



## An assessment of the non-target effects of copper on the leaf arthropod community in a vineyard

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### ABSTRACT

Bordeaux mixture has been used for more than 150 years in viticulture and other agricultural applications because it is the most effective fungicide against grape downy mildew. However, the copper present in these mixtures is not degradable and can have environmental consequences. Even though the effect of Bordeaux mixture on downy mildew is well known, the non-target effects of this fungicide on leaf arthropod communities are poorly understood. In this study, we simultaneously monitored the direct effect of Bordeaux mixture on three grapevine leaf diseases and five leaf arthropods (beneficial and pest species) in the field over a four-month time period. We found a strong interaction between sampling year and treatment for all tested organisms. Overall, the presence of copper generally decreased both the occurrence of disease and densities of leaf arthropods. Thus, copper reduces some pest densities but also biological agent densities which might interfere with biological control. The effects and results presented here should prove valuable when implementing future crop management strategies and pest control procedures.

### 1. Introduction

For more than 150 years, copper and its derivatives (e.g. Bordeaux mixture) have been massively and systematically used as a fungicide against downy mildew; historically in viticulture and more recently in orchards and potato farming (Dagostin et al., 2011; Llorens et al., 2000; Martins et al., 2014a,b). The Bordeaux mixture was developed after the devastating introduction of the grape downy mildew (*Plasmopara viticola*) from North America to Europe at the end of the 19th century. It has been intensively used in wine-growing since that period (Gessler et al., 2011). As copper is the only treatment effective against this disease, it is also the only treatment permitted in organic farming. However, copper is not degradable: its accumulation does not only alter water quality but spreads throughout the food chain as well, resulting in tangible consequences for human health (Ashish et al., 2013; Tóth et al., 2016). Despite being an important micronutrient for the grapevine, accumulation of copper in soil and subsequently within plants can cause numerous morphological (e.g. foliar chlorosis, shorter and thicker roots) and physiological abnormalities (e.g. oxidative stress, reduced capacity of the roots to acquire nutrients and water) (see Brunetto et al., 2016 for a review). Thus, the use of copper in viticulture is a double-

edged sword, where protecting grapevine plants from diseases also reduces both grapevine productivity (Brunetto et al., 2016).

The effects of copper on grapevine and the grape downy mildew are now widely studied and recognized (Brunetto et al., 2016; Gessler et al., 2011), but its effects on non-target arthropods (beneficial and/or pest) are under-investigated. This is surprising, as copper treatments are applied directly onto the external surfaces of leaves, where arthropods then come into contact with this non-specific substance. Contact with this fungicide could affect either positively or negatively their life history traits. For instance, it has been found that sulfur application against the grape powdery mildew (*Uncinula necator*) negatively affected the survival and parasitism success of the beneficial parasitoid *Anagrus erythroneurae*, while its target – the western grape leafhopper (*Erythroneura elegantula*) – was not affected by the fungicide (Jepsen et al., 2007). This severely limits the effectiveness of biological control of this pest species. Conversely, in citrus farming, the exposure of two beneficial species of coccinellid beetles to copper-sulfate fungicides does not affect their life history traits (Michaud and Grant, 2003) at all. Copper applications have even been shown to positively influence arthropod fecundity, and has been associated with outbreaks of the citrus red mites, *Panonychus citri* (Kim et al., 1978), a major pest species. In

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forest ecosystems, a small concentration of copper has a positive effect on saphrophagous oribatid mite communities while the abundance and number of these mites drop down in high copper concentrations (Skubala and Kafel, 2004). In Australian vineyards, high pesticide use has been shown to decrease the number of native arthropods living in the canopy and the ground while it increases the prevalence of invasive species (Nash et al., 2010). Finally, it has been found that the effect of copper treatment on the “Flavescence Dorée” vector, the American grapevine leafhopper (*Scaphoideus titanus*) depends upon the vineyard where they came from: leafhoppers originating from a vineyard with high doses of copper are more likely to stay and tolerate plants grown with high copper doses (Anatole-Monnier, 2014). Thus, fungicides in general but copper specifically appears to have strong effects on vineyard communities. This highlights the importance of understanding the effects of copper upon the ecological interactions of multiples species, both beneficial and pest, with the goal better informing future pest management strategies.

More than just affecting the wider arthropod community in a vineyard, the application of copper and its derivatives as a fungicide could also impact other leaf diseases such as the grape powdery mildew *U. necator* and the black-rot *Guignardia bidwellii*. To the best of our knowledge, no study investigated the effects of copper on other leaf diseases than the downy mildew. Most studies investigated the effect of a mix of plant protection products targeting both diseases and insects (Beers et al., 2016; Bruggisser et al., 2010; Di et al., 2016). As such, it is difficult to understand the sole effect of copper on leaf diseases.

