https://doi.org/10.17221/40/2020-PSE

Effects of silicon amendments on grapevine, soil and wine

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Citation: Schabl P., Gabler C., Kührer E., Wenzel W. (2020): Effects of silicon amendments on grapevine, soil and wine. Plant Soil Environ., 66: 403–414.

Abstract: Replacing Bordeaux broth and synthetic fungicides by less invasive approaches of fungal disease control remains a challenge for both conventional and organic viticulture. Silicon (Si) application has been proposed as a viable alternative for the control of pathogens and other stresses in agriculture. In a three-year field trial, we tested the effect of foliar and soil application of colloidal silicon on its availability in vineyard soil and pants, the performance and quality of yield, and finally, the control of powdery mildew for grapevine cv. Grüner Veltliner. Soil application of colloidal silicon increased plant-available Si, but only foliar application increased the total silicon concentrations in leaves, yield, and cluster weight. Moreover, the wine produced from the silica-treated grapes were ranked better in sensory evaluations. Our findings provide evidence for the potential of at least partially replacing conventional fungicides, rendering viticulture more sustainable in terms of soil protection and biodiversity. Silicon applications are low in costs and comply with the principles of organic wine production.

Keywords: *Vitis vinifera*; colloidal silicon; plant nutrition; biostimulant; wine quality

Exposed to suboptimal conditions, abiotic and biotic stresses can be limiting to one or several resources of the plant (Keller 2015). Although not traditionally thought of as an important element to the life cycle of plants, silicon (Si) is found at concentrations from 1 to 100 g/kg, which is equivalent to or even exceeding plant tissue concentrations of other macronutrients (Epstein 1994). However, silicon is not considered as an essential element according to the classical definition of essentiality (Arnon and Stout 1939). Silicon is regarded as one of the most beneficial elements that increase plant resistance against various abiotic and biotic stresses. It has been shown to improve plant cell wall strength and structural integrity, to improve drought and frost resistance, to decrease lodging potential (Currie and Perry 2007), and to boost plant's natural pest and disease-fighting systems (Rodrigues and Datnoff 2007). Silicon has been shown to improve plant vigor and physiology by improving root mass and density as well as increasing above-ground plant biomass and crop yields (Epstein 2009, Liang et al. 2015, Debona et al. 2017).

Silicic acid (H_4SiO_4) is the only known precursor of silicon compounds in biota, and plants take up aqueous, uncharged silicic acid through their roots (Ma and Yamaji 2006). The ability of plants to accumulate silicon varies greatly between species. Silicon uptake is passive for dicotyledons, and its transport in the xylem is largely determined by the transpiration rate (Ma and Takahashi 2002). Silicon can be deposited in any plant part within or between cells or as part of the cell wall with the formation of discrete silica bodies known as phytoliths. Once deposited, they are immobile and cannot be translocated to new growing leaves. Following plant senescence, plant silicon dissolves in the soil solution and either cycle through biota or is leached into waterways (Cooke and Leishman 2011).

Supported by the Austrian Research Promotion Agency (FFG) through the Research Studio Austria FERTI-MINE, Project No. 844744.

https://doi.org/10.17221/40/2020-PSE

Plants deprived of silicon are often structurally weaker and more prone to abnormalities of growth, development, and reproduction. Silicon is the only nutrient that is not detrimental when collected in excess (Epstein 1994). The mechanical barrier formed by silicon polymerisation below the cuticle and in the cell wall was the first proposed hypothesis to explain how silicon reduces or impedes fungal penetration. However, new insights suggest that silicon effects on plant resistance may also occur through mediated host plant resistance mechanisms against pathogen infection (Rodrigues et al. 2015). Plants supplied with silicon exhibit potentiated activation of the phenylpropanoid pathway resulting in increased total soluble phenolics and lignin. Only root applications of silicon potentiate plant defense responses such as the activities of peroxidases, polyphenol oxidases, β-1,3-glucanases, and chitinases and the transcription of defense-related genes occurs faster and with greater output (Rodrigues et al. 2015).

Previous studies showed that the supplement of silicon to grapevine can increase the efficiency of the photochemical reactions in photosystem II (Qin et al. 2016), whereas Liang et al. (2015) state that silicon might play an important role in protecting the photosynthetic machinery and improve salt tolerance. The studies of Bowen et al. (1992), Reynolds et al. (1996), and Lafos (1995) showed reductions of powdery mildew in response to silicon application in greenhouses but emphasised large differences between cultivars. Blaich and Grundhöfer (1997) showed significant varietal differences only for the inter-specific hybrid grape cv. Regent, which accumulated about 20% more silicon than *Vitis vinifera* cultivars. The results from Blaich and Grundhöfer (1998) show silica to be essential for a powdery mildew tolerance but provide evidence that Oidium susceptibility of cultivars cannot be overcome by supplementary silica fertilisation in the field. Farouk et al. (2017) found that spraying Roumy Ahmar grapevine with silicon controlled mildew disease in grapevine and improved its growth. Furthermore, in the wine-making industry, silicon can lower the risk of off-flavors like H_2S in wines, and silica sol is used as a fining agent for clarification purposes. Silicon has the potential to reduce stress while the material cost is deemed low and potentially falls into the guidelines for organic winegrowers as a natural substance (Tubaña et al. 2015).

Apart from the potential of Si application in fungal disease control, Bordeaux broth or synthetic fungicide application remains the common practice in viticulture. While synthetic fungicides bear the risk of negative impacts on the biodiversity of fish, invertebrates, primary producers and non-target fungi (Zubrod et al. 2019), Bordeaux broth has been a substantial source of Cu accumulation in vineyard soils (Brun et al. 2001, Chaignon et al. 2003). As the latter is still being used in conventional and organic viticulture, Cu accumulation is expected to continue and eventually exceed critical concentrations for multifunctional soil use.

In this study, we test the efficiency of silicon applications (soil fertilisation and foliar spraying) to increase the tolerance against abiotic and biotic stress, with emphasis on the control of powdery mildew, *Erysiphe necator*, in grapevine *V. vinifera* L. cv. Grüner Veltliner, a major cultivar in Austria, in a field trial. We hypothesise that Si application can substantially increase resistance and therefore reduce the amounts of fungicides required for fungal disease control.

