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Efficacy of reduced copper dosages against *Plasmopara viticola* in organic agriculture

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ABSTRACT

The grapevine is the most widely cultivated and economically important fruit species worldwide. Downy mildew produced by *Plasmopara viticola,* which occurs throughout the wide, is one of the most destructive of all grapevine diseases. In organic farming, copper is the only product effective against this pathogen accepted under European organic farming regulation EC 889/2008. Recently, due to its high environmental impact, the use of copper in organic farming has been limited by legislation to a yearly maximum of 6 kg/ha and its use will probably be even more restricted in the future. For this reason, the average dose recommended by pesticide producers of about 1 kg/ha per treatment should be revised. The aim of this work was to provide scientific support to effectively use reduced copper concentrations. A leaf disk assay was used to determine the minimum copper dosage that provided effective control. Dosages higher than 5 mg Cu/m^2 (of grapevine leaf material) did not prove to be more effective than the dosage of 5 mg Cu/m^2 itself. With a post-infection disk assay, copper treatments made 1 h after a simulated infectious rain, were also confirmed to give the same efficacy as preventive copper treatments. The efficacy of preventative low copper doses was validated in field trials. In the field, the rates of 200 and 400 g Cu/ha (equivalent to 5 and 10 mg Cu/m^2, respectively) was able to significantly reduce downy mildew (72–89% efficacy), confirming results obtained from leaf disks assays. Adjusting from higher copper spray rates, to those between 200 and 400 g Cu/ha should provide sufficient control depending on the infection pressure. This spraying regime should also enable viticulturists to remain under the current annual limit of 6 kg/ha and likely under lower limits that might be proposed in future regulations.

1. Introduction

Grapevine (*Vinis vinifera* L.) downy mildew is caused by the oomycete *Plasmopara viticola* (Berk & Curt) Berl. & de Toni, and is one of the most widely distributed and serious grapevine diseases worldwide (Gessler et al., 2011; EGTOP, 2014).

Under the presence of leaf wetness and warm temperatures, the sporangia of *P. viticola* release flagellate zoospores that swim toward the stoma within the water film on the lower surface of the leaf. When they encounter the stomata they lose their flagella, attach and encyst (Kiefer et al., 2002). Subsequently, they form a germ tube that reaches into the substomatal cavity, where it dilates into a vesicle. Afterwards, a primary hypha emerges, and develops a mycelium that spreads within the leaf tissue (Jürges et al., 2008). Thereafter, the development takes place within the host leaf, impeding curative approaches to pathogen control.

Since the discovery of the efficacy of copper against downy mildew by Millet in the nineteenth century till the first successful greenhouse trials with non-copper products in the middle of the twentieth century (Gessler et al., 2011) broad-spectrum contact fungicides (multi-site) like copper, were the only compounds available for controlling *P. viticola*. Today, copper remains the most important fungicide in organic agriculture because it is a non-synthetic compound with a wide activity spectrum (Dagostin et al., 2011). Long-term application and runoff from treated

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plants, however, have caused extensive copper accumulation in soils (Komárek et al., 2010), causing environmental problems, resulting in laws which limit its use. The present European regulation sets a maximum limit of 6 kg Cu/ha per year or a maximum of 30 kg Cu/ha per 5 years (CE n° 354/2014). In some countries (e.g. Netherlands, Denmark), copper use in agriculture is forbidden, and in other countries there is a lower quantitative limit (e.g. 3 kg Cu/ha per year in Germany). Further quantitative limitations in legislation are likely for the future. The approval period for copper products in part A of the annex to Reg. 540/2011 expires 31 January 2018 (EU n° 85/2014).

In different commercial copper formulations, the percentage of copper content can vary widely, as well as the recommended dose. As seen in Table 1, in Italy the average doses range depending on the product and the type of copper, from 693 g Cu/ha for hydroxide to 1575 g Cu/ha for copper oxide, while the overall average dose of 111 copper products available in Italy to control *P. viticola* in 2014, was found around 1 kg Cu/ha per treatment.

In the Trentino region (northern Italy), roughly 12–14 copper sprayings are done annually for downy mildew in years with normal weather conditions. Following recommended dosages proposed by the producers of copper-based fungicides (the average dose being 1 kg Cu/ha), 12–14 kg Cu/ha per year would be used, twice the limit set by EC Regulation 354/2014 (6 kg Cu/ha per year). Therefore, according to the current European regulation and the data in Table 1, we can note that recommended product doses are excessive for organic growers in Trentino, Italy, and regions with similar downy mildew pressure.