In the present study, we investigated simultaneously the direct effect of copper on grapevine leaf diseases and arthropods over four months in 2014 and 2015. This experiment was performed directly in open vineyards, as only field experiments can provide the definitive assessment of the environmental impact of a certain pesticide and its consequences on naturally-occurring populations. We monitored the effects of exposure to Bordeaux mixture upon three of the most common grapevine diseases in French vineyards and five species of arthropods. We predicted a lower occurrence of grape diseases in the copper treatments due to its direct and indirect effects upon plant physiology. Similarly, the density of pest arthropods might be lower in copper treatments because of the direct toxic effect of copper and the decrease of the grapevine quality. Indeed, the stress caused by copper on the plant can also weaken grapevine defenses and consequently increase the density of pests, as predicted by the “Plant Vigor Hypothesis” (Price, 1991). Finally, predators density should follow the density of their prey (Price et al., 1980).

## 2. Material and methods

### 2.1. Vineyard plot and diseases management

This study was conducted at the experimental vineyard of INRA Research Center ‘la Grande Ferrade’ (Villeneuve d’Ornon, France; 44°47’25.1”N 0°34’36.7”W) in a vineyard plot planted in 1991 with Merlot vines (clone 181). The management of this vineyard is a double Guyot training system where each vine stock has two fruiting arms along a main wire trained in opposite directions. Leaves and tops were removed every 14 days, if necessary. The area of the plot is 1257 m<sup>2</sup> containing 16 rows of 42 vine stocks (total of 672 vine stocks) and a density of ca. 5700 vine stock/ha. The distance between rows is 1.60 m, with 1.10 m between vine stocks. The soil was bare under the vine stocks with grass strips in the middle of the rows. Pesticides against the grape powdery mildew, the black rot and *Phomopsis* cane were applied three times in the vineyard between the vine phenology stage of 66 and 88 (Lancashire et al., 1991) (Table 1). In this experiment, only five rows were used. In each of the five rows tested, the two outermost vines were not used because of potential effects from the surrounding grape plots.

**Table 1**

Pesticides (active compounds) used on the vineyards according to the pest targeted. C: *Phomopsis* cane; P: Powdery mildew (*U. necator*) and R: Black-rot (*G. bidwellii*). Three pesticide applications were done in the whole vineyard between the crop stage 66 and 88.

Pesticide active compounds	Pest	Doses
Fosetyl-Aluminium 50% + folpel 25% + cymoxanil 4%	P	3 kg/ha
Mancozeb 46.5% + cymoxanil 4%	C; R	3 kg/ha
Meptyldinocap	P	0.6 l/ha
Metiram 64% + cymoxanil 4.8%	R	2.5 kg/ha
Tebuconazole	R; P	0.4 l/ha

