Express Pest Risk Analysis for

"*Citrus yellow vein clearing virus*"

PRA area: Italy (Campania region)

Performed according to EPPO Standard PM5/5 (1) "Guidelines on Pest Risk Analysis"

Approved by National Plant Health Committee on 25/26 June 2024

Summary[1] of the Express Pest Risk Analysis for "*Citrus yellow vein clearing virus*"			
PRA area: Italy (Campania region)			
Describe the endangered area: *(see question 14)* Possible economic impact on Campania and other citrus-growing Italian regions: Sicily, Apulia, Calabria, Basilicata and Sardinia. These regions all share common environmental conditions favouring the presence of the vectors.			
Main conclusions The risk of entry, both in Campania and other Italian areas, is considered moderate. The major risk factor is represented by the dispersal of viruliferous insect vectors, whereas the movement of plants for planting constitutes a minor risk as it is sufficiently guaranteed by the current reference phytosanitary regulatory framework. The economic, social and/or environmental impact of the possible establishment and spread of CYVCV in the Italian territory is difficult to evaluate due to the poor scientific knowledge available for the environments of the Mediterranean basin. . In Campania region, symptomatic infected plants mainly belong to *Citrus* x *limon* species and were reported in private gardens or small citrus orchards. Prompt phytosanitary measures, consisting of extended surveys in the Campania territory, are strongly recommended, giving priority to the coastal areas of Sorrento and Amalfi, where many lemon orchards are present, endowed with high economic, social and environmental added-value. Such measures will support the early detection of possible new outbreaks; their implementation is also recommended in other citrus-growing Italian regions and EPPO countries. Taking into account the potential spread rate of the virus and the limited damage reported so far, which affects only *Citrus* x *limon* plants, it is likely that eradication measures may now have higher costs than actual economic losses induced by the virus.			
Phytosanitary risk for the *endangered area* *(Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document)*	High ☐	Moderate X	Low ☐
Level of uncertainty of assessment *(see section 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)*	High ☐	Moderate X	Low ☐
Other recommendations: • *Inform EPPO or IPPC or EU* • *Inform industry, other stakeholders* • *State whether a detailed PRA is needed to reduce level of uncertainty (if so, state which parts of the PRA should be focused on)* • *Specify if surveys are recommended to confirm the pest status* • *State what additional work/research could help making a decision*			

[1] The summary should be elaborated once the analysis is completed

Express Pest Risk Analysis:

Citrus yellow vein clearing virus

(Pest name)

Prepared by: Anna Taglienti, Andrea Gentili, Luca Ferretti e Francesco Faggioli **(**CREA - Research Centre for Plant Protection and Certification); Carmela Carbone, Valentina Murano e Raffaele Griffo (Regional Plant Protection Organization of Campania); Maria Minutolo, Maria Cinque e Daniela Alioto (University of Naples "Federico II" - Department of Agriculture)
Date: 18 June 2024

Stage 1. Initiation

Reason for performing the PRA: *(e.g. interceptions, outbreak*)
The pest is not known to occur in the EU territory. In 2024, CYVCV was detected in some private gardens and commercial orchards in different areas of Campania region (provinces of Naples, Caserta and Salerno).

PRA area: Italy (Campania region)

Stage 2. Pest risk assessment

1. Taxonomy

- Regno viruses and viroids (1VIRUK)
- Category Riboviria (1RIBVD)
- Category Orthornavirae (1ORTVA)
- Phylum Kitrinoviricota (1KITVP)
- Class Alsuviricetes (1ALSVC)
- Order Tymovirales (1TYMOO)
- Family Alphaflexiviridae (1AFLXF)
- Genus Potexvirus (1POTXG)
- Subgenus Mandarivirus (1MANRG)
- Species Potexvirus citriflavivenae - Citrus yellow vein clearing virus (CSYV00)
- Synonym: citrus yellow vein agent

2. Pest overview

• *Biology*: Citrus yellow vein clearing virus (CYVCV, *Potexvirus citriflavivenae*) has structural and genomic features common to the *Mandarivirus* subgenus. Elongated, flexuous viral particles are observed; the ssRNA genome is 7531 nt long and organized in 6 ORFs.
CYVCV is transmitted by grafting and contaminated tools; such transmission modes may cause both long-range spread by asymptomatic propagation material, and diffusion in the field, the latter mainly caused by insect vectors.
The virus was frequently detected in seeds, but seed transmission is not ascertained to date (Zhou et al., 2015).
Virus particles are mainly localized in phloematic tissues.

