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Is there any influence of biodynamic preparation 501 on the physiological activity of grape leaves cv. Cesanese d'Affile?

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Abstract

Background Biodynamic agriculture is a management approach that aims to reduce the reliance on agrochemicals for production by emphasizing the use of specific natural preparations. A 2-year field trial spanning 2019–2020 was conducted in an established vineyard (*Vitis vinifera* L., cv. Cesanese d'Affile) to elucidate the impact of the cow horn silica biodynamic preparation (BD-501) on leaf vine physiology, potential resistance via chitinase activity, and analysis of secondary metabolites. The vineyard under biodynamic management was divided into two plots: one treated with BD-501 (BD-501) and the other untreated (BD). Throughout the vine growth season, measurements of carotenoid and chlorophyll levels, polyphenols, and chitinase activity were taken around key phenological phases (BBCH scale). During the ripening phase, a fluorometer was employed to assess chlorophyll fluorescence in the leaves.

Results Leaves treated with BD-501 exhibited elevated concentrations of polyphenols and increased chitinase activity during the later phenological phases. In contrast, the untreated BD samples demonstrated high values primarily in the central phase of the observation period but not consistently throughout. At the time of harvest, chlorophyll concentration and quantum yield exhibited no statistically significant differences. BD-501 triggered a distinct response in terms of potential defense mechanisms (elevated polyphenols and chitinase activity) during the veraison phase. However, conversely, lower levels of chlorophylls and carotenoids were observed.

Conclusions Nevertheless, a further round of experimental work is required to thoroughly comprehend the regulatory mechanisms behind this adaptive response and to ascertain the efficacy of BD-501.

Keywords Biodynamic viticulture, Horn silica, Chitinase, Chlorophylls, Enzymatic activity

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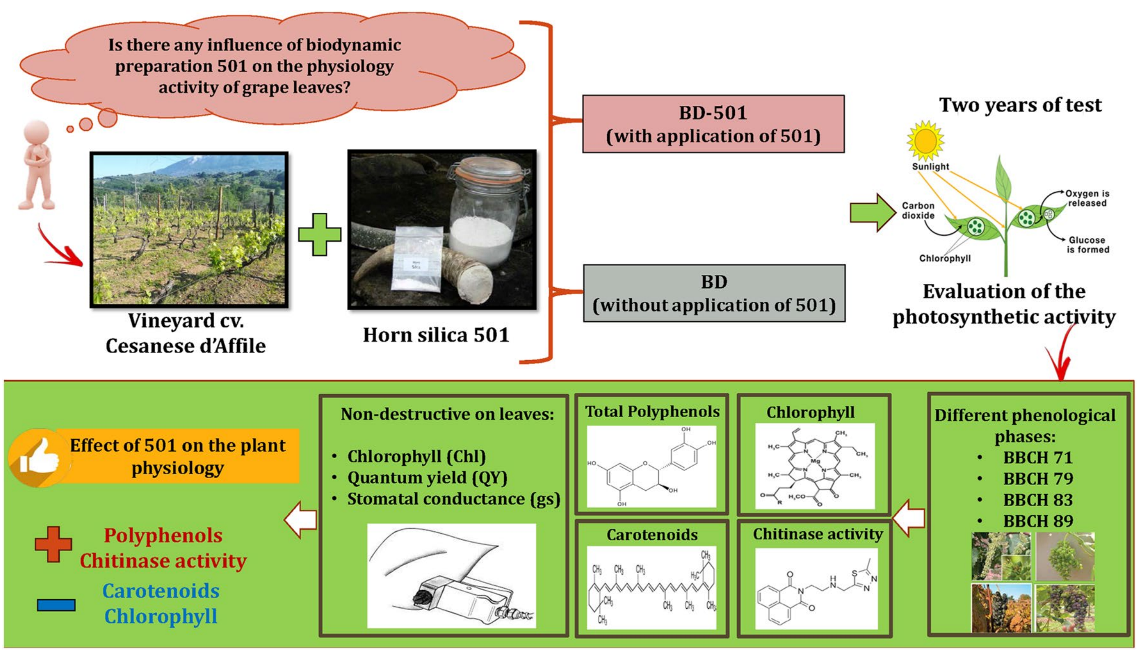
Graphical Abstract