### 2.2. Experimental design and sampling

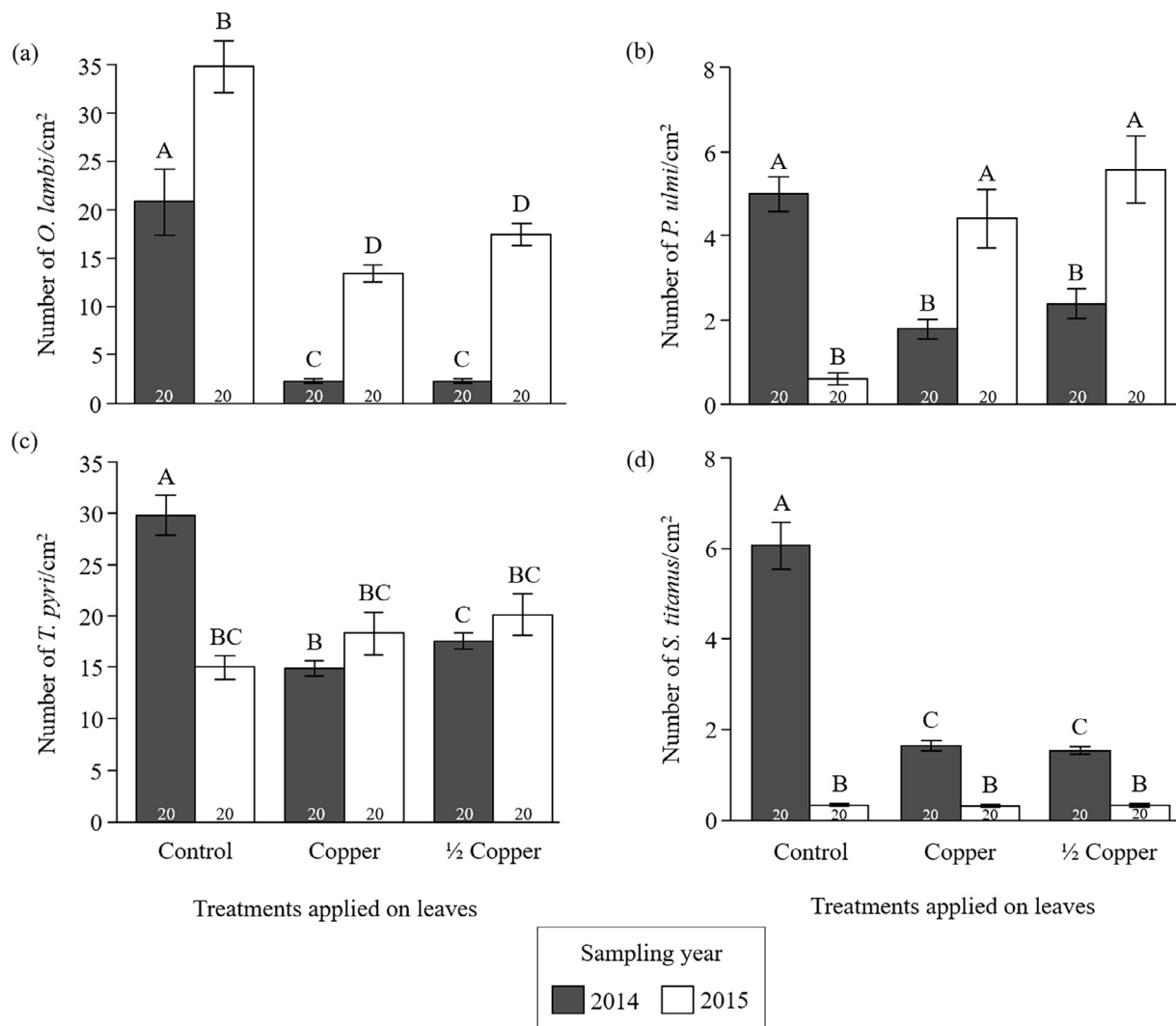
In order to assess the effect of Bordeaux mixture in vineyards, we measured the occurrence of disease symptoms as well as the density of five key species of arthropods present on the leaves. Data were collected on seven dates – corresponding to different vine phenology – from May to August in both 2014 and 2015 (a total of 14 replicates). The same rows and vine stocks were used in the two years of sampling. We used the Bordeaux mixture “Bouillie Bordelaise RSR® dispers® NC” comprised of 20% copper sulfate (Table 1). This mixture is commonly used when growing grapevines and other crops, in both conventional and organic farming. Vine stocks were exposed either to (1) full dose of Bordeaux mixture (‘copper’) used in conventional management (3.750 kg/ha of Bordeaux mixture RSR), (2) half dose of Bordeaux mixture (‘½ copper’) (1.875 kg/ha of Bordelaise mixture dispers® RSR) or (3) no Bordeaux mixture as a control treatment (‘control’). To avoid any contamination from the copper treatments, the control was geographically separated from the two copper treatments. Copper treatments were applied every 15 days from May to August in order to maintain the right copper concentration and avoid copper washout by rainfall.

As both diseases and arthropods can be affected by the different environmental conditions between years (Vogelweith and Thiéry, 2017), we recorded precipitation, temperature and humidity from May to end of August. In 2014, a monthly average of 2.10 mm of rain fell in the vineyard, the mean temperature per month was 19.14 °C (minimum 14.48 °C; maximum 24.48 °C) and the humidity per month was 72.47% (minimum 48.33%; maximum 93.69%). In 2015, a monthly average of 0.65 mm of rain fell in the vineyard, the mean temperature per month was 21.60 °C (minimum 14.89 °C; maximum 27.67 °C) and the humidity per month was 65.64% (minimum 38.67%; maximum 92.62%).

#### 2.2.1. Grapevine arthropods on leaves

Three mite species (*Orthotydeus lambi*, *Panonychus ulmi* and *Typhlodromus pyri*), the leafhopper *Scaphoideus titanus* and the harvestman *Phalangium opilio* were monitored as described in Vogelweith and Thiéry (2017). We focused on these five species because of their common occurrence in European and French vineyards, and of their status as pests and/or beneficial species (Sentenac, 2011).