• *Host plants:* Positive, symptomatic lemon plants and positive, asymptomatic sweet orange plants were found in the PRA area.
Many *Citrus* species are reported as CYVCV hosts, but severe symptoms are reported only in lemon and sour orange (main hosts). Recently, a single case of natural infection was reported in grapevine in Turkey. Also, artificially inoculated grapevine plants showed severe symptoms as shortened internodes, leaf chlorosis and necrosis, and reduced leaf size. The following herbaceous hosts are also reported: *Malva sylvestris, Ranunculus arvensis, Sinapis arvensis* and *Solanum nigrum.*

• *Symptoms:* Infected lemon plants in the PRA area showed yellow vein clearing of leaves. Symptoms of CYVCV are mainly reported in lemon and sour orange.

Yellow vein symptoms include strong yellow vein clearing and dropsy, leaf distortion and occasionally ringspots and vein necrosis. Symptom remission is observed in the summer (Liu et al., 2020). Severely diseased plants show decay and a quantitative and qualitative decrease of fruit yields. CYVCV can also cause fruit deformation. In most sweet orange, pomelo and tangerine varieties CYVCV is latent, thus not expressing symptoms. Generally, symptomatology may vary greatly depending on variety, genotype, viral strains and environmental conditions.

• *Detection and characterization:* molecular and serological diagnostic tests are described in the literature, the latter based on ELISA or, for *in situ* testing, on immunostrip. To date, none of these has been validated. For samples in the PRA area, the diagnosis has been performed by total nucleic acid extraction from leaf tissue and RT-PCR amplification of RNA using primer pairs CYVCV-F/ CYVCV-R (Chen et al., 2014) and CYVR-07F/ CYVR-07R (Meena et al., 2019). Sanger sequencing was then performed on the amplicon obtained from a lemon sample with primer pairs CYVCV-F/CYVCV-R, and BLAST analysis of the sequence confirmed the identity of the virus.

3. Is the pest a vector? **Yes** ☐ **No** X

4. Is a vector needed for pest entry or spread? **Yes** X **No** ☐

Aphis aurantii (TOXOAU), *Aphis gossypii* (APHIGO), *Aphis spiraecola* (APHISI) and *Dialeurodes citri* (DIALCI) are known vectors of CYVCV and are present in the PRA area. Moreover, *A. craccivora* (APHICR) is reported as an experimental vector from *Citrus* spp. to legumes.

5. Regulatory status of the pest

The virus is included in the EPPO Alert List.

6. Distribution

Continent	***Distribution*** *(list countries, or provide a general indication, e.g. present in West Africa)*	*Provide comments on the pest status in the different countries where it occurs e.g. widespread, native, introduced….)*	***Reference***
Africa	Absent		EPPO database
America	USA (California)	Restricted distribution	EPPO database
Asia	China (9 Provinces), India (3 States), Pakistan	India: restricted distribution	EPPO database
Europe	Turkey	Eradicated pest, no longer present	EPPO database
Oceania	Absent		EPPO database

7. Host plants /habitats* and their distribution in the PRA area

Host Scientific name (common name) / *habitats**	**Presence in PRA area** (Yes/No)	**Comments** (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)	***Reference***
Gen. *Citrus*	Yes	Major host (*Citrus* x *aurantium*, *Citrus* x *limon*), host (other *Citrus* spp.)	EPPO database
Fortunella sp.	Yes	Host	
Vitis vinifera (grapevine)	Yes	Host	
Phaseolus vulgaris (bean)	Yes	Experimental host	
Vigna unguiculata (black-eyed pea)	Yes	Experimental host	
Malva sylvestris (wild mauve)	Yes	Wild host	
Ranunculus arvensis (ranunculus)	Yes	Wild host	
Sinapis arvensis (wild mustard)	Yes	Wild host	
Solanum nigrum (black nightshade)	Yes	Wild host	

8. Pathways for entry

Possible pathways *(in order of importance)*	**Short description explaining why it is considered as a pathway**	Pathway prohibited in the PRA area? Yes/No	Pest already intercepted on the pathway? Yes/No
Plants for planting (except seeds, bulbs and tubers) with or without soil attached	Transmission through infected propagation material is ascertained	No	No
Natural spread, hitchhiking	Horizontal transmission by insect vectors present in the PRA area is ascertained	No	No

Rating of the likelihood of entry	*Low* ☐	*Moderate* X	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* X	*High* ☐

9. Likelihood of establishment outdoors in the PRA area

The pathogen is considered able to establish in most of the Italian territory, as many host plants and vectors *(Aphis spiraecola, A. craccivora, A. aurantii, A. gossypii e Dialeurodes citri)* are present. Thus, environmental conditions in most parts of Italy, particularly in citrus-growing regions, are potentially favourable to the establishment of the virus. Nonetheless, long-range transmission is mainly due to infected propagation material, whose production and movement are performed under strict phytosanitary regulation. Moreover, the control of aphid vectors, particularly in citrus-growing areas, is already carried out for preventing the spread of other important pathogens of citrus (e.g., citrus tristeza virus - CTV). The above mentioned measures concur in decreasing the risk of establishment of the virus.