MATERIAL AND METHODS

Field experiment. The trial was conducted at a vineyard in Landersdorf (community Krems) of the School of Viticulture and Horticulture in Krems, Austria. For each treatment, 48 plants of *Vitis vinifera* cv. Grüner Veltliner (scion: SO4) was used. The completely randomised blocked design consists of 6 treatments, including (1) Si application to soil; (2) foliar application of Si; (3) combined soil and foliar application of Si; (4) control for soil application; (5) control for foliar application, and (6) conventional treatment using common plant protection agents. Details of the treatment schemes are presented in Tables 1–3. Each treatment comprised 4 replicates with 12 plants spaced at 3 × 1 m. The experimental site was selected based on prior analysis of plant-available and amorphous silicon fractions. In 2016 we applied 0.7 kg LUDOX® TM-50 colloidal silica (colloidal silica 50 wt% suspension in H_20, Sigma Aldrich, USA) suspended in 4 L water from an on-site well, and split into 6 proportions during the growth season (Table 1), to the vineyard soil within a pouring ring of 0.4 m in diameter (~0.126 m^2) around each specimen. The total application corresponds to ~163 g Si per specimen, and this equals around 544 kg Si per hectare. An equivalent amount of water was applied to the control (Table 1). In 2017, the same amount of the water-suspended silica product was applied at the beginning of the growing season in one single batch to the subsoil (30–60 cm) through 30 cm deep holes

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Table 1. Description of the silicon (Si) treatments and their controls

	2016			2017			2018		
	BBCH[a]	water volume (L)	(g Si/L)	BBCH[a]	water volume (L)	(g Si/L)	BBCH[a]	water volume (L)	(g Si/L)
Soil application of Si (colloidal silica)	17	192	13.6	17	192	40.8	–	–	–
	57	192	5.4						
	73	192	5.4						
	77	192	5.4						
	81	192	5.4						
	83	192	5.4						
Foliar application of Si (colloidal silica)	17	5	3.3	14	7	3.3	14	7	3.3
	57	6	3.3	17	8	3.3	16	8	3.3
	73	8	6.5	19	10	6.5	19	10	6.5
	77	8	6.5	57	10	6.5	59	10	6.5
	81	8	6.5	63	10	6.5	60	10	6.5
	83	10	6.5	73	12	6.5	73	12	6.5
				77	12	6.5	77	12	6.5
				79	15	6.5	81	15	6.5
				81	15	6.5	85	15	6.5
				85	15	6.5	85	15	6.5
Combined soil and foliar application (colloidal silica)	See application soil and foliar application treatment.								
Control for soil application (water)	The same application dates and water volumes were used in this treatment as in the soil application treatment except no Si was applied.								
Control for foliar application (water)	The same application dates and water volumes were used in this treatment as in the foliar application treatment except no Si was applied.								

[a]phenological development stages according to the BBCH-scale for grapevine

around each vine with a diameter of 0.4 m (Table 1). In 2018, no additional fertilisation was applied due to enough silicon availability as determined by the monitoring of plant-available, and Si contained in amorphous minerals (see below). This treatment is hereafter termed "soil application."

In 2016 the foliar application of Si was sprayed 6 times onto the grape canopy, with ~23 g of LUDOX® TM-50 colloidal silica suspended in water, obtaining final Si concentrations of 3.3 (first two applications in 2016) and 6.5 g Si/L (otherwise), respectively per specimen. This equals a total amount of 428 kg Si/ha. In 2017 and 2018, the foliar application of Si was sprayed 10 times, using ~62 g of the colloidal silica suspended in water, obtaining Si concentrations of 3.3 (first two applications) and 6.5 g Si/L (otherwise) per specimen. This equals a total amount of 1 154 kg Si/ha. The control was treated using the same scheme, but with water (Table 1). In all years, conventional fungicides were sprayed according to the common plant protection schedule of the school of viticulture, according to a good farmer's practice protocols (Table 3). Every year, sampling, measurements, and assessments were done at similar phenological development stages according to the BBCH-scale for grapevine (Lorenz et al. 1995).

Soil sampling and analysis. Soil samples from 0–30 cm (topsoil) and 30–60 cm depth (subsoil) were taken three times during each vegetation period (BBCH 0, 73, 85). Potentially plant-available silicon (hereafter termed PASi) in soil was analysed using a modified 0.01 mol/L $CaCl_2$-extraction method of Haysom and Chapman (1975). Two g of air-dried soil (< 2 mm) were mixed with 20 mL of the 0.01 mol/L $CaCl_2$ solution (Merck, Germany, 99.0–102.0%) in a tube and were shaken for 16 h in an overhead shaker. Silicon contained in amorphous minerals, including phytoliths (hereafter termed ASi) was extracted according to Georgiadis et al. (2015). To this end, a 0.2 mol/L sodium hydroxide (Merck, Germany, ≥ 97.0%) solution was added to soil at a ratio of 1:400

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Table 2. Maintenance of all silicon treatments and their controls, the product wettable sulfur Stulln (Nufarm, Austria, 796 g S/kg) and Cuprozin progress (Spiess-Urania, Germany, 250 g Cu/L) were used. The fungicide Aktuan Gold (BASF, Austria, 350 g/kg Dithianon, 150 g/kg Dimethomorph) was used once due to high infections of *Plasmopara viticola* in 2016

	BBCH[a]	Water volume (L)	(g S/L)	(g Cu/L)
2016	68	8	5.6	0.83
	73	10	Aktuan Gold 4.0	
	81	12	5.6	0.83
	85	12	5.6	0.83
2017	69	8	5.6	0.83
	73	10	5.6	0.83
	75	12	5.6	0.83
	79	12	5.6	0.83
2018	59	8	8.0	0.83
	65	10	8.0	0.83
	75	12	8.0	0.83

[a]phenological development stages according to the BBCH-scale for grapevine

before shaking for 120 h in an overhead shaker. All samples were analysed in two replicates. Extracts were filtered with folded filters (Munktell Ahlstrom, grade: 14/N, 80 g/m^2) and analysed colorimetrically with a Varian DMS 200 UV visible spectrophotometer at wavelength 810 µm. This analysis is based on the absorptiometric measurement of solutions of reduced β-molybdosilicic acid (Morrison and Wilson 1963).