Briefly, two species were directly identified on site: *P. opilio* and *S. titanus*. The occurrence of harvestmen – presence/absence on a vine stock – was visually counted on each vine stock and 20 vine stocks were assessed per treatment at each data collection. Then, the number of leafhoppers was visually counted on five randomly chosen leaves per vine stock, directly in the vineyard. Twenty vine stocks per replicate were assessed. After these measurements, five leaves per vine stock (total of 100 leaves sampled for each treatment per replicate) were randomly sampled, removed and brought to the laboratory for mite species determination (Bolland et al., 1998; Collyer, 1982; Zhang et al., 2001). Each leaf was brushed with a mite brush and all mites were collected, counted and identified with a binocular microscope (magnification 20×). We determined the average number of each species per vine stock; identifying and focusing on three major mite species



**Fig. 1.** Effect of copper dose and sampling year on the density of arthropods (individual/cm<sup>2</sup>) on grapevine leaves: (a) *P. ulmi* ( $\pm$  s.e.); (b) *O. lambi* ( $\pm$  s.e.); (c) *T. pyri* ( $\pm$  s.e.) and *S. titanus* ( $\pm$  s.e.). Dark grey bars represent the sampling year 2014 and white bars the sampling year 2015. The numbers in the bars represent the number of vine stocks sampled per replicate. Different letters indicate significant differences ( $p \leq 0.05$ ).

during this monitoring: *Orthotydeus lambi*, *Panonychus ulmi* and *Typhlodromus pyri*.

The number of primary and secondary branches, as well as the number of leaves on those branches, were counted on each vine stock and at each replicate. Each sampled leaf was transversely measured with a ruler. We obtained the number of leaves on each vine and the average size of the leaves (precision  $\pm 0.01$  mm). Thus, we corrected the number of leafhoppers and mites by both the number and the size of the leaves to obtain a density in cm<sup>2</sup>. The vine phenology was recorded at each replicate following the BBCH-scale for grapevines (Lancashire et al., 1991).

### 2.2.2. Grapevine diseases on leaves

We monitored three of the most common grapevine diseases present on leaves in French vineyards: the grape downy mildew (*Plasmopara viticola*), the grape powdery mildew (*Uncinula necator*) and black-rot (*Guignardia bidwellii*).

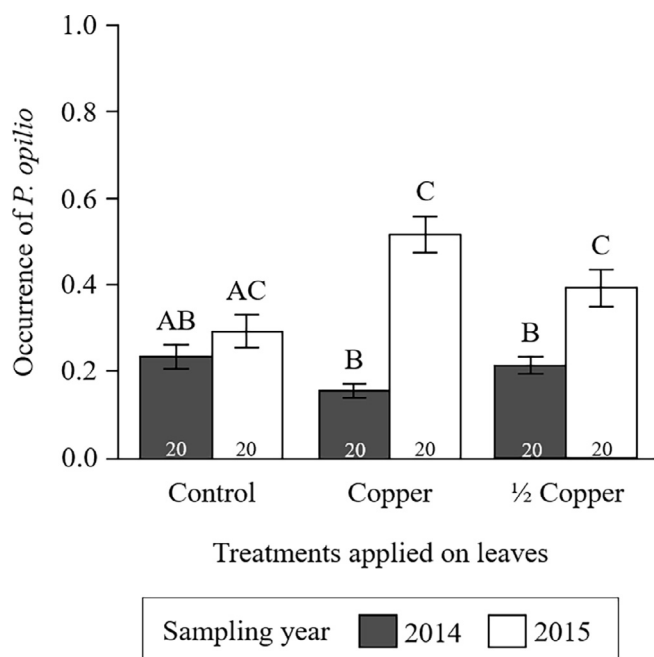
As described in Vogelweith and Thiéry (2017), we estimated the occurrence of each disease by randomly recording the presence/absence of symptoms on 15 leaves per vine stock. Twenty vine stocks per treatment were assessed. An average presence of each disease on each vine stock was then calculated.

### 2.3. Statistical analysis

All the statistical analyses were conducted using the software R v3.4.3 (R Core Team, 2018) loaded with the packages car v3.0-0 (Fox and Weisberg, 2011), lme4 v1.1-17 (Bates et al., 2015) and MASS v 7.3-50 (Venables and Ripley, 2002). The corrected densities of mites and leafhoppers on leaves were analyzed using linear mixed effect models (lmer), in which treatments (control, copper and 1/2 copper) and year (2014 or 2015) were entered as explanatory categorical factors, while grapevine phenology was entered as a random effect. To fulfil homoscedasticity and Gaussian distribution, these models were computed using square root-transformed densities for *O. lambi* and *S. titanus*, and log + 1-transformed densities for *P. ulmi* and *T. pyri*. The occurrence of harvestmen on leaves was tested using a generalized linear mixed model (glmer, with binomial distribution) and the variables mentioned above.