Rating of the likelihood of establishment outdoors	*Low* ☐	*Moderate* X	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* X

10. Likelihood of establishment in protected conditions in the PRA area

Rating of the likelihood of establishment in protected conditions	*Low* ☐	*Moderate* X	*High* ☐
Rating of uncertainty	*Low* X	*Moderate* ☐	*High* ☐

11. Spread in the PRA area

- Natural spread: flights and wind dispersal of insect vectors.
- Human-assisted spread: movement of plants for planting, propagation material and rootstocks, contaminated tools.

The virus is associated with many *Citrus* species and transmitted by insect vectors present in Italy. Under experimental conditions, the transmission rate of *Dialeurodes citri* on sour orange is around 39% after 6 months from the exposition to the viruliferous vector (Zhang et al., 2019).

Moreover, CYVCV can spread through grafting and movement of propagation materials and rootstocks, or by contaminated equipment and tools. Given that no treatments are available against CYVCV, the best preventive strategy is based on the control of vectors and sanitization of equipment and tools.

Rating of the magnitude of spread	*Low* ☐	*Moderate* X	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* X

12. Impact in the current area of distribution

Citrus spp. are economically important crops in the Mediterranean basin, and are cultivated both for fruit production and ornamental purposes. CYVCV is the aetiological agent of a disease which negatively impacts on production, by decreasing plant fitness and marketability of fruits (Chen et al., 2014; Li et al., 2017). In case of severe disease, a decay is observed and the malformation of fruits cause yield losses up to 50-80% (Zhang et al., 2019). The detection of CYVCV in grapevine in Turkey may potentially broaden the risk induced by the virus also to this crop.

The economic, social and environmental impact of the possible establishment and spread of CYVCV in the Italian territory is difficult to evaluate, taking into account the absence of the pathogen in the Mediterranean Basin, leading to poor scientific knowledge in such environment. To date, symptoms were mainly observed in *Citrus* x *limon* plants, which are economically important in the current area of distribution of the virus.

Rating of the magnitude of impact in the current area of distribution	*Low* ☐	*Moderate* X	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* X	*High* ☐

13. Potential impact in the PRA area

Will impacts be largely the same as in the current area of distribution? Yes

If No

Rating of the magnitude of impact in the area of potential establishment	*Low* ☐	*Moderate* ☐	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ☐	*High* ☐

14. Identification of the endangered area

Likely economic impact in other citrus-growing regions in Italy: Sicily, Apulia, Calabria, Basilicata and Sardinia.

15. Overall assessment of risk

The risk of entry in new areas in Campania region and the rest of Italy is moderate. The main factor is the diffusion of viruliferous insect vectors, whereas the movement of plants for planting constitutes a minor risk as it is sufficiently guaranteed by the current reference phytosanitary regulatory framework. The economic, social and/or environmental impact of the possible establishment and spread of CYVCV in the Italian territory is difficult to evaluate due to the poor scientific knowledge available for the environments of the Mediterranean basin. In Campania region, symptomatic infected plants mainly belong to *Citrus* x *limon* species and were reported in private gardens or small citrus orchards.

Prompt phytosanitary measures consisting of extended surveys in the Campania territory are recommended, prioritizing the coastal areas of Sorrento and Amalfi, where many lemon orchards with high economic, environmental and social added-value are present. Such measures will help in the early detection of possible new outbreaks; their implementation is also recommended in other citrus-growing Italian regions and EPPO countries.

Stage 3. Pest risk management

16. Phytosanitary measures

Given the detection of CYVCV in Campania region in 2024 in different citrus species in private gardens and commercial orchards in the provinces of Naples, Caserta and Salerno, the adoption of phytosanitary measures is prompted to improve surveys in the Campania territory, in order to establish the actual distribution of the virus and better evaluate further measures to be adopted. Meanwhile, it is recommended that other regional Plant Protection Organizations in Italy, which may potentially be concerned, set up appropriate control measures and establish the actual distribution of the virus in Italy. Taking into account the potential spread rate of the virus and the limited damage reported to date, only on *Citrus* x *limon* plants, it is likely that eradication measures may have higher costs than actual economic losses induced by the virus.

17. Uncertainty

The main sources of uncertainty within this risk assessment are due to the absence of the virus in the Mediterranean Basin; hence, the features of establishment and spread associated with the Italian climatic and environmental conditions cannot be inferred by similar occurrences in other countries.

The main sources of uncertainty within the risk management is the poor knowledge of the actual distribution of the virus in the PRA area. To address uncertainties, extended surveys would be needed.

18. Remarks

The recommended phytosanitary measures do not apply to those Italian regions nor EPPO countries where citrus growing is not concerned.

Once the analysis has been completed, a summary should be prepared
(see the summary box at the beginning of the Express PRA)

19. REFERENCES

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Appendix 1. Relevant illustrative pictures (for information)

Photo 1	*Photo 2*
	
Photo Prof. D. Alioto	*Photo Prof. D. Alioto*