Leaf and root sampling and analysis. Samples from mature and young leaves were taken at three times during the vegetation period. Only healthy leaves were collected, and an interval of seven days after the last foliar application was allowed for before sampling. At every sampling date, 20 mature and 40 young leaves were taken from the bunch zone. Whereas mature leaves differed in their leafage among sample times during a vegetation period, young leaves were at the same developmental stage. Root samples were collected only at the end of the growing seasons of 2017 and 2018 from the subsoil (30–60 cm).

Total Si concentrations in plant materials were determined according to Kraska and Breitenbeck (2010). Leaves and roots were washed with deionised water, dried at 65 °C for 48 h in an oven and ground using a Retsch ball mill to pass a 20-mesh screen. Silicon was extracted by adding 80 µL octyl alcohol (Sigma Aldrich, USA, anhydrous, ≥ 97.0%) to reduce foaming and 2 mL 30% of H_2O_2 (Acros, USA, stabilised, 50 wt%). After 90 min in the oven at a temperature of 95 °C, 4 mL of a 50% *w*/*v* NaOH solution (Merck, Germany, ≥ 97.0%) was added, and the samples returned to the oven for 5 h at 95 °C. To facilitate the formation of monomeric silicic acid, 1 mL ammonium fluoride (Sigma Aldrich, USA, 99.99%) was added, and vials were filled up with water to obtain a final volume of 50 mL. Samples were analysed colorimetrically with a Varian DMS 200 UV visible spectrophotometer at wavelength 810 µm (modified from Morrison and Wilson 1963).

Disease assessment. Infections of powdery mildew (*E. necator*) were documented during the period according to EPPO standard PP 1/4(4) *Uncinula necator* (EPPO 2001). To assess the percentage of leaf surface and affected bunch area, the following scale was used to class-divide the different levels of infection: 1 – no disease; 2 – < 5%; 3 – 5–10%; 4 – 10–25%; 5 – 25–50%; 6 – 50–75%; 7 – > 75%. Out of these classes, the indicators "disease incidence" and "disease severity" were calculated (EPPO 2001). Four hundred leaves and 200 clusters were assessed in each treatment. The assessment of powdery mildew was on August 11, 2016. In 2017 and 2018, no fungal assessment could be performed due to a lack of symptoms.

Analytical parameters for quality of grapes, must and wine. For the analysis of the grape quality parameters, 30 berries in four replicates were picked at the end of the growing season, crushed and analysed using Fourier transform infrared spectroscopy (FTIR, OenoFoss, FOSS, Hilleroed, Denmark). The must weight, density, acidity, pH-value, amount of tartaric, malic, acetic and gluconic acid and the amount of alpha-amino nitrogen were obtained from this analysis. The same analysis was done with samples of the pressed must during microvinification (see below).

The grapes were harvested in each year according to good practice standards. During manual harvesting, the total cluster weight per vine, the weight per cluster, and the health conditions were classified at a range from 1 to 5, where 1 is the best category.

The fermentation process was monitored by daily measurements of the density with an Oscillating U-tube (Anton Paar, Austria). After the fermentation, samples of the wines were analysed again by FTIR for measuring the density, alcohol, titratable acidity, pH value, malic acid, tartaric acid, volatile acidity, the content of glucose and the total dry matter.

https://doi.org/10.17221/40/2020-PSE

Table 3. Description of the common plant protection treatment

	BBCH 14[a]	BBCH 53[a]	BBCH 57[a]	BBCH 69[a]	BBCH 73[a]	BBCH 75[a]	BBCH 79[a]	BBCH 81[a]
2016	Polyram WG (0.8 kg/ha) Kumulus (4.8 kg/ha) Topas (0.15 L/ha)	Polyram WG (0.8 kg/ha) Prosper (0.5 L/ha)	Veriphos (2 L/ha) Delan 700 WG (0.4 kg/ha) Karathane Gold (0.6 L/ha) Vivando (0.2 L/ha)	Veriphos (3 L/ha) Delan 700WG (0.4 kg/ha) Luna Experience (0.375 L/ha)	Aktuan Gold (1.25 kg/ha) Legend Power (1.4 L/ha)	Enervin (3 kg/ha) Kumar (5 kg/ha)	Aktuan Gold (1.5 kg/ha) Kumar (5 kg/ha)	Cuprozin progress (1.6 kg/ha) Veriphos (4 L/ha) Kumar (5 kg/ha)
2017	Netzschwefel Stulln (3.0 kg/ha) Dithane Neo Tec (1.6 kg/ha) Topas (0.16 L/ha)	Nautile (0.8 kg/ha) Veriphos (2 L/ha) Systhane (0.12 L/ha) Netzschwefel Stulln (3.0 kg/ha)	Delan Pro (2.2 L/ha) Topas (1.6 kg/ha) Netzschwefel Stulln (3.0 kg/ha)	Mildicut (2 L/ha) Legend Power (0.8 L/ha)	Vincare (1.5 kg/ha) Veriphos (3 L/ha) Delan WG (0.6 kg/ha) Cidely (0.3 L/ha)	Enervin (3.0 kg/ha) Collis (0.64 L/ha) Delan WG (0.8 kg/ha) Teldor (1.0 kg/ha)	Aktuan Gold (1.5 kg/ha) Legend Power (1.5 L/ha) Karathane Star (0.6 L/ha)	Kupfer Fusilan (2.5 kg/ha) Vivando (0.32 L/ha)
2018	Dithane Neo Tec (3.2 kg/ha) Netzschwefel (3 kg/ha) Prosper (0.6 L/ha)	Folpan 500SC (1.2 L/ha) Veriphos (3 L/ha) Netzschwefel (3 kg/ha) Dynali (0.6 L/ha)	–	Enervin (3 kg/ha) Sercadis (0.24 L/ha)	Aktuan Gold (1.5 kg/ha) Dynali (0.65 L/ha)	Delan Pro (4 L/ha) Vivando (0.32 L/ha)	Enervin (4 kg/ha) Prosper (0.8 L/ha)	Cuprozin progress (1.6 L/ha)

