediterranean Plant Protection Organization (EPPO) included this virus in the Alert List in 1999 (1999/134) and later removed it in 2001 (2001/116). The virus is still mentioned in the EPPO Database as CiSV, ID CISV00. The virus is also mentioned in the 2015 Plant Health Australia Citrus Biosecurity Plan (Plant Health Australia Ltd., 2015). However, it is not considered as a threat to the citrus industry. The author of the first report of the virus is not aware of any further work on the identification or characterization of the virus nor of the availability of the plant material for further analysis (Jean-Pierre Thermoz, personal communication).

Bergamot vein yellowing, Greece

Bergamot vein yellowing was first reported on bergamot (*Citrus aurantium* subsp. *bergamia* (Risso & Poit.) Wight & Arn. ex Engl., syn. *Citrus bergamia*) trees in Greece showing bright yellowing of the mid and lateral veins (Protopapadakis et al., 2002). Different citrus hosts were graft-inoculated with buds from the symptomatic bergamot trees, and the symptoms were reproduced in bergamot seedlings 14 months after inoculation, indicating that the disease was graft-transmissible. The symptoms were also reported to be more severe at 32°C compared to below 25°C. However, the vein yellowing symptoms were not reproduced in any of the other citrus species inoculated. While the symptoms observed on the originally reported trees were similar to yellow vein disease reported in California, USA (Weathers, 1960), which has since been attributed to citrus yellow vein-associated virus, the incubation time and the reaction of different citrus hosts distinguished the two disorders. Other symptoms observed on grafted citron seedlings such as epinasty, drooping leaves, and yellow blotch led the authors to suspect the presence of different viroids. They subsequently tested for and confirmed the presence of citrus exocortis viroid and hop stunt viroid, without ruling out the presence of additional viroids. Since the original report, the only other mention of the disease has been a brief allusion to the original report under “Other virus and virus-like diseases” in a book chapter describing bergamot diseases (Agosteo et al., 2013). Further efforts have been non-conclusive, and it is unknown if any isolates are available (Grazia Licciardello and Antonino Catara, personal communication).

Bergamot gummosis, Italy

Bergamot gummosis, which is characterized by gum exudate oozing from small pustules and cracks on the bark during spring and autumn months that are washed away by winter rains, has been observed in Italy on bergamot trees for over a century (Catara et al., 1980). The disorder is of unknown etiology, but there has been some discussion on whether it could be a viral disease (Terranova and Cutuli, 1975; Terranova et al., 1978-1979; Terranova et al., 1984; Catara et al. 1980; Catara et al., 1984). The disorder was not shown to be graft-transmissible and bergamot trees have been shown to exude gum in reaction to many biotic and abiotic stresses (Matarese Palmieri et al., 1979). Since the disease was localized in some areas with different pedoclimatic conditions it was hypothesized that pedoclimatic factors were directly or indirectly involved with the symptomatology (Catara et al. 1980; Catara et al., 1984). The most recent appearance of bergamot gummosis in the literature is in a book chapter describing bergamot diseases, which states that it is widespread in bergamot groves in the province of Reggio Calabria, Italy (Agosteo et al., 2013). Further work attempting to attribute bergamot gummosis to a viral pathogen was inconclusive.

Bud knot, Italy

A third virus-like disorder of bergamot, named ‘bud knot’, was found widespread in the three main varieties of bergamot in Italy (Catara et al. 1968). The symptoms were bud enlargement, shortened internodes, yellowing and corking of the leaf veins, and dieback of the stems and branches. La Rosa et al. (1984) investigated its etiology by transmission and propagation tests, histology, and in-vitro culture and found no evidence of transmissibility. Bergamot seeds of the Femminello variety produced some seedlings with vein corking and uneven growth. *In-vitro* culture of affected tissues revealed an irregular, undifferentiated tumor-like growth. The results suggested the disorder was a propagable, non-infectious disorder of a possible genetic origin. No conclusive information is available. However, bud knot is still randomly observed in some old orchards, which makes it possible to collect and analyse the symptomatic plant material further (Grazia Licciardello and Antonino Catara, personal communication).

Citrus disorder with symptoms similar to those caused by citrus crinkly leaf virus, Cuba

In 1977, a graft-transmissible citrus disorder was reported in Cuba, causing various symptoms in three citrus varieties: Dancy mandarin, Valencia sweet orange, and Marsh grapefruit (*Citrus ×aurantium* L. var. racemosa (Risso) ined.). These trees were located in the citrus-growing areas of Jagüey Grande (Matanzas province) and Sola (Camagüey province) (Perez et al., 1977). The described symptoms included dwarfing with varying degrees of severity, tiny leaves, vein clearing, and leaf malformation so severe that it prevented recognition of the original citrus species (asymmetry, sinuous margins,

central vein bifurcation, and blisters). Indicator plants inoculated in transmission assays using leaves and bark from the diseased plants exhibited symptoms similar to those caused by the citrus crinkly leaf virus (CCLV). Vein clearing, oak leaf-like symptoms, leaf deformation, bark necrosis, and short internodes were observed from five weeks up to two-three months, and occasionally up to five and half months after inoculation. Mechanical transmission to citrus and herbaceous plants using sap from the diseased plants was ineffective, which distinguished it from CCLV (Matos & Pérez, 1980; Matos et al., 1980). Despite attempts, the potential pathogen could not be eliminated through *in-vitro* shoot-tip grafting using different shoot-tip sizes. However, two isolates of this suspected pathogen were obtained: one from Dancy mandarin with mild symptoms and another from Marsh grapefruit with severe symptoms, both free of the viroids present in the original source (González et al., 1977). Subsequently, 33 citrus cultivars susceptible to infection with the isolates obtained by *in-vitro* shoot-tip grafting were identified (Perez et al., 1981). Pathogen inactivation was possible through heat treatment at 40°C in a controlled temperature chamber (Peña, 2001). The crinkly leaf-like pathology observed in Cuba exhibits distinctive characteristics: broad diversity of symptoms, lack of sanitation through in-vitro shoot-tip grafting, and non-mechanical transmission. Although the disease is no longer present in citrus orchards, these isolates are preserved in a greenhouse at the Instituto de Investigaciones en Fruticultura Tropical (IIFT), allowing for further studies to gather new information on this disorder and clarify its phantom status as associated with a known or novel citrus pathogen.

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