The occurrence (presence/absence on each leaf) of each disease (*P. viticola*, *U. necator* and *G. bidwellii*) was also tested using a generalized linear mixed model (glmer, with binomial distribution). In these models, treatments and years were entered as explanatory categorical factors, while grapevine phenology was entered as a random effect.

When applicable, pairwise comparisons between treatments or years were tested using Tukey contrasts.



**Fig. 2.** Effect of copper dose and sampling year on the occurrence of *P. opilio* ( $\pm$  s.e.) on grapevine leaves. Dark grey bars represent the sampling 2014 and with bars the sampling year 2015. The numbers in the bars represent the number of vine stocks sampled per replicate. Different letters represent significant differences ( $p \leq 0.05$ ).

### 3. Results

#### 3.1. Grapevine arthropods on leaves

All five species of arthropods monitored within this study were affected by the interaction between treatment and sampling year (Figs. 1 and 2; Table 2). The density of the beneficial mite *O. lambi* was higher in the control treatment compared to both copper and 1/2 copper treatments (Fig. 1a). Their density was also higher in 2015 compared to 2014 (Fig. 1a). By contrast, the density of the pest *P. ulmi* was higher in the control treatment in 2014, while it was higher in both copper treatments in 2015 (Fig. 1b). The density of the predatory mite *T. pyri* was also higher in the control treatment in 2014 compared to 1/2 copper and copper treatments, whereas there was no difference in the density between the treatments in 2015 (Fig. 1c). In 2014, the density of the leafhopper *S. titanus* was three times higher in the control treatment compared to both copper treatments, while there was no difference in 2015 (Fig. 1d). Finally, the occurrence of the harvestmen *P. opilio* was higher in copper treatments compared to the control treatment in 2015 whereas there was no difference in 2014 (Fig. 2).

#### 3.2. Grapevine diseases on leaves

All the three-species of diseases monitored were also affected by the interaction between treatment and sampling year (Fig. 3; Table 3). In

**Table 2**

Effect of the treatment (control, copper or 1/2 copper) and the sampling year (2014 or 2015) on leaf arthropods.

	<i>Orthotydeus lambi</i>		<i>Panonychus ulmi</i>		<i>Typhlodromus pyri</i>		<i>Scaphoideus titanus</i>		<i>Phalangium opilio</i>	
	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
Treatment	<b>298.69</b>	<b>&lt; 0.0001</b>	<b>20.60</b>	<b>&lt; 0.0001</b>	<b>69.21</b>	<b>&lt; 0.0001</b>	<b>118.94</b>	<b>&lt; 0.0001</b>	1.05	0.591
Year	<b>613.17</b>	<b>&lt; 0.0001</b>	3.09	0.079	1.19	0.275	<b>232.32</b>	<b>&lt; 0.0001</b>	<b>70.70</b>	<b>&lt; 0.0001</b>
Treatment * Year	<b>9.08</b>	<b>0.011</b>	<b>193.29</b>	<b>&lt; 0.0001</b>	<b>9.21</b>	<b>0.010</b>	<b>49.82</b>	<b>&lt; 0.0001</b>	<b>24.54</b>	<b>&lt; 0.0001</b>

Significant *P*-values are in bold.

2014, the occurrences of grape downy and powdery mildew were higher in the control treatment compared to the two copper treatments, while there was no difference in 2015 (Fig. 3a and b). Finally, the occurrence of black-rot *G. bidwellii* was higher in the control treatment compared to 1/2 copper and copper treatments, but the difference was smaller in 2015 than 2014 (Fig. 3c).