[a]phenological development stages according to the BBCH-scale for grapevine

Microvinification. In 2016 and 2017, grapes from the combined treatment of silicon fertilisation and spraying and the common plant protection treatment were micro-vinified. In 2018, the control spray treatment was included for better comparison and insights into fermentation processes. The procedure for the microvinification started by adding 30 mg SO_2/kg to the grapes before destemming and pressing them in a 250 L Wottle press with an output of 55–60%. 150 mg/hL gelatin (Erbsöh, Germany) was added to remove tannins and 4.5 mL/hL Trenolin® Super DF (Erbslöh, Germany), a pectinase, was added. Overnight sedimentation at 15 °C and separation of 34 L of clear must to a carboy were completed. The selected yeast strain *Saccharomyces cerevisiae* (var. bayanus) EC 1118 Lalvin (Eaton Electric GmbH, Bonn, Germany) was added to the must, and the carboy was cooled at 18 °C. In 2018, 1 mL of LUDOX® TM-50 colloidal silica ("SI" Sigma Aldrich, USA) was added to one treatment before the fermentation. The end of fermentation was determined by Paar Oscillating U-tube. After cooling down to 7 °C over two days, 75 mg/hL SO_2 was added, and the wines were removed from the lees. The wines were filtered by sheet filtration with K 150 filter layers and were stored at 7 °C 3 months until filled into 0.5 L bottles.

Sensory evaluation. For the assessment of differences in the micro-vinified wines, a triangle test was performed in March and September 2017 and 2018. During the test, the panelists were presented with one different and two alike samples. The panelists rated the wines according to a 20-points rating system for color, odor, taste, and the overall impression of the wine. In 2019, the quality and sensory properties of wines from vintage 2018 were assessed in a ranking method. The four micro-vinified wines were ranked by the panelist according to their impression of the wine quality from 1st to 4th place. The number of assignments to a certain rank is multiplied by the rank (1 for 1st place, 2 for 2nd place, etc.). The product of this multiplication indicates the quality of the wine. During all tastings, all samples were presented to the panelists at once, and the panelist were instructed to taste the samples from left to right. The combinations were randomised across the panelists.

Statistical analysis. Statistical analysis of the data was made with the software IBM SPSS Statistics 23. All data were tested on normal distribution and homogeneity of variance. Since all requirements were met, a one-way ANOVA post hoc multiple comparison Tukey test was used to determine significant differences between the treatments.

https://doi.org/10.17221/40/2020-PSE

Table 4. Initial characteristics of the experimental vineyard soil in Krems, Landersdorf, soil samples were taken on March 18, 2016

Property	Plant-available silicon	Amorphous silicon	pH	Sand	Silt	Clay	Carbonate content	Organic carbon	Nitrogen	C/N ratio
	(mg/kg)			(g/kg)						
Topsoil (0–30 cm)	35.4	1 250	7.43	100	672	228	1 850	19.3	1.4	13.8
Subsoil (30–60 cm)	33.1	1 370	7.55	89	758	153	192	16.4	1.0	16.4

RESULTS AND DISCUSSION

The response of soil silicon to silica applications. The initial concentrations of Si associated with the amorphous minerals in soil (ASi) in 2016 before soil applications were 1 250 (topsoil) and 1 370 mg/kg (subsoil), respectively (Table 4). Compared to literature data, the experimental soil ranks in the lower range (Tubaña et al. 2015). ASi was instantly almost doubled to ~2 000 in response to topsoil Si application in 2016 with a subsequent further increase to ~5 700 mg/kg by the end of the growing season 2018 (Figure 1). In the subsoil, ASi peaked at ~4 900 mg/kg shortly after soil application in 2017, followed by a decrease to ~3 500 mg/kg in the subsequent sampling, and a steady increase to ~4 700 mg/kg hereafter until the end of the experiment in autumn 2018 (Figure 1). Very similar patterns were observed in the combined soil and foliar application, with slightly larger ASi concentrations, compared to the soil application treatment, particularly in the topsoil (Figure 1). A tendency of increasing ASi concentrations was also observed in control for both topsoil and subsoil; however, these changes are small compared to those in the Si application treatments and can be explained by the inherent spatial heterogeneity of the experimental plots and related uncertainties of soil sampling (Figure 1). Our data indicate that the colloidal silicon applied in this study was only partly extractable within the year of application. Based on the application rate of 0.7 kg Si per plant and the soil volume directly treated (0.12 m^2 to ~0.3 m depth), and assuming a soil mass of ~500 kg/m^2 in this upper 0.3 m layer, the applied

