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SHEEP GRAZING ORGANIC VINEYARDS AND ORCHARDS: WHAT ABOUT COPPER POISONING?

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Abstract: Sheep have long been allowed to graze in orchards and vineyards during winter, generating benefits for both breeders and wine or fruit growers. This practice, which had become scarce because of agricultural specialization, is recently regaining popularity. However, concerns have raised about the potential risk of Chronic Copper Poisoning (CCP) of sheep – particularly in the context of organic agriculture, which widely uses Cu as a fungicide. CCP is driven by the long-term, symptomless Cu accumulation in the liver, potentially leading to a hemolytic crisis that generally triggers animal death within 48h. Our study aimed at evaluating both the quantity and dynamics of Cu in the cover vegetation of vineyards and orchards, and the potential harmful effects of Cu on the health of sheep that graze therein. Our results show that i/ Cu content and assimilability is high in the studied plots, and may lead to CCP; ii/ surprisingly, sheep show only slight signs of ongoing CCP.

Introduction: Sheep exposed to copper (Cu) endure long-term accumulation of this element in the liver, called Chronic Copper Poisoning (CCP), which is accompanied with almost no visible symptoms. When very high liver Cu concentrations are reached, a hemolytic crisis can occur, leading to death of the animal within a few days ((NRC), 2005; Suttle, 2010).
Up to the mid-XXth century, sheep were commonly allowed to graze in vineyards and orchards, where they were at risk of enduring CCP because of the use of Cu as a fungicide (Delas et al., 1959). Ovine-mediated herb management in perennial crops has almost disappeared since then, but it is regaining great interest as it can allow savings on mechanical or chemical interventions, while providing unexploited sources of forage for sheep. In this context, concerns have raised about the possible CCP undergone by the sheep that graze in vineyard plots – especially in organic agriculture, where Cu still is the main technically available option against many fungal diseases (Andrivon et al., 2018).

Material and methods: During winter 2017 and 2018, respectively 16 and 12 crossbred Merino x Mourerous, 8 months-old female sheep were allowed to graze in organically-managed vineyard plots in the area of the Clairette de Die wine production in Drôme, France. Unfavorable weather conditions in 2017 impeded the collection of valuable data, which therefore were used as preliminary results. In the 2018 experimental campaign, the grazing period extended for 56 days from November 15th (T_0) to January 10th 2019 (T_f), in two different vineyard plots, for a total surface of 3.1 ha.

Blood samples were collected from all sheep at T_0, T_f, and at the time of plot changing (T_0 + 42 days); samples were also collected after the end of the grazing period, at T_f + 12, 39, and 63 days. Blood was collected using heparin plasma tubes, and plasma was stored at -4°C after centrifugation, until determination of Cu, Mo and Fe was performed by ICP-OES. Determination of Glutamate Dehydrogenase (GLDH), Gamma-Glutamyl Transferase (γ-GT), and Aspartate Aminotransferase (AST) were also performed by spectroscopic methods.
Samples of the vegetation cover were collected for Cu, Mo and S content determination, beginning after the end of all Cu treatments. In plot 1, samples were collected from vegetation located both in the inter-rows and under the vine rows, while the latter was impossible in plot 2.

Results:

Cu in soil and plants

The soils of the studied plots happened to be moderately contaminated by Cu, with values ranging from 13.1 ± 6.1 to 32.0 ± 5.8 mg Cu/kg soil DM (Komárek et al., 2010).
Applied Cu doses during the year 2018 were 3.29 and 5.93 kg Cu /ha, respectively in plots 1 and 2. All Cu-based fungicide applications were over on August, 6th.
Cu concentration in the vegetation cover decreased significantly before sheep were allowed to graze, ending at values of 27.9 ± 0.8 and 36.7 ± 15.9 mg Cu/kg DM (in plots 1 and 2 respectively) at the beginning of the pasture period (Fig.1, full lines). Cu concentrations up to 98.0 ± 17.5 mg Cu/kg DM were found in the vegetation under the vine rows. Vegetation that had been cut to ground after the last Cu treatment harbored concentrations of 9.2 ± 0.6 and 11.8 ± 1.0 mg Cu/kg DM after 77 and 112 days, in plots 1 and 2, respectively (Fig.1, dashed lines).

Blood parameters of sheep

Despite great inter-individual variations, GLDH activity in plasma of sheep significantly ($P<0.1$) increased during and after Cu exposure, starting from 6.75 ± 2.3 to reach 16.1 ± 8.2 U/L on T_f+14 days. In the meantime, Mo concentration in the plasma underwent a dramatic decrease, quickly reaching the limit of detection (<10 ppm); it eventually increased strongly at the end of the pasture time, to reach concentrations 3 times higher than the starting value (Fig.2). Cu plasma concentration was not significantly affected (data not shown).

