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Surveillance, symptomology, and status update of three emerging viruses in Trentino region in Northern Italy

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Trentino region
Northern Italy

pome fruits

grapevine

stone
fruits

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emerging viruses detected in Trentino

Luteovirus mali Apple luteovirus 1, ALV1

Cherry necrotic rusty mottle virus, CNRMV

Trichovirus pinovitis Grapevine Pinot gris virus GPGV

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Apple luteovirus 1, (ALV1)

A new luteovirus and/or three known viruses were found in trees with Rapid apple decline with HTS in Pennsylvania

In 2022 reported on apple in Belgium, but the trees showed no visible symptoms of viral infection

Genus Luteovirus in the family Luteoviridae

- Fontdevila Pareta, N., Lateur, M., Steyer, S., Blouin, A.G. & Massart, S. (2022) First reports of Apple luteovirus 1, Apple rubodvirus 1 and Apple hammerhead viroid infecting apples in Belgium. New Dis Rep. 45, e12076. Fontdevila Pareta

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Apple luteovirus 1, (ALV1)

‘Rapid Apple Decline’ (RAD) is a newly emerging problem of young, dwarf apple trees in the Northeastern USA.

Trunk necrosis, cracking and canker before collapse in summer

First report of dieback in Trentino: 1996-1997 (Pertot and Vindiminan, 1998), symptoms increasing in the last years (2009) specially in young stressed plants (2-5 years)

Apple decline in Trentino region

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- Initial symptoms: stunted growth and chlorotic leaves
- Most affected plants collapse and die during the growing season

Apple decline in Trentino region

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- Cracking, necrosis of the bark and cankers appear mainly in the lower part of the trunk and on the graft union
- Blister bark and brown discoloration are also noted

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Apple luteovirus 1, ALV1

2018-2025

apple cultivar,
new accession
M9 rootstock.

Apple decline
(symptomatic and
asymptomatic)

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Apple luteovirus 1, (ALV1)

- A total of 60 apple plants were analysed in 2018-2025, wood and leaves, different period
- No viral symptoms were observed on the trees
- A causal association between apple tree decline in Trentino and the presence of Luteovirus has not yet been established
- Further investigation of its distribution and etiological role are necessary

detection

RT-PCR using virus-specific primers

- ALuDetF6
- AGCCAATGATTGTAT TCGACGTG
- ALuDetR6
- AGCTCTCTTCTAATGT GCGGAAC
- 478 bp

The thermal cycling conditions for RT-PCR

- 94 °C for 2 min,
- 35 cycles of 94 °C for 30 s, 55–60 °Cs) for 1 min and 68 °C for 40 s final extension at 68 °C for 5 min.

Sanger sequencing confirmation

- Liu, H., Wu, L., Nikolaeva, E. et al. Characterization of a new apple luteovirus identified by high-throughput sequencing. Virol J 15, 85 (2018). https://doi.org/10.1186/s12985-018-0998-3

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Cherry necrotic rusty mottle virus (CNRMV)

Genus Robigovirus, family Betaflexiviridae

Reported from various European countries, North America, Chile, Japan, Korea, and New Zealand

First report in Trentino region in 2019

- Fry & Wood 1973; Isogai et al. 2004; Sabanadzovic et al. 2005; Fiore & Zamorano 2013; Noorani et al. 2013; Zhou et al. 2013; Cho et al. 2014; EPPO 2016a

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Cherry necrotic rusty mottle virus, CNRMV

In May 2019
in sweet cherry
orchards ↘

in cv Mariant
Giant Red/Gisela 5
1 year after plantation ↘

Sympotoms
appearing 3-6 weeks
after bloom ↘

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Symptoms

- bark symptoms in the form of shallow necrotic areas, general bark necrosis, deep gum pockets and shallow gum blisters can appear.

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Symptoms

- Leaves of symptomatic plants showed brown angular necrotic spots, the center of which can drop out giving a shot-hole appearance.

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Symptoms

- Smaller spots can have a purplish coloring

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detection

Reverse transcription (RT)-PCR Li&Mock 2005

- CGRMV1 (5'-CCTCATTCACATAGCTTAGGTTT-3')
- CGRMV2 (5'-ACTTTAGCTTCGCCCCGTG-3') from both CGRMV and CNRMV viruses.

