A non-destructive method for quantifying Cu, monitoring its residues, and downy mildew incidence on grapevine leaves after the application of copper-based antifungals

Fateme Sadeghian(1), Giovanni Mian(1), Piergiorgio Comuzzo(1), Lucilla Iacumin(1), Roberto Zanzotti(2), Oscar Giovannini(2),Gianni Trioli(3), Nicola Pio Torraco(1), Emilio Celotti(1)

(1) Department of Agricultural, Food, Environmental and Animal Science, University of Udine, via delle scienze 206, Udine (UD), 33100.

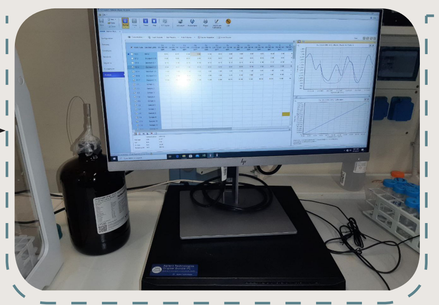
(2) Fondazione Edmund Mach, via E. Mach 1, San Michele all'Adige (TN)(3) Vinidea srl, Ponte dell'Olio (PC)

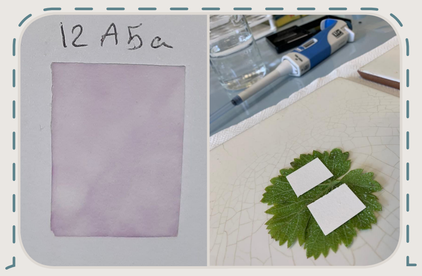
Perennial crops such as grapevine are often sprayed with copper-based antifungals against one of the most important diseases in viticulture, downy mildew (DM), which is caused by the biotrophic oomycete *Plasmopara viticola*. Although the European Union has limited the use of copper-based fungicides in organic farming since 2002, the likely negative effects on the environment, human health and wine quality, as well as soil contamination from diffuse sources in some agricultural regions, should be taken into account. The aim of this work, funded by the INTAVIEBIO project (PSR - FVG), was 1) to minimise the copper use by studying the variance of Cu coating on vine leaves and 2) to develop a non-destructive and rapid method to detect Cu on treated leaves using a new chemical probe that can detect the presence of Cu by image analysis.

This study was carried out on five organically managed vineyards in north-eastern Italy. Copper-based fungicides were applied to the canopy at different times during two growing seasons. The Cu dose applied to the grapevine leaves ranged from 1.00 to 3.6 kg ha^{-1}

The leaves were collected and washed with a 1% nitric acid solution (HNO_3)

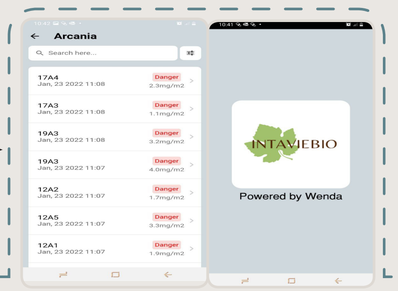
Cu concentration was analysed in the laboratory using ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectroscopy)





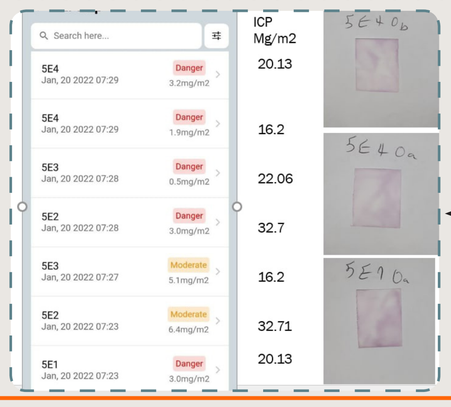
For direct Cu analysis of the leaves, we also used Cuprotesmo™ papers, which are particularly sensitive to the presence of copper salts and turn pink in the presence of the chemical (the colour changes when the paper is moistened).

Images of the coloured papers were taken with a smartphone camera. The Cu concentration data obtained from a mobile device application developed in the INTAVIEBIO project and that measured with ICP-AES were compared on the same leaves.



The Cu concentration data obtained from a mobile device application developed in the INTAVIEBIO project (by image analysis)and that measured with ICP-AES were compared on the same leaves.

Monitoring Plasmopara viticola Conducted from June to August. Percentage data collection for single adult leaf through visual observation (diffusion and severity) Data processing for each vineyard reported with Meteorology data (rain) and ICP -AES data.



The data demonstrate that this approach (once well developed) can be used as a non-destructive, rapid and minimal knowledge required method for Cu measurement by farm managers. Mean values of accumulated Cu on leaves ranged from 1.67 to 7.91 (µg/*cm*²), with different Cu concentrations on young and old leaves. Consequently, the effectiveness of Cu protection against DM and the correlation between concentration and disease incidence were evaluated. A method defined for copper on leaves by image analysis with corresponding datapreviously obtained from ICP-AES (young and adult). Climatic changes have increased the difficulty in preventing fungal infections. This has led to an increasing need for effective and rapid tools to evaluate an intervention. The average value of copper from preventive coverage (≥ 3.53 µg /*cm* ²) determined in this work is theoretically a parameter that would ensure adequate protection while meeting regulatory limits and minimising environmental impact.In this context, an app based on mathematical models is developed to define active residual copper thresholds on the leaf required to protect the crop. In this context, our work is a preparation for the development of this app, the initial results of which are promising.