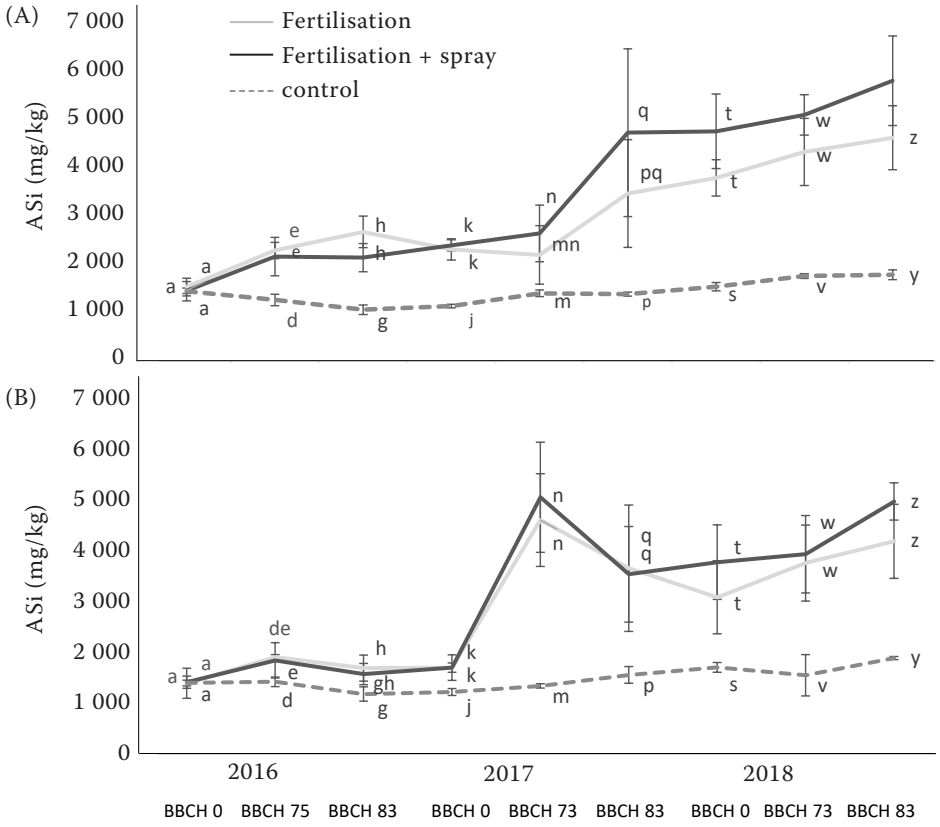

Figure 1. Silicon (Si) in amorphous minerals (AS) of (A) topsoil and (B) subsoil. One-way ANOVA post hoc multiple comparison test was used to determine differences between the treatments, different letters above columns indicate significant differences between treatments, α = 0.05, at the same time point of analysis, error bars represent standard deviation of the mean, $n = 4$

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Si should have increased the ASi by ~2 600 mg/kg both in the topsoil and subsoil. Considering the ASi concentration in control at the end of the experiment as initial concentration, this corresponds to an ASi concentration in the topsoil of ~4 400 mg/kg after application if all added Si were extractable by NaOH. This mass balance almost perfectly complies with the measured ASi concentration at that time in the soil application treatment (Figure 1). Similarly, the measured ASi concentration by the end of the growing season 2018 closely matches the expected value in the subsoil (Figure 1). The lower extractabilities at the previous sampling dates suggest that the applied colloidal silica required some time to fully transform into amorphous, NaOH-extractable forms.

The plant-available silicon fraction (PASi) responded like amorphous silicon, indicating a positive effect of fertilisation. Starting from concentrations slightly above 25 (topsoil) and 20 (subsoil) mg Si/kg, respectively, PASi steadily increased in the soil application and the combined soil and foliar treatment (Figure 2). Upon termination of the experiment, PASi reached ~75 (topsoil) and almost 60 mg/kg (subsoil) in the soil application treatment, with slightly larger concentrations in the combined treatment. In the same period, PASi in control increased in the second half of the growing season 2016 to ~40 and 35 mg/kg in topsoil and subsoil, respectively, and remained at this level until termination of the experiment (Figure 2). This noticeable increase of PASi in the control treatment in 2016 may be related to a combination of high temperatures and frequent rainfalls during this period, accelerating mineralisation and the release of Si from phytoliths. In the subsequent years 2017 and 2018, prolonged drought periods did not allow for rapid mineralisation and further mobilisation of Si, conserving the PASi level obtained in the previous year. Plant uptake rates in the dry years 2017 and 2018 were too low to substantially deplete the PASi back to the initial levels. As PASi is known to respond to soil pH, we explored our data for a correlation with pH; however, in the calcareous experimental soil, pH is well buffered, and no relation was observed (data not shown).

The application of colloidal silica substantially increased PASi (measured in 0.01 mol/L $CaCl_2$) from concentrations near or even below the upper limits of deficiency established for sugar cane (Haysom and Chapman 1975) and rice (Narayanaswamy and

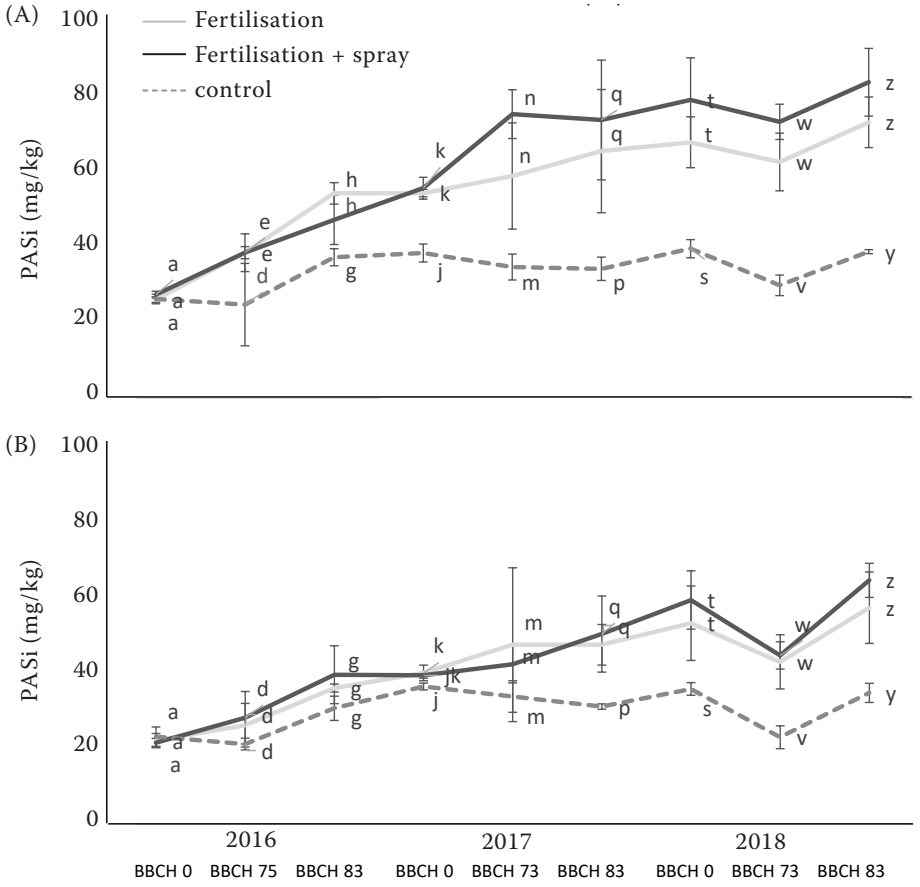

Figure 2. Analysis of plant-available silicon in (A) topsoil and (B) subsoil, according to Haysom and Chapman (1975) and Liang et al. (2015). One-way ANOVA post hoc multiple comparison test was used to determine differences between the treatments, different letters above columns indicate significant differences between treatment, α = 0.05, at the same time point of analysis, error bars represent standard deviation of the mean, *n* = 4

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Prakash 2009, Tubaña et al. 2016) to values that are safely above any likely deficiency range. This is supported by the fact that grapevine, in contrast to rice and sugar cane, is not a Si accumulator, indicating lower Si demand. This increase of PASi during the experimental period is closely related to that of ASi.

