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Efficacy of kaolin treatments against *Drosophila suzukii* and their impact on the composition and taste of processed wines

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Summary

***Drosophila suzukii* is a very polyphagous species that can also tack and develop in a great variety of grape cultivars. In Switzerland, the control of *D. suzukii* mainly relies on prophylactic measures and kaolin, a white inert aluminosilicate mineral who's particles stick to the leaf surface and form a physical barrier that help to reduce ovipositions by the pest. Here we present a synthesis of our recent insights on the efficacy of kaolin against *D. suzukii* as well as on the chemical and sensory properties of the wines vinified from kaolin treated grapes. In autumn 2016, kaolin (Surround WP®) was applied in 23 field trials on various cultivars located in various winegrowing regions of Switzerland. Overall, kaolin achieved an average efficiency of 54 % and no significant differences could be observed between kaolin applications at 1 % and 2 % with 56.8 % and 57.1 % efficacy, respectively. At the higher concentration, the preventive and curative strategy were also nearly as effective with efficacies at 67.4 % and 50.3 %, respectively. In addition, a field experiment was set up on the red grape cultivar 'Mara' in 2015. This experiment revealed that three applications of kaolin at 1 % or 2 % did neither affect fermentation nor the usual chemical properties of kaolin treated wines compared to the untreated control. However, aluminum concentration within wines increased with the applied dosage of kaolin but the measured aluminum levels were 38-times lower than the maximal German threshold of 8 mg·L^{-1}. Moreover, tasters were also not able to distinguish the aroma and the taste of wines processed from kaolin treated grapes from the untreated control. We therefore conclude that kaolin applications are effective against *D. suzukii* and do not cause any major risks to the environment, to wine quality and to human health.**

K e y w o r d s : Spotted wing drosophila; *Vitis vinifera;* organoleptic tests; aluminosilicate mineral; aluminum.

Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), commonly called Spotted wing drosophila, is a very polyphagous species that has become a major pest ever since it has spread around the world. Thanks to the serrated ovipositor of females, the insect can attack and develop in a wide range of wild and cultivated fruits (Walsh *et al.* 2011, Cini *et al.* 2012, Asplen *et al.* 2015). Although past experiences indicate that grapes have to be considered as a secondary host plant, eggs can be laid in a great variety of cultivars (Ioriatti *et al.* 2015, Entling *et al.* 2019). These eggs might trigger the development of sour rot when suitable weather conditions allow for the development of the responsible yeasts and acetic acid bacteria (Rombaut *et al.* 2017, Ioriatti *et al.* 2018). Since 2014, *D. suzukii* is also causing damages in Swiss vineyards (Linder *et al.* 2015). Swiss authorities responded quickly to this new threat and approved temporarily the use of insecticides such as pyrethrins and spinosad. Their use is, however, restricted and winegrowers are reluctant to apply them prior to harvest due to residues issues. The control therefore relies on prophylactic measures, in particular on a good aeration and lighting of the grape zone (Linder *et al.* 2018, Knapp *et al.* 2019). Kaolin, a white inert aluminosilicate mineral, could be an alternative to chemical insecticides (Glenn *et al.* 1999). Its particles stick to the leaf surface and form a physical barrier, which helps to reduce the damage of various vineyard arthropods pests (Isaacs *et al.* 2004, Tubajika *et al.* 2007, Sleezer *et al.* 2011, Maier and Williamson 2016, Pease *et al.* 2016, Tacoli *et al.* 2017a, Tacoli *et al.* 2017b). However, the influence of multiple kaolin treatments applied just prior to vintage on the chemical composition and organoleptic properties of processed wines is poorly documented (Coniberti *et al.* 2013, Lobos *et al.* 2015, Ferrari *et al.* 2017). Although kaolin is used as a fining agent, it contains aluminum, which has raised concerns about its presence in the food chain and its impact on human health (Stahl *et al.* 2011, Bondy 2016, Stahl *et al.* 2017). This paper presents a synthesis of our recent insights on the efficacy of kaolin against *D. suzukii* as well as on the chemical and sensory properties of the wines vinified from kaolin treated grapes.