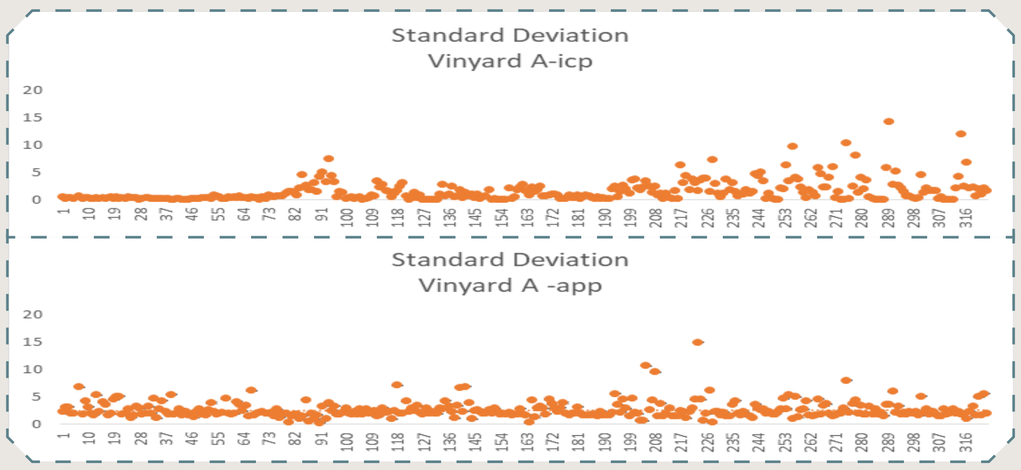
Table 1- The Average Infection Reduction value in five studied vineyards during two vegetative seasons.

Vineyards	Copper Residual Maximum (µg/cm2)	Infection Maximum (%)	Linear Correlation (R^2)	Total range	Average *Infection Reduction Value* (µg/cm²)
A	5.49	13.4	0.98		
B	2.9	6	0.99		
C	1.67	5	0.25	1,67 - 7,91	≥ 3,53
D	7.91	15	0.89		
E	5.08	20	0.001		

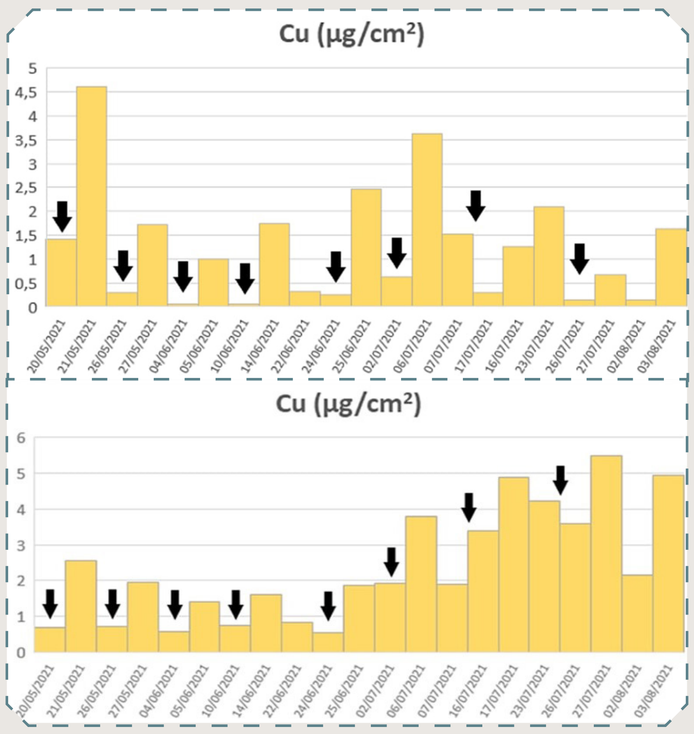
The average Infection Reduction Value is an important index that can hypothetically be achieved on a single-leaf vineyard over time.

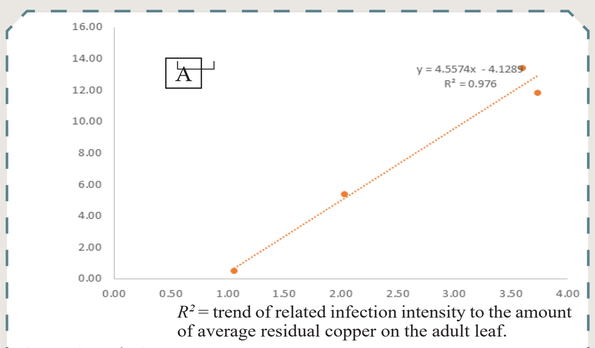
Each agronomic situation is different from the others.

R^2 of two vineyards was very low.



Totally 972 photos were tested by the mobile application(developed by image analysis based on photos have taken from cupertesmo TM paper) and compered by ICP-AES data (Fig, B, C, D, and E)





R^2 = trend of related infection intensity to the amount of average residual copper on the adult leaf.

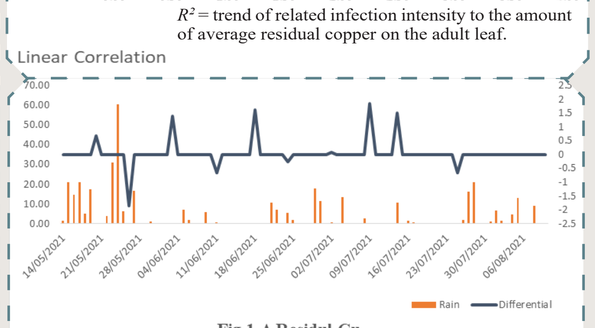


Fig 1-Δ Residul Cu

Δ Cu =output index of copper washed out with rain. (Strongly influenced by rain (Δ negative near a rain)