Here we used colloidal silica to minimise fertilisation effects of other elements contained in most materials suitable for Si fertilisation. In practice, minerals such as calcium metasilicate ($CaSiO_3$) and fertilisers based on secondary raw materials (e.g., basic oxygen furnace (BOF) and Linz-Donawitz (LD) slags) could be used. We could recently show that the Si release kinetics of colloidal silica are slow compared to those of several other materials, including the ones mentioned above (Duboc et al. 2019). This is in line with our current findings.

Silicon uptake in plant tissues. Silicon concentrations in mature leaves showed similar seasonal changes in 2016, 2017, and 2018 (Figure 3A,B,C). In the two treatments with the foliar application (foliar and combined), throughout each season, the mature leaves showed increased silicon concentrations compared to the control of the foliar application and the Si application to soil. In 2016, an additional pronounced effect was found in the combined soil and foliar application treatment.

Silicon concentrations in the young leaves of the years 2016, 2017, and 2018 show a more differentiated pattern (Figure 3D–F). At the beginning of the growing season, the foliar concentrations remained comparably low in each year. Whereas a steep increase was observed during the season 2016 up to 12 000 mg Si/kg. Foliar Si in young leaves remained

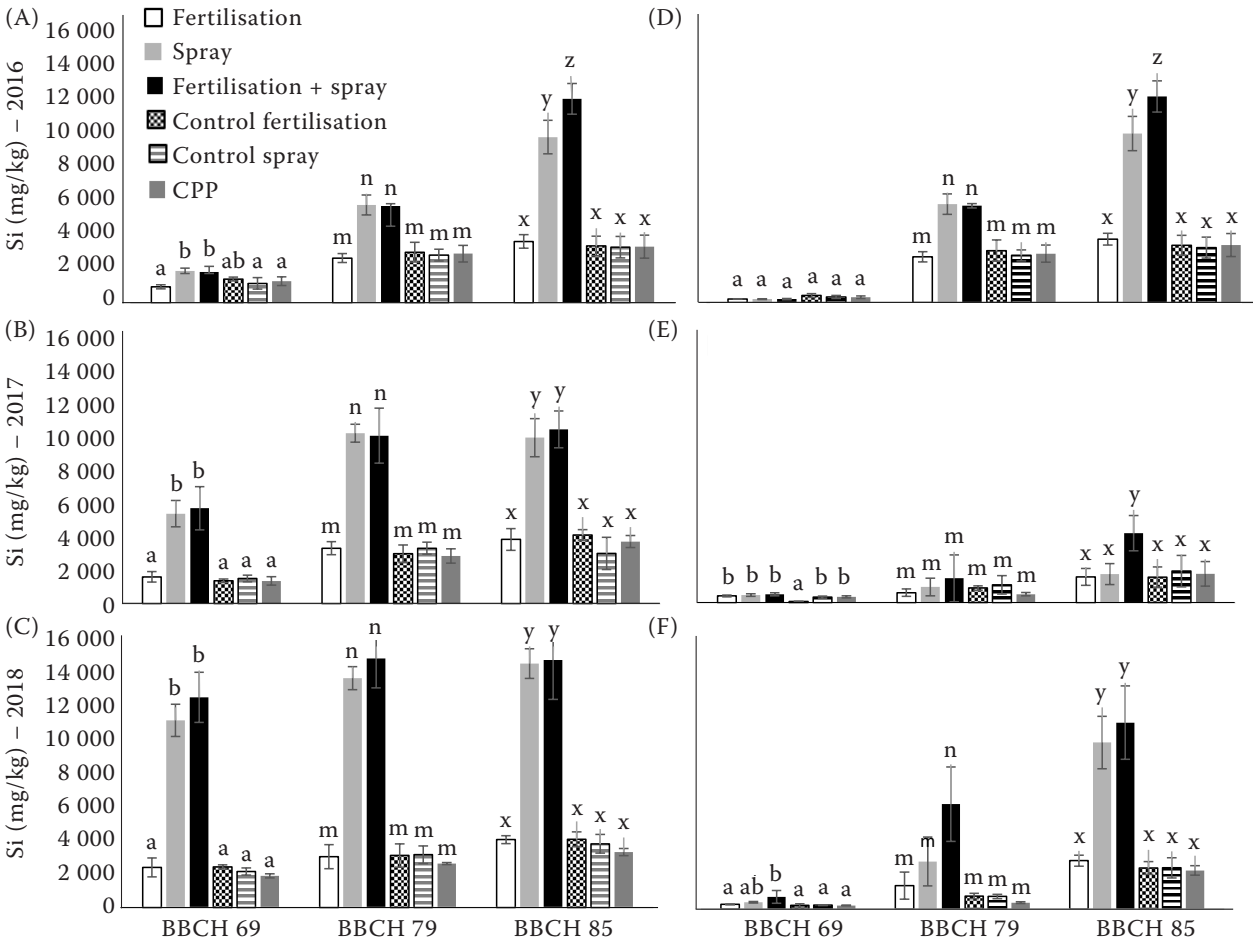

Figure 3. Analysis of total silicon (Si) content of mature (A, B, C) and young leaves (D, E, F), according to Kraska and Breitenbeck (2010) and Morrison and Wilson (1963). One-way ANOVA post hoc multiple comparison test was used to determine differences between the treatments, different letters above columns indicate significant differences between treatment, α = 0.05, at the same time point of analysis, error bars represent standard deviation of the mean, n = 4; CCP – common plant protection

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below 2 000 mg/kg in 2017 throughout the growing season. In 2018, the foliar concentrations increased again to values near 10 000 mg/kg. Both treatments with foliar application exceeded the control and fertilisation treatments by up to > 8 000 mg Si/kg. The combined foliar and soil treatment showed significantly larger Si concentrations in young leaves than the foliar treatment (Figure 3D–F). Such a benefit from Si application could not be found in the soil application treatment in the absence of a foliar application. It appears that the strengthening of the leaf cuticula and cell walls by Si impregnation in response to the foliar application can enhance uptake and translocation of Si from soils, possibly through enhanced transpiration transport of Si upon improved drought stress and related opening of the stomata (Keller 2015).