### 4. Discussion

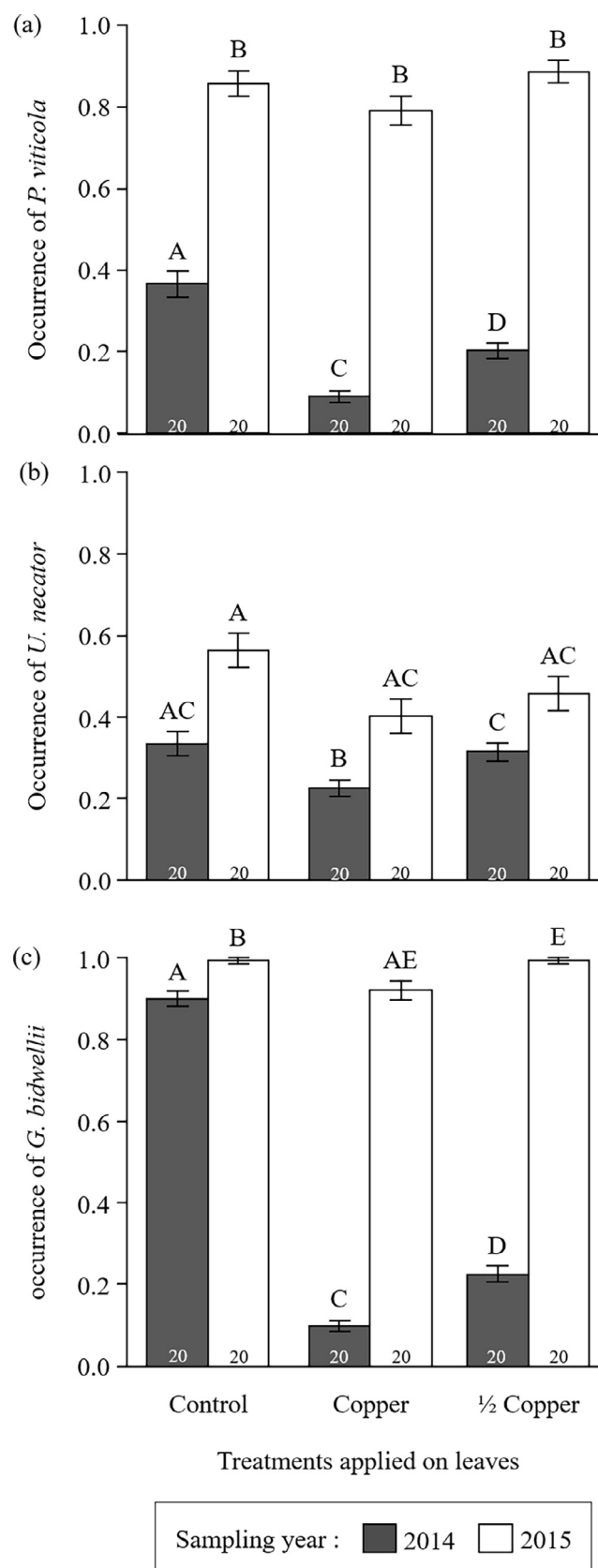
The aim of this study was to determine the non-targeted effects of copper treatments, applied as Bordeaux mixture, upon beneficial/pest arthropod species and different grapevine diseases under natural vineyard conditions. The Bordeaux mixture had a non-targeted effect on both arthropods and diseases, strongly modulated by sampling year, as indicated by all interactions. There are four, non-mutually exclusive explanations for such effects of copper upon leaf organisms.

Firstly, the general decrease of arthropods densities (mites and leafhopper) and grapevine diseases on leaves (mostly in 2014) might be due to the direct effect of copper. Copper is an essential micronutrient involved in a wide array of critical physiological processes, such as wound healing and protection against reactive oxygen species (Kaplan and Maryon, 2016). However, beyond a certain concentration accumulated in the body – depending on the organisms considered – it can be highly toxic. For instance, high concentration of copper can cause DNA damage in freshwater invertebrates (Bernabò et al., 2017), reduce growth and increase mortality in *Apis mellifera* (Hymenoptera: Apidae) (Di et al., 2016) and inhibited *Beauveria bassiana* (Hypocreales: Ophiocordycipitaceae) germination and sporulation up to 50% (Martins et al., 2014a,b). Thus, it is not surprising to find a decrease in arthropod densities, and diseases within the two copper treatments, in 2014.

Secondly, copper can also have an indirect effect across trophic levels, producing a cascading effect upon species within the ecosystem (Fig. 4). Indeed, by reducing grapevine diseases (mainly the grape downy and powdery mildews), copper treatments reduce the resources available for mycophagous mites such as *O. lambi*, diminishing their density and subsequently the densities of their predators such as *T. pyri* (Fig. 4). Indeed, Duso et al. (2005) have shown, in vineyards, that the abundance of the mycophagous mite *Tydeus caudatus* (Acari: Tydeidae) was positively correlated to the spreading of the grape downy mildew symptoms and ultimately to the predatory mite *Paraseiulus talbii* (Acari: Phytoseiidae). *P. viticola* can also be an alternative food source for generalist phytoseiid mites (i.e. *T. pyri*) in absence of prey (Pozzebon and Duso, 2008) (Fig. 4). By reducing *P. viticola*, copper can then induce a switch in the diet of *T. pyri* and, then, modify trophic interactions. Similarly, by reducing the density of *S. titanus*, copper can affect *P. opilio*'s diet which will have to find alternative preys (Fig. 4).