Background

Alternative agronomic practices, such as organic and biodynamic agriculture, have been supported by growing consumer awareness of environmental degradation in agriculture and the significance of food quality in connection to human health [1, 2]. Generally speaking, biodynamic agriculture differs from traditional organic management, in the use of specific fermented preparations proposed by Rudolf Steiner (1861–1925) which are claimed to stimulate the soil nutrient cycle and enforce the efficiency of leaf activity and optimal evolution of compost enhancing both soil and crop quality [2, 3]. These treatments, which are applied to crops, compost, and soil, have been shown to enhance the quality of crops and animals while promoting an effective nutrient cycle. Sustainable agro-ecosystems with biodynamic management are thought to have positive environmental benefits in terms of their energy efficiency [3–5].

As regards wine quality from biodynamic management of vine and vinification, some papers have been published especially from Italian group comparing the quality of conventional and biodynamic produced wines [6–9]. In vineyard, some studies were conducted to better understand the effects of the preparations/method on vine physiology and on yield [5, 10, 11]. Regarding vine physiology response, it was found a better enzymatic activity of chitinase and β-1,3-glucanase in the biodynamic method [10], while regarding the yield, similar values were found when compared with organic management [4, 5, 12]. Other studies [12–14] showed that berries from vine biodynamic cultivation had higher level of soluble solids, total phenols and anthocyanins in red grape, and in white grape no difference on soluble solids, pH and total acidity was detected.

Another study [11] approach was made through the application of the high resolution nuclear magnetic resonance spectroscopy (HR-NMR) to reveal differences between the metabolomes of organic and biodynamic red wines, with a multivariate analysis. It confirmed the presence of specific differences that are statistically meaningful.

Biodynamic agriculture is managed according to Demeter standards, to produce products labeled Demeter or Biodynamic®. To obtain authorization to use the trademark, Regulation 834/0715 must be respected, replaced by Reg. 889/0816, relating to organic production, the Demeter standards of production, processing and distribution and labeling [15].

Silica (Si) is the second most present element on the planet, following oxygen, and makes up 26–27% of the earth's crust [16]. In the soil, it normally occurs in the form of silicic acid (H_4SiO_4), and similarly to potassium, calcium and other elements, is considered essential in plant nutrition [17, 18]. In plants it is absorbed and reaches concentrations in a range that varies between 1 and 10% of the dry matter, except for Equisetaceae' plants which highest values are reached in [19]. Si may be incorporated into the cell wall by cross-linking with other wall constituents such as hemicelluloses, pectins, and phenols in the endo- and exo-dermal tissues of roots [20, 21]. Si is transported from the soil to the plant as silicic acid which is often limiting in the soil [22], two Si transporters, Lsi1 and Lsi2, are responsible and are located in the absorbing root [23–25]. Lis6 transporter, a homolog of Lsi1, is needed to unload silicic acid from the xylem throughout all the plant cells [26, 27].

Many reports presented Si as a prosperous beneficial plant nutrient for stimulating the growth and yield of different crops [28–30]. Therefore, Si has important vital roles in alleviating the adverse negative impacts of drought stress [17, 31, 32]. Silica is widely accepted that it has two key processes in contribution to stress resistance: (i) physical and mechanical protection from Si deposited in the cell wall, which reduces leaves stomatal conductance and transpiration; (ii) a biochemical reaction causing metabolic changes [33].

Another function of Si is correlated with its activity of inhibiting insect damage [28, 34, 35]. Therefore, Si is one possible remedy to reduce the intensive use of pesticides, since it is a natural environment-friendly alternatives, that pose no threat to the human health and environment [18, 36].

In plants, Si after the absorption and accumulation is subsequently transported to the outer part of the cell wall of the epidermis or in the trichomes of the leaves. Moreover, it is documented that Si promotes cells division and cells enlargement, controls the physiological processes and metabolic activities such as membrane permeability, water and nutrients uptake, photosynthesis, transpiration and phytohormones biosynthesis [31, 37, 38].

The underlying research postulated that the utilization of Preparation 501, derived from horn silica, might impact the physiological activity of vine leaves while potentially inducing a resistance mechanism. This investigation spanned 2 years and was conducted within a vineyard that had been under long-term biodynamic management.