Roots grown in Si-amended soil contained significantly higher levels of silicon compared to the control for soil application (Figure 4). In 2017, the Si concentration in the roots of the Si-amended treatments increased by ~25% from ~2 000 (control) to slightly > 2 500 mg/kg. In 2018 an increase by ~50% from ~3 000 to slightly > 4 500 mg/kg was observed. As the increase in the control from 2017 to 2018 cannot be related to a concomitant increase of PASi during this period (Figure 2), we assume that the general growth conditions (weather conditions, pathogen pressure) could explain this difference. The increased Si concentrations in roots can be clearly related to the associated increase in PASi in the subsoil (Figure 1B). No root samples were taken in 2016. The results of this trial show that grapevines can take up the applied silicon through leaves and roots.

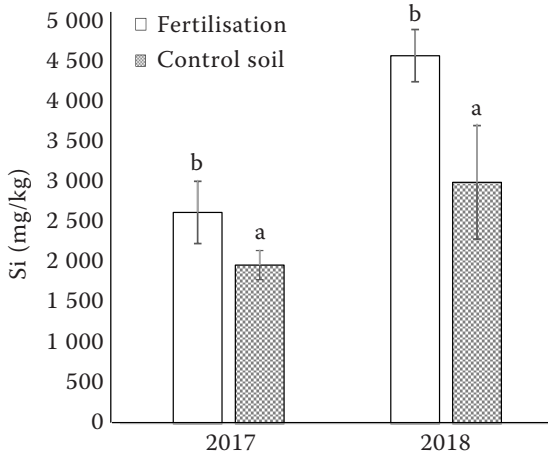

Figure 4. Analysis of total silicon (Si) content of roots according to Kraska and Breitenbeck (2010) and Morrison and Wilson (1963). One-way ANOVA post hoc multiple comparison test was used to determine differences between the treatments, different letters above columns indicate significant differences between treatment, $\alpha = 0.05$, at the same time point of analysis, error bars represent SD of the mean, $n = 4$

Disease assessment. In 2016, the disease incidence of *E. necator* was 51.7% in the foliar application control treatment (Figure 4A). The foliar Si treatment and the common plant protection treatment (CPP) tended to show the lowest incidence with 31.5% and 38.1%, respectively. The disease severity of powdery mildew was 20% in the foliar application control treatment (Figure 5B). The foliar Si application and the CPP treatment again tended to display the lowest severity of 10% and 9.5%, respectively. However, the observed differences in both assays were not statistically significant. In 2017 and 2018, no disease assessment could be performed due to a lack of symptoms owing to the mostly dry weather conditions.

Out data indicates that grapevine transports Si to susceptible young leaves when the risk of fungal disease is high. The early season 2016 was warm with frequent mild rainfalls, which benefits *E. necator* by enhancing its spore dispersal. In this situation, the Si concentrations in the young leaves quickly approached high values similar detected in mature leaves, i.e., 12 000 mg/kg. In contrast to the young leaves, the mature leaves had time to accumulate silicon from the beginning of the growing season by receiving several foliar silicon applications. This suggests that the grapevine actively transports Si to susceptible young leaves to protect them.

In 2017, a very hot year with 47 days above 30 °C and 7 days even exceeding 35 °C, the infection risk was very low, and no infections could be detected over the whole season (2016: 37 days above 30 °C, 1 day above 35 °C; 2018: 39 days above 30 °C, 10 days above 35 °C). Temperatures outside the optimal range of 10–31 °C limit the development of *E. necator*, and temperatures higher than 35 °C kill it outright (Keller 2015). Accordingly, the Si concentrations in the young leaves remained below 1 500 mg/kg due to benign conditions. Epstein (2009) refers to such situations: "Silicon plays an astonishingly large number of diverse roles in plants and does so primarily when the plants are under stressful conditions, whereas under benign conditions its role is often minimal or even nonexistent." The year 2018 started with optimal warm and wet conditions for powdery mildew infections. However, in June, high temperatures above 30 °C up to

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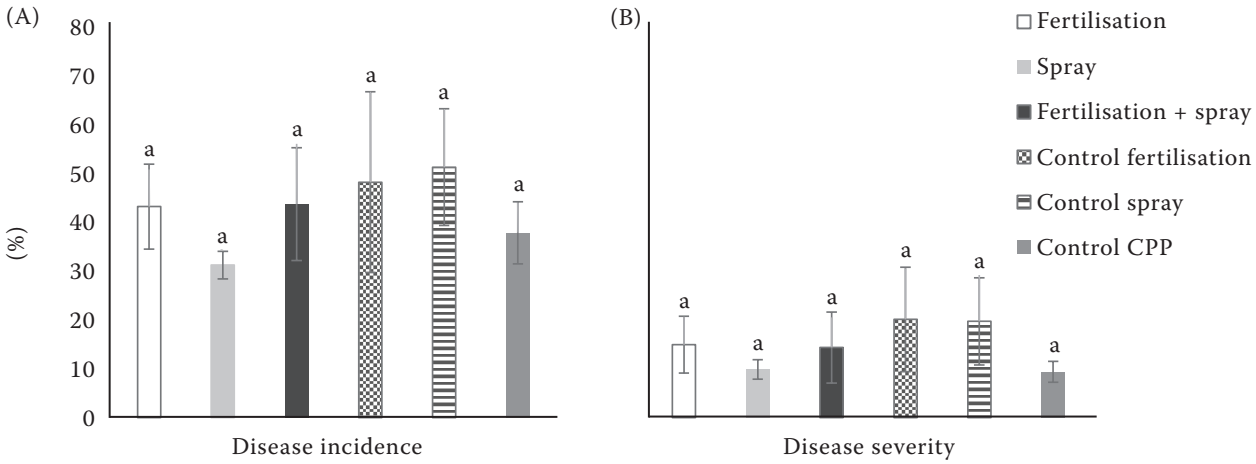