Thirdly, the negative effect of Bordeaux mixture on the densities of *P. ulmi* and *S. titanus* in 2014 might be related to changes caused by copper on grapevine metabolism. Indeed, accumulation of copper in vineyard soils can lead – to a lesser extent – to a higher concentration in roots and aerials part of the plant (Brun et al., 2001); reducing mineral nitrogen, sugar and protein contents, and altering leaf characteristics such as light absorption and photosynthesis (Martins et al., 2014a,b; Moutinho et al., 2001). Thus, sap-feeding (e.g. *S. titanus*) and phytophagous mites (e.g. *P. ulmi*) might be affected by such changes; either





**Fig. 3.** Effect of copper dose and sampling year effect on the occurrence of grapevine diseases on leaves/vine stock: (a) Grape downy mildew (*P. viticola*;  $\pm$  s.e.); (b) Grape powdery mildew (*U. necator*;  $\pm$  s.e.) and (c) Black-rot (*G. bidwellii*;  $\pm$  s.e.). Dark grey bars represent the sampling 2014 and white bars the sampling year 2015. The numbers in the bars represent the number of vine stocks sampled per replicate. Different letters represent significant differences ( $p \leq 0.05$ ).

by being repelled by the plant or by the consumption of poor quality food. These results are consistent with those obtained by [Anatole-Monnier \(2014\)](#) who found a decrease of *S. titanus* densities with increased copper concentration.

In addition, once the concentration of copper in the soil exceeds the concentration needed for the plant, the latter will be affected. Such an accumulation of copper in the soil can then explain the similar effect of the copper and 1/2 copper treatments on grapevine diseases and leaf arthropod densities.

Fourthly, predators such as *P. opilio* and to a lesser extent *T. pyri* appeared to be less affected by copper compared to the other organisms examined in this study. We propose two possible explanations for this observation. First, it has been postulated in the literature that predators may be better able to regulate their internal metal concentrations ([Liess et al., 2017](#)). Secondly, contrary to their prey (e.g. phytophagous arthropods) which directly eat plant material with copper inside and outside, predators consume copper indirectly through their prey, meaning that heavy metals exposure might be reduced ([Poteat and Buchwalter, 2014](#)).

Finally, our results appear to be highly dependent on sampling year. Contrary to 2014, all diseases monitored, *T. pyri* and *S. titanus* and *P. opilio* densities were not affected by copper treatments in 2015. Also, their presence/densities were very variable between sampling years. Variation in diseases and arthropods densities between years has been reported multiple times in vineyards ([Delière et al., 2015](#); [Duso et al., 2005](#); [English-Loeb et al., 2007](#); [Moreau et al., 2010](#); [Vogelweith and Thiéry, 2017](#)). In 2015, the temperature was higher with a lower humidity level and less rainfall compared to 2014. Such climatic conditions should reduce these fungal diseases which require high humidity. However, these conditions may also have caused a hydric stress, weaken the plant and then favored diseases and *P. ulmi* despite the copper treatments. At the opposite, arthropods relying on the plant quality, such as *S. titanus*, may have been repel.

Nonetheless, we have to keep in mind that our study focused on three pathogens and five arthropods but other key species, such as spiders, parasitoids and ground organisms, could also respond differently to copper. This study places the first building block, which can be further expanded upon by investigating the direct effect of copper ingestion on arthropods life history traits and the subsequent effects this has upon higher trophic levels.

In conclusion, Bordeaux mixture has a strong effect on targeted and non-targeted insect species. These results also show the importance of environmental conditions, and highlights the interplay between environment and crop management. In the long term, copper accumulation might change arthropod communities by adversely affecting some species and not others, potentially opening the door for the arrival of invasive species. Elucidating and understanding the ecological dynamics of copper in fungicides is essential to the success of pest management, and should be considered in future experimental set-ups. The potential negative effects of Bordeaux mixture – a common fungicide used in organic farming – on beneficial organisms or biological agents such as *B. bassiana* ([Martins et al., 2014a,b](#)) should be taken into account prior to crop management for more efficient pest control, and to avoid seasonal and economical losses.

## 5. Declarations of interest

None.