Reverse transcription (RT)-PCR Fiore N. 2013

- CNRM-CPF (GAGTGTGTGTGAGCTTTCAAGTT)
- CNRM-CPR (TTCGCCCCGTGTTGTAAAAC) specific for CNRMV

Sanger sequencing method

- Sequence identity was analysed using the BLAST algorithm. Sequences available in GenBank

- Li R., Mock R. (2005): An improved reverse transcriptionpolymerase chain reaction (RT-PCR) assay for the detection of two cherry flexiviruses in Prunus spp. Journal of Virological Methods, 129: 162-169
- Fiore N., Zamorano A. (2013): First report of Cherry green ring mottle virus and Cherry necrotic rusty mottle virus in sweet cherry (Prunus avium) in Chile and South America. Plant Disease, 97: 1122

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Grapevine Pinot gris virus (GPGV)

- Trichovirus pinovitis Grapevine Pinot gris virus GPGV
- Members of the Trichovirus genus of the Betaflexiviridae family
- GPGV, first reported in Italy in 2012
- Been found in all the major grapevine-growing areas of the world

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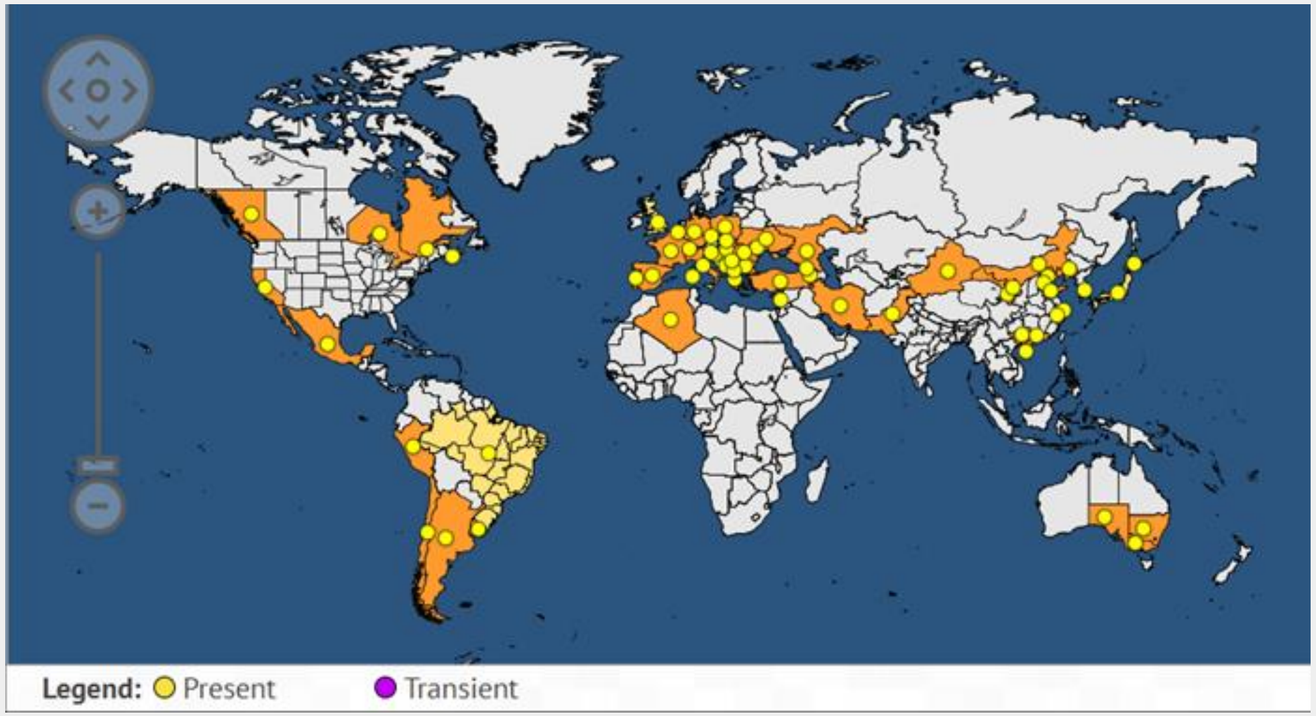
Distribution



- EPPO Global Database

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Grapevine Pinot gris virus GPGV