Figure 5. (A) Disease incidence and (B) disease severity of powdery mildew on August 11 on clusters, 2016, according to EPPO PP 1/4(4) *Erysiphe necator*. One-way ANOVA post hoc multiple comparison test was used to determine differences between the treatments, different letters above columns indicate significant differences between treatment, α = 0.05, at the same time point of analysis, error bars represent standard deviation of the mean, *n* = 4; CCP – common plant protection

even 38 °C stopped all ongoing infections. No symptoms of mildew could be assessed on the plants at the end of the season. Therefore, we assume that penetrating fungi at the beginning of the season induced Si accumulation in the young leaves as a protective measure (Figure 3). These findings support the results from Blaich and Grundhöfer (1998), showing that Si can play a crucial role in the grapevine to defend itself from a powdery mildew infection.

Yield and quality parameters of grapes, must and wine. In 2016, the health status of the grapes was generally poor due to mildew infections. No significant differences between the number of clusters per vine could be detected throughout all years. The weight per cluster and total yield were significantly higher in the combined treatment of soil and foliar amendments in 2016 and 2017 compared to the common plant protection treatment (Table 5). In 2018, no such effect could be seen. The analytical parameters of the berries and the must (density, °Brix, titratable acidity, pH value, malic acid, tartaric acid, and yeast available nitrogen) did not show differences between the treatments nor did the fermentation curves deviate between the treatments (data not shown).

The yield data show that the weight per cluster and the overall yield increased in the combined soil and foliar treatment in 2016 and 2017. The observed yield increment may be related to the beneficial effects of Si, including growth promotion and alleviation of biotic and abiotic stresses.

Table 5. Health status, grape clusters per specimen, and weights per cluster at harvest, *n* = 4

Grapes	Harvest date	Health rating	Clusters per vine	Weight per cluster (g)
2016				
Combined soil and foliar application of Si	September 20	3	22.4 ± 6.4	293.1 ± 66.5 *
Control CCP	September 20	2	21.7 ± 5.3	178.4 ± 46.5 –
2017				
Combined soil and foliar application of Si	September 12	1	15.3 ± 5.5	307.1 ± 57.1 *
Control CCP	September 12	1	12.8 ± 5.1	213.8 ± 33.4 –
2018				
Combined soil and foliar application of Si	September 4	1	30.0 ± 8.6	197.9 ± 80.1
Control CCP	September 4	1	21.3 ± 5.5	263.4 ± 88.5
Control	September 4	1	31.4 ± 7.4	179.9 ± 53.6

Health rating: 1 – excellent; 2 – good; 3 – satisfactory; 4 – poor; 5 – total yield loss; CCP – common plant protection

https://doi.org/10.17221/40/2020-PSE

Table 6. Results of sensory triangular test, percentage indicates right answers which could detect differences between compared wines, a higher number of points stands for higher quality

Wine Rating	Triangular test 1st tasting	Ranking	Triangular test 2nd tasting	Ranking
2016				
Combined soil and foliar application of Si	Right choice 90.9%		Right choice 46.2%	
Control CCP	14.7 points	1	15.6 points	1
	13.2 points	2	14.5 points	2
2017				
Combined soil and foliar application of Si	Right choice 63.6%		Right choice 33.3%	
Control CCP	15.2 points	1	15.0 points	1
	14.4 points	2	14.6 points	2
	Ranking method			
	1st tasting		2nd tasting	
2018				
Combined soil and foliar application of Si	29 (4 × 1, 5 × 2, 2 × 3, 3 × 4)	1	20 (3 × 1, 4 × 2, 3 × 3)	1
Control CCP	47 (2 × 1, 1 × 2, 1 × 3, 10 × 4)	3	32 (2 × 1, 2 × 3, 6 × 4)	4
Control	32 (3 × 1, 4 × 2, 7 × 3)	2	27 (3 × 1, 1 × 2, 2 × 3, 4 × 4)	3
Control + Si	32 (5 × 1, 4 × 2, 4 × 3, 1 × 4)	2	21 (2 × 1, 5 × 2, 3 × 3)	2

CCP – common plant protection

Sensory evaluation. In the four tastings in 2016 and 2017, the panelists rated the wine from the silicon treatment higher (Table 6).

At the first tasting of the wine from 2018, the best wine was the control treatment with silicon added before fermentation, followed in a tie by the combined soil and foliar application of Si and the control treatment. The common plant protection treatment was ranked in third place. The results show that there is a positive effect from silicon amendments to the quality of the wine. However, the very similar analytical parameters of the wines after microvinification such as density, alcohol, titratable acidity, malic acid, tartaric acid, volatile acidity, glucose, and pH value cannot explain the consistent higher rating of the wine from the silicon treatment in the sensory evaluation. We suppose that silicon amendments in the vineyard results in lower environmental stress for the plants, which in turn increases the availability of one or several resources for better sustenance of the clusters (Keller 2015). This fosters the nutrition of the yeast, which needs sources of carbon, nitrogen, phosphorus, and trace elements (Spencer et al. 1997). Better nutritional conditions during fermenting processes can improve the sensory profile of wines. Furthermore, the results from 2018 suggest that silicon also has a direct effect on the fermentation. Further research must be done to clear if a better clarification of the must is responsible for this improved quality of the wine or if there is an interaction between the winemaking yeast and silicon.

Together with the results from previous greenhouse studies, our field experiment provides evidence for the potential of replacing at least part of the common fungicides by foliar Si treatments. Silicon applications are crucial from a farmer's perspective due to its improvement on the yield and sensory properties of the wine.

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Received: January 21, 2020
Accepted: June 29, 2020
Published online: August 18, 2020