The mode of action of copper products is linked to copper ions (Cu^{2+}) that are able to enter the pathogen spore, denaturing proteins and blocking various enzymes (Montag et al., 2006a). Copper-based fungicides have preventative action and therefore the treatments are done before an infective rain, by closely observing weather forecasts (Gisi, 2002). There is a documented lag phase between the release of the *P.viticola* zoospores and the penetration of the stomata by the pathogen (Gessler et al., 2011). Consequently, timely treatments after infectious rains could be effective as well.

The objective of this work is to study the efficacy of reduced copper doses (lower than the dosages recommended on product labels) against *P. viticola* in order to adapt to legislative restrictions. In addition, to test the effectiveness of timely treatments of copper after the start of the infection period.

2. Materials and methods

2.1. Preventive treatment with copper

2.1.1. Pathogen material

The *Plasmopara viticola* (Berk. & Curtis) Berl. & DeToni population comes from sporulated leaves collected in an untreated vineyard sited near to the laboratory (GPS N 46.184474; E 11.124458) in Summer 2014 and cultivated in a greenhouse on *Vitis vinifera* cv. Pinot Noir grapevines. The lower surface of the leaves was infected by spraying with an aqueous suspension of sporangia (2–5 × 10^5 sporangia/ml) until runoff. Inoculated plants were transferred to a growth chamber and kept in the dark for 24 h at 25 ± 0.5 °C and at 95–100% relative humidity (RH). The plants were then transferred to the greenhouse (25 °C, 60–80% RH, and natural light regime), where they were kept for 7 days (incubation period). After the incubation period, plants were placed overnight (12 h) in a growth chamber at 25 ± 0.5 °C and at 95–100% RH to promote *P. viticola* sporulation.

2.1.2. Plant material

For all greenhouse experiments, material came from 3 year-old plants of susceptible grapevine variety, *V. vinifera* cv. Cabernet Sauvignon grafted on Kober 5BB rootstock, grown in 25-liter pots with standard soil, four plants per pot. Plants were kept under natural conditions until the start of the vegetative season, when they were transferred under a tunnel to avoid natural *P. viticola* infection. In the tunnel, 6 sulphur treatments were made to avoid powdery mildew infection.

1.9 cm diameter leaf disks were cut from said material using a cork-borer and placed, abaxial side up, in 8.5 cm-diameter plastic Petri dishes containing 4 layers of filter paper moistened with 6 ml distilled water, following a widely used protocol described by Staudt and Kassemeyer (1995). 5 Petri dishes with 5 leaf disks each were used per treatment. The experiment was repeated.

2.1.3. Copper treatment

The different concentrations of pure copper were chosen based on a study conducted by Mescalchin et al. (2011) that analysed the amount of copper metal on leaves after spray treatments using different sprayers with a dose of 500 g Cu/ha of Bordeaux mixture. The minimum value of copper detected was 1.5 mg Cu/m^2 and the maximum detected 16.2 mg Cu/m^2, depending on the position of the leaf on the vine. For these reasons the initial doses (mg Cu/m^2 leaf) were set at 2.5, 5, 7.5, 10, 12.5 and 15. Obtaining such deposition on leaf disks by means of the Potter spray tower required administering solutions with the following empirically derived concentrations: 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 g Cu/L.

Leaf disks were treated with Bordeaux mixture (Poltiglia Disperss, Cerexagri, with 20% copper content) in the various concentrations cited above using the Potter tower (Burkard Scientific Co., Uxbridge, UK). After which, the Petri dishes were left open in order to let the leaf disks dry before proceeding with the inoculum. The amount of copper (Cu mg/m^2) deposited on the disks was calculated based on the post- and pre-treatment weight difference, the concentration of the sprayed solution and area of the treated surface.

2.1.4. Inoculation

The inoculum was prepared collecting *P. viticola* sporangia from previously infected plants (see *2.1.1 Pathogen material)* by washing grapevine leaves that had freshly sporulating lesions with cold (4 °C) distilled water. The sporangia were counted with a

Table 1
Absolute minimum, absolute maximum and average dose recommended on the label of copper products indicated to control downy mildew of grapevines in Italy in 2014 (111 products analysed).

g Cu/ha	Type of Copper Salt					
	Hydroxide	Oxychloride	Tribasic Sulphate	Bordeaux Mixture	Oxide	Overall Average
Number of Products	22	48	15	25	1	
Absolute Minimum	300	352	420	75	1275	
Absolute Maximum	1400	3000	1158	2400	1875	
Average Dose	693	1145	763	1491	1575	1065