Material and Methods

F i e l d e f f i c a c y a g a i n s t *D . s u z u k i i* : In autumn 2016, kaolin (Surround WP®) was applied in 23 field trials on various cultivars located in various winegrowing regions of Switzerland (Tab. 1). The grape zone was treated

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Table 1

Oviposition and reduction of egg-laying rates in the 23 kaolin trials conducted all over Switzerland in 2016

Site (Swiss Canton)	Cultivar	Rate	Timing*	% Oviposition		% Efficacy
				Control	Treated	
Salenstein (TG)	Regent	1 %	P	6	0	100
Aesch (BL)	Garanoir			2	0	100
Aesch (BL)	Dunkelfelder			64	26	59.4
Muttenz (BL)	Dunkelfelder			69	23	66.7
Wintersingen (BL)	Cabernet Dorsa			14	4	71.4
				31 ± 32.7	10.6 ± 12.8	79.5 ± 19.2
Malans (GR)	Pinot noir	1 %	C	10	10	0
Fläsch (GR)	Pinot noir			8	8	0
				9 ± 1.4	9 ± 1.4	0
Neunforn (TG)	Garanoir	2 %	P	7	3	57.1
Salenstein (TG)	Regent			6	0	100
Schlattingen (TG)	Marechal Foch			18	2	88.9
Frümsen (SG)	Pinot noir			2	2	0
Frümsen (SG)	Cabernet Jura			15	2	86.7
Frümsen (SG)	Gamaret			3	0	100
La Tour-de-Peilz (VD)	Divico			8	0	100
La Tour-de-Peilz (VD)	Cabernet Jura			24	14	41.7
La Tour-de-Peilz (VD)	Galotta			0	4	0
				9.2 ± 8.1	3 ± 4.4	67.4 ± 41.5
Gordola (TI)	Merlot	2 %	C	2	0	100
Giornico (TI)	Merlot			2	2	0
La Neuveville (BE)	Regent			2	2	0
Villeneuve (VD)	Dunkelfelder			18	12	33.3
Ollon (VD)	Cabernet Jura			22	4	81.8
Malans (GR)	Pinot noir			10	3	70
Fläsch (GR)	Dunkelfelder			8	6	25
				9.1 ± 8.2	4.1 ± 3.9	50.3 ± 40

* P: Preventive; C: Curative.

based on a theoretical water volume of 1200 L·ha^{1} and at a rate of 1 or 2 % of kaolin (e.g. 12 to 24 kg·ha^{-1}) either shortly before (= preventive) or after (= curative) the first eggs were laid. Winegrowers applied kaolin once with various types of sprayers. Prior to harvest, treated plots were compared to untreated controls by monitoring the oviposition rate on 50 berries randomly picked from 50 bunches per plot. The efficacy of the treatments was then expressed as the percentage of reduction in egg-laying. The data obtained were statistically analyzed with either unparametric Wilcoxon Signed-Ranks Tests for paired samples or unparametric Mann-Whitney U Tests for unpaired values.

Composition and taste of kaolin treated wines: In 2015, a field experiment was conducted in four lines of the red grape cultivar 'Mara' in Nyon (Switzerland) in order to evaluate the impact of kaolin on the chemical composition as well as on the taste of wines. Kaolin (Surround WP®) was applied at a rate of 1 or 2 % and compared to an untreated control. The three different treatments were applied in a completely randomized block design. In particular, we divided the four lines of 'Mara' in three parts of 50 to 60 grapevines, which resulted in four replicates for each of the three treatments. Applications were conducted only in the defoliated grape zone using a backpack sprayer (Birchmeier M125) at a volume of 1200 L·ha^{-1} on August 11, 18 and 26. No egg-laying by *D. suzukii* was observed in this field experiment and grapes were harvested 13 d after the last application of kaolin.