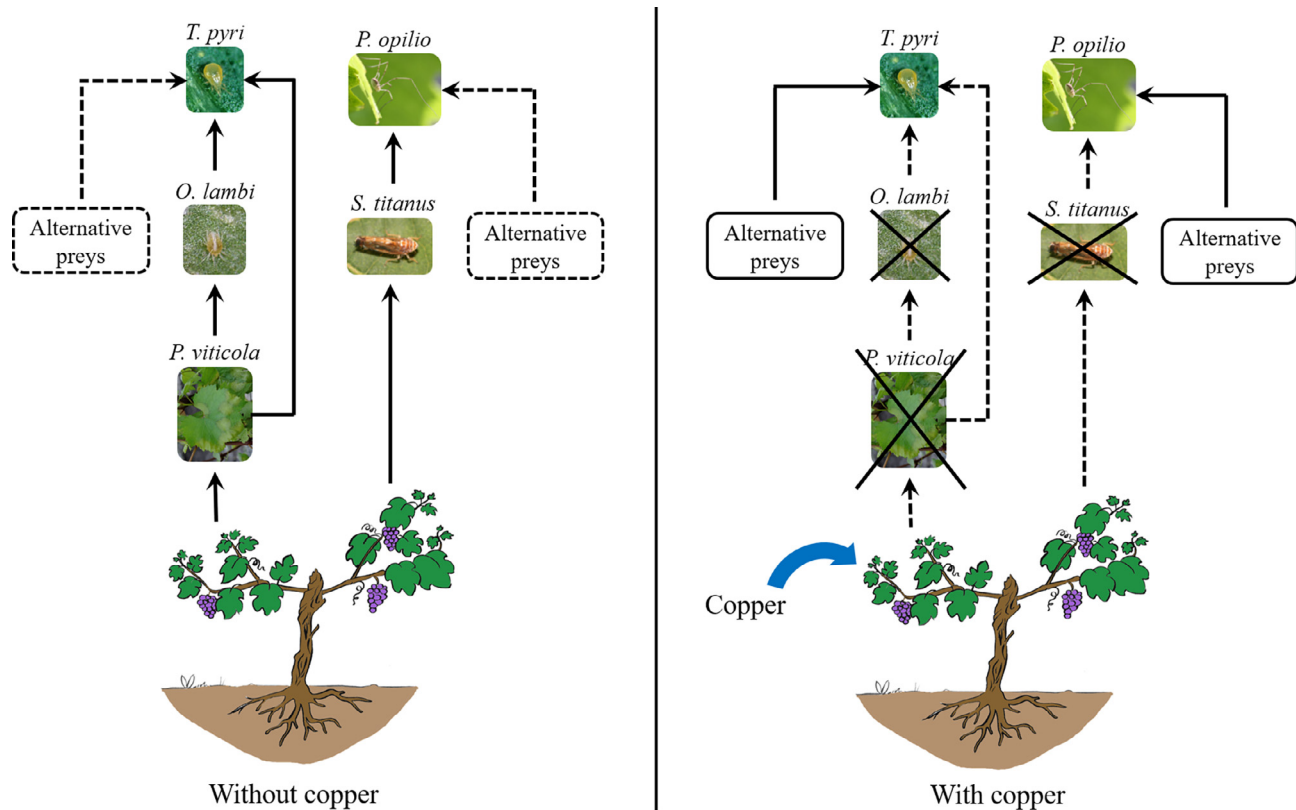
## 6. Author contribution statement

FV and DT conceived and designed research. FV conducted experiments. FV analyzed data. FV and DT wrote the manuscript. All authors read and approved the manuscript.

**Table 3**  
Effect of the treatment (control, copper or ½ copper) and the sampling year (2014 or 2015) on the occurrence of diseases on grapevine leaves.

	<i>Plasmopara viticola</i>		<i>Uncinula necator</i>		<i>Guignardia bidwellii</i>	
	F	P-value	F	P-value	F	P-value
Treatment	<b>49.46</b>	<b>&lt; 0.0001</b>	<b>61.77</b>	<b>&lt; 0.0001</b>	<b>79.98</b>	<b>&lt; 0.0001</b>
Year	<b>78.92</b>	<b>&lt; 0.0001</b>	<b>8.93</b>	<b>0.002</b>	<b>66.76</b>	<b>&lt; 0.0001</b>
Treatment * Year	<b>46.36</b>	<b>&lt; 0.0001</b>	<b>6.67</b>	<b>0.035</b>	<b>13.63</b>	<b>0.001</b>

Significant P-values are in bold.



**Fig. 4.** Schematic diagram explaining the indirect effect of copper on trophic interactions. The left part represents trophic interactions in a vineyard without copper applications and the right part, those in a vineyard with copper applications. The black arrows represent the connection between two trophic levels (i.e. eaten/parasitized by), the dotted arrows, the potential connection between two trophic levels, and the crosses, the species negatively affected by copper.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.biocontrol.2018.08.011>.

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