Colomerus vitis

latent and symptomatic infections

mixed infectionability to infect non-Vitis

mixed infection

Symptoms

- leaf distortion

Symptoms

- mottling

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Symptoms

- mottling

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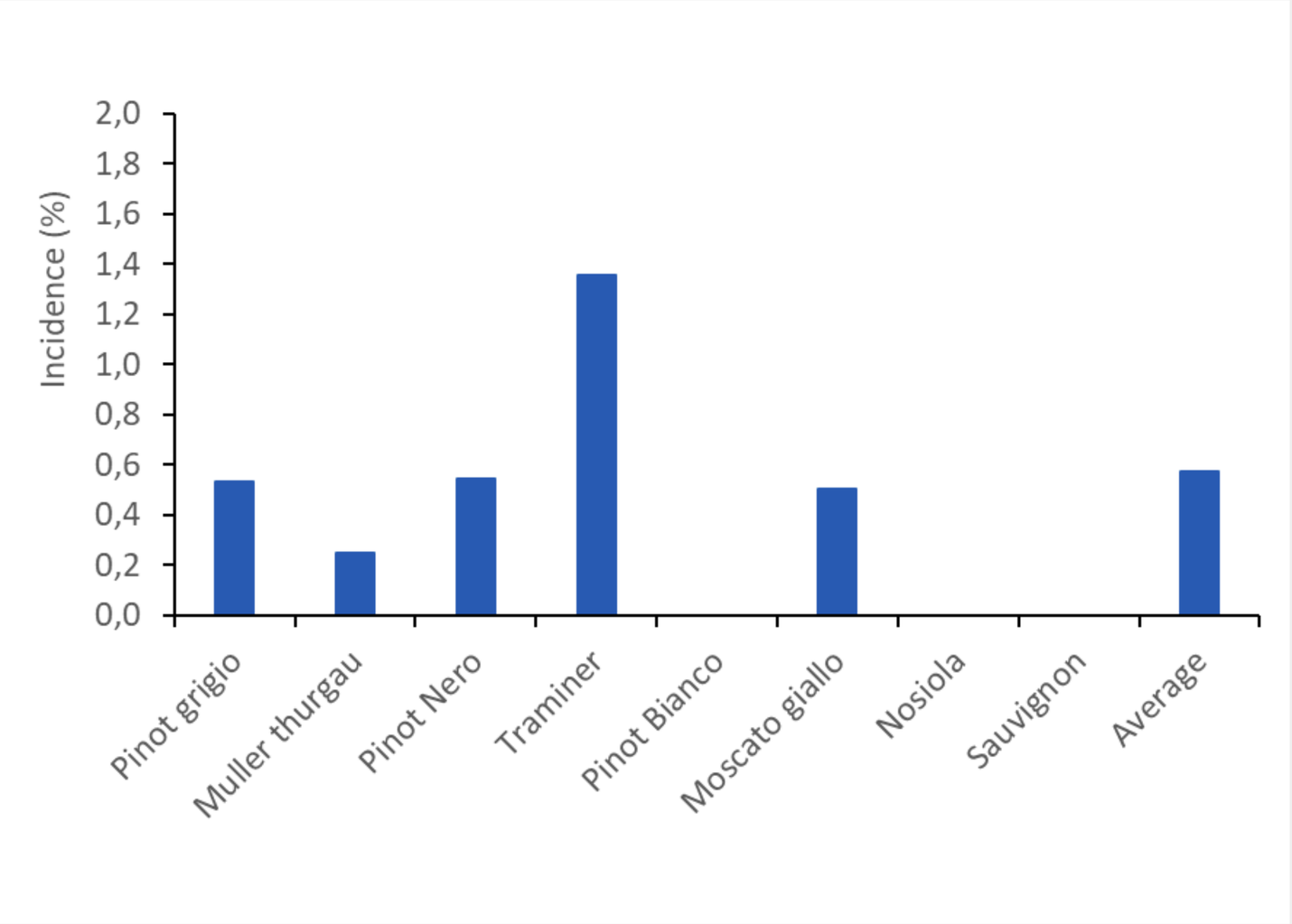
Symptoms

- affected vines had reduced number and weight of the berries ottling

2024_Monitoring in vineyards by visual assessment of symptoms



110 vineyards, 22000 plants

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Symptoms

- certified Propagating Material

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Symptoms

- certified Propagating Material

detection

REAL TIME PCR

- DTU N°17 (1)
- RT-PCR
- Real Time RT-PCR
- validation data
- One step RT-PCR (Ratti et el., unpublished) CP gene
- GPGVTaqF: CATTTCTGTGAGGATAGGTGTCATG
- GPGVTaqR: CAGACATAAGTGAACGGCCAATAT
- Sonda GPGV: FAM-AGGCCTTTCAATTCA

ELISA TEST

- double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)
- with the equipment, protocols, and reagents of BIOREBA AG (Reinach, Switzerland)
- wood samples

detection_propagation material

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		ELISA	RT- Real Time-PCR	CT (average)
DF02006	certified propagation material	+	+	26,01
DF02007	certified propagation material	+	+	23,85
DF02334	certified propagation material	+	+	24,62
DF00150	certified propagation material	+	+	27,89
DF00151	wood samples	-	-	-
DF00152	wood samples	-	-	-
DF00153	wood samples	+	+	33,22
DF00154	wood samples	+	+	24,27
DF00155	wood samples	-	-	-
DF00156	wood samples	-	-	-
DF00157	wood samples	+	+	23,61
DF00158	wood samples	+	+	27,54
DF02303	certified propagation material	-	+	26,4
DF02321	certified propagation material	+	+	23,99
DF02322	certified propagation material	+	+	24,5

Preliminary screening ELISA vs RT Real Time PCR accuracy 93.33% in wood

High incidences of GPGV in certified propagation material

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in conclusion

This result proved the urgency of continuous and close monitoring of the phytosanitary status of planting material.

Cases like these indicate the importance of investments in preventive measures to continuously monitor plants' phytosanitary status with official methods.

The monitoring of phytosanitary status will present us with new challenges in the future.

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Thank you