At harvest, the four replicates of each variant were pooled together and processed in a classical microvinification. The chemical properties of wines were analyzed at filling by a mid-infrared spectrophotometer (FOSS Winescan). Determination of aluminum content in wine samples was performed in 2018 in triplicate using an in-house method. For this purpose, a wine sample of 50 g was concentrated to dryness (150°C; 30 min) and left to react with 5 mL of 65 % HNO_3 at room temperature overnight. The reaction mixture was heated to 104 °C for one hour and then left to cool. Three Teflon vessels were filled each with 1 mL of the cold reaction mixture. The digestion was performed in a pressure digestion system (Berghof DAB II) at 150 °C for 2 h. The clear solution was then quantitatively transferred with ultrapure water (Milli-Q) to a 20 mL quartz flask. This solution was measured directly with a Microwave Plasma-Atomic Emission Spectrometer (MP-AES) to determine the aluminum concentration. The measurements were performed on an Agilent MP-AES 4200 instrument (Agilent Technologies) equipped with an OneNeb nebulizer, a double pass glass cyclonic spray chamber and an autosampler. The nebulizer flow was 0.8 L·min^{-1}. The aluminum content was measured at 396.152 nm with 394.401 nm as the control wavelength. Quantification was performed using a calibration curve (0 / 0.25 / 0.5 / 1.0 / 1.5 / 2.0 mg·L^{-1}) prepared with a standard solution of $Al(NO_3)_3$ (Merck, Darmstadt; Aluminum-Standard-Solution, $Al(NO_3)_3$, 1000 mg·L^{-1} Al).

Two months after bottling, wines were tasted in a two-out-of-five discrimination test by a panel of 12 judges in order to examine if the highest dose of kaolin could be distinguished organoleptically from the untreated control. A second panel of 12 tasters established the sensory profiles of the three wines. They rated the sensory descriptors on a continuous linear scale ranging from 1 (bad/weak) to 7 (excellent/high). Data were recorded using Fizz software (Biosystemes ®, Couternon, France) and thereafter analyzed with 1-way ANOVAs.

Results and Discussion

Field efficacy against *D. suzukii*: Although the trials were conducted in vineyards planted with sensitive cultivars and/or in situation of high infestation risk, oviposition rates in untreated controls varied from 0 % to 69 % (Tab. 1 and Fig. 1). Although the efficacy of kaolin applications ranged from 0 to 100 %, they significantly reduced egg-laying by *D. suzukii* compared to the untreated control ($W = 5.5$, $P < 0.001$). However, no significant differences

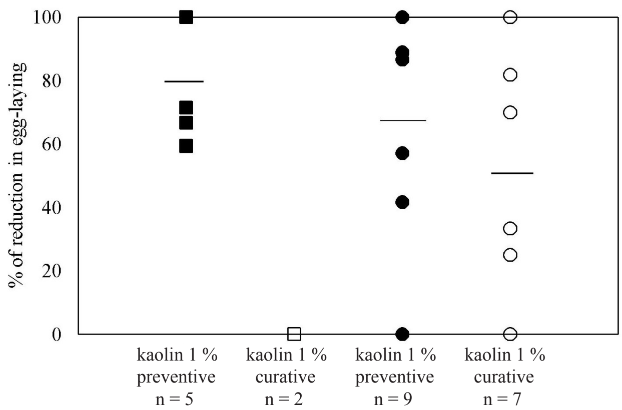

Fig. 1: Percentage of reduction in egg-laying in the kaolin trials 2016. Horizontal lines represent the mean efficacy.

could be observed between kaolin applications at 1 % and 2 % with 56.8 % and 57.1 % efficacy, respectively ($U = 55$, $P = 0.97$). Over all trials, the preventive strategy tended to be more effective than the curative strategy with efficacies of 69.4 % and 34.5 %, respectively ($U = 32.5$, $P = 0.054$). This difference in efficacy dropped, however, considerably when only field trials with 2 % kaolin were considered with 67.4 % efficacy for the preventive and 50.3 % for the curative strategy ($U = 21.5$, $P = 0.31$, Tab. 1). We therefore conclude that despite similar efficacy, the 2 % concentration seems to be more appropriate since it provides a better adhesion after rainfall and consequently a higher persistence on grapes. Moreover, it also seems to be appropriate to wait with the first application of 2 % kaolin until the first eggs of *D. suzukii* are observed on a cultivar of similar sensibility towards this novel pest in the region in order to avoid systematic applications of kaolin at veraison.