Discussion: In our experiment, Cu concentrations in the cover vegetation largely decreased before sheep were allowed to graze in the vineyard plots. This decrease may be caused by washing off during rain events, but most of this phenomenon likely occurs very soon after the end of fungicide application, while the remaining copper has a very long lifetime on the leaves (Molot and Gaimon, 2004). On the other hand, plant uptake of Cu from the soil was significant but moderate, in agreement with the values of pH, CEC, organic matter content (data not shown), and Cu soil content, that were globally unfavorable to Cu transfer to plants. This means that most of the observed Cu decrease was linked to plant growth, which induced the dilution of the trace elements deposited on the surface of the cover vegetation.
Cu contents in the cover vegetation were sufficiently high to make plausible the advent of a CCP phenomenon, exceeding the values of 15 or 17 mg/kg DM that are frequently considered as a threshold ((NRC), 2005). Based on the Mo and S contents, a calculation of the absorbable Cu in forage has been proposed (Suttle, 2010); in the present case, it indicates that pasture times as short as 60 days could lead to levels of liver Cu corresponding to high risk of triggering a hemolytic crisis (data not shown).
Despite this, sheep did not harbor clinical signs of copper toxicosis, and additionally showed moderate values of GLDH contents in the plasma, indicative of Cu liver accumulation in its starting phase, most probably lower than 100mg Cu/kg

liver WW (Humann-Ziehank et al., 2001). Still, the strong and sudden decrease of Mo plasma content observed upon Cu exposure can be interpreted as a sign of entering the ‘danger zone’ of hypercuprosis. Since Mo is an antagonistic element towards Cu assimilation, we believe that its disappearance from the plasma reflects its mobilization to prevent Cu storage in the liver. At the end of the pasture time in the vineyard, the Mo plasma concentration rises to very high levels, which might reveal an adaptation mechanism of the sheep to mitigate the effect of high Cu exposure.

The drawback between high Cu content in the herbage, and moderate health effects on the ewes, may be linked to a possible tolerance of this sheep breed regarding CCP, or to an initially elevated Mo supply which could have played a protective role.

Future work will focus on confirming that sheep grazing in vineyards during winter can be safe in other conditions (sheep breed, type of soil, etc.); on deciphering the mechanisms by which upregulation of Mo uptake could help mitigate ongoing CCP; and on evaluating the potential long-term Cu accumulation in the liver of sheep from one year to the other.

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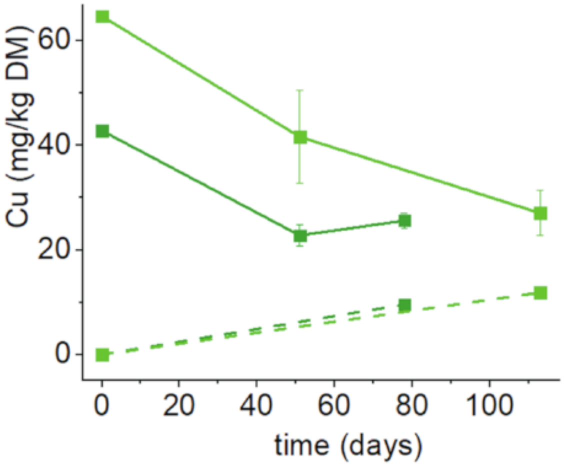

Fig.1: Measurements of the total Cu concentration in the cover vegetation of vineyard plots 1 and 2 (dark and light green, respectively), illustrating the evolution of Cu content after the end of the last treatment (full lines), and Cu uptake from the soil in places where vegetation had been cut to ground (dashed lines).

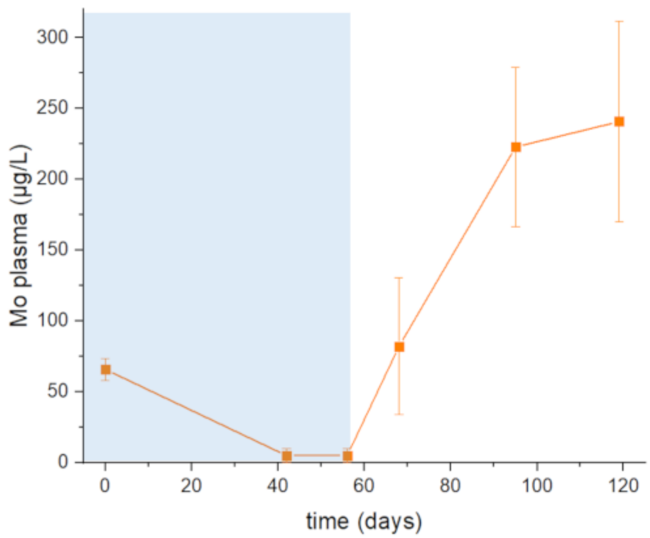

Fig. 2: Evolution of the Mo plasma concentration of sheep during (blue panel) and after grazing in the vineyard plots. Error bars indicate standard deviations (n=12)