Composition and taste of kaolin treated wines: The application of kaolin did neither affect the start nor the duration of the fermentation. Processed wines treated three times with kaolin could also not be distinguished chemically from the untreated control and the measured chemical properties were similar among the three wines (Tab. 2). However, kaolin treatments affected the aluminum content in wines and its concentration increased with the applied dosage of kaolin. Although no limit values are defined for the maximum aluminum concentration permitted within wines in Switzerland, with 0.21 mg·L^{-1} the maximum value was well below the German threshold of 8 mg·L^{-1} (WeinV 1995). The two-out-of-five tests showed that the kaolin did neither alter the aroma nor the taste of wines since the 12 panelists were unable to discriminate the wine processed from grapes treated with 2 % kaolin from the untreated control (0 correct responses, $P > 0.99$). Alike, there were no significant differences in the rating of the main organoleptic descriptors by the second panel (Fig. 2).

Table 2

Average values of chemical properties for control and kaolin treated 'Mara' wines

Composition	Untreated control	Kaolin 3 x 1 %	Kaolin 3 x 2 %
pH	3.59	3.59	3.64
Total acidity (g·L^{-1})	5.0	4.9	4.8
Volatile acidity (g·L^{-1})	0.5	0.5	0.5
Ethanol (% Vol.)	13.7	13.6	13.9
Anthocyanins (mg·L^{-1})	945	903	936
Free SO_2 (mg·L^{-1})	39	40	39
Total SO_2 (mg·L^{-1})	66	67	65
Aluminium (mg·L^{-1})	0.083	0.184	0.211

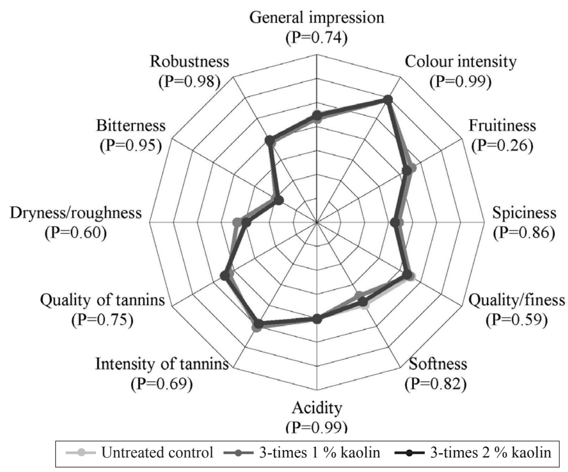

Fig. 2: Organoleptic properties on a scale from 1 to 7 (1 = bad/weak, 4 = satisfactory, 7 = excellent/high) for the control and the two kaolin treated 'Mara' wines from the field experiment in Nyon in 2015. In brackets: *P*-values of the ANOVA for the descriptors rated by the 12 tasters.

Conclusion

Approved by organic farming, a careful application of kaolin in the grape zone provides a satisfactory efficacy that is comparable to the one of conventional insecticides. It does, however, neither cause residue nor resistance issues. Moreover, kaolin has a negligible impact on predatory mites (unpublished data) and parasitoids (Pease *et al.* 2016). It therefore presents a valuable alternative to conventional insecticides in the control of *D. suzukii*. Although Coniberti *et al*. (2013) noticed some slight differences in the composition and sensory attributes in kaolin treated 'Sauvignon Blanc', the red cultivar 'Mara' used in our study remained unaffected. Even though kaolin treatments increased aluminum levels in wines, the amounts stayed well below the fixed threshold values. We therefore conclude that kaolin applications are effective against *D. suzukii* and do not cause any major risks to the environment, to wine quality and to human health.

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