Methods

Vineyard management and climatic conditions

The experimental site was an old vineyard located in Piglio (Frosinone, Lazio), Italy (41° 49′ 45″ 84 N, 13° 7′ 23″ 88 E, 430 m a.s.l) planted in 1965 with cv. Cesanese d'Affile (*Vitis vinifera* L.), grafted onto 1103 Paulsen, trained to guyot. Tested vineyard was on a slope, with south/north and downhill oriented rows, vine were spaces at 2.2 m × 1.2 m, with a density of 3787 plants/ha. Agriculture in the Piglio area is characterized by a hilly mountainous territory with the presence of very rich soil. Cesanese d'Affile is the only red variety authorized in Lazio region suitable to produce PDO wines. Starting in 2012, this vineyard was managed and certified as organic in accordance with EC Regulation 834/2007. In 2019 and 2020 seasons, the number of buds (10–12) for vine was adjusted by winter pruning. The herbaceous species fava bean (*Vicia faba*), subterranean clover (*Trifolium subterraneous*), pea (*Pisum sativum*), and annual ryegrass (*Lolium multiflorum*) were sown in the rows at the conclusion of each vegetative season in both treatments. No irrigation was applied in both seasons. Using organic products permitted by EC Regulation 1112/2002, the entire plot was treated to manage diseases and pests in the same way. Treatment consisted mainly of copper (an average of 5 kg/ha year) and sulphur (an average of 7 kg/ha year), enabling control of fungal pathogens.

Temperatures during the vegetative season of 2019 were higher than the seasonal norm, with August seeing the highest temperature peaks of 35 °C. The average relative humidity (RH) ranged from 40 to 70% from bud bursting to harvest; the highest values were noted in May (85%), which was unexpected, and the lowest (33%) in August, which was expected. The total rainfall (250 mm) from bud burst to harvest was concentrated mainly in May and at ripening time, September. In 2020, there was an increase of the temperatures over the average in March (average T: 23 °C) with a subsequent lowering in April where freezing and cold occurred in the lowland areas, but it did not affect the vineyards of the experimentation. July and August were very hot (many days at 35 °C) with no rain. The RH from bud burst to harvest varied from 30 to 82% with higher peaks in July and in September. The total rainfall (330 mm), registered from bud burst to harvest, mainly occurred in spring.

Experimental design

In 2019, the surface of 0.4 ha was divided in two large uniform blocks with similar soil characteristics [The 0–0.4 m soil layer was silty clay loam (Aquic Haplustepts, fine, mixed, mesic), sub-alkaline (pH 8.0), and contained 14.0 g/kg of total organic carbon, 1.1 g/kg of total nitrogen, and 7.4 mg/kg of available phosphorus]. Each block was used to test a specific manure cultivation method: (i) BD-501 biodynamic farming, based on biodynamic management with the application of the 501 born silica preparation according to Demeter standards (biodynamic certification organism; (ii) BD based on the same management but without the application of the BD-501. Each treatment was provided on 4 plots (2 plot per type of treatment), containing 30 monitored vines each, for a total of 120 vines. Between the two blocks two rows of vines were left not considered in the experimentation.

The biodynamic preparations consisting of cattle manure-horn (500) and horn silica powder (501) were produced by Azienda agricola biodinamica Carlo Noro (Labico, Italy). The 500 and 501 preparations were prepared as indicated by Demeter protocol (Biodynamic Federation Demeter International E.V. Brandschneise 1D-64295 Darmstadt, DE). The latter preparation, which was the focus of the experimental work, was sprayed on vines with a backpack atomizer, with low water usage nozzle, during early morning, without any breeze or wind. In 2019, 2020 three applications of preparation 500 have been applied during spring period in the entire vineyard. Three applications of preparation 501 in both blocks have been supplied one during spring and two during summer, only in block named BD-501. The blocks without the treatment of 501, named BD were used as control to assess the effect of the preparate. The application times were chosen according to environmental conditions and plant phenological condition. 6 leaves per plant were sampled on the top, middle and bottom of the canopy of the grapevine. In total 60 plant per treatment (BD-501 or BD), for a total of 360 leaves during each phenological phase were used. Sampling was carried out in 3 different phenological phases: at the beginning of berry growth (BBCH 71), at cluster closure (BBCH 79) and when the berries were partially red (BBCH 83).

Chemical and enzymatic analyses

Leaves sampling was carried out in the three different phenological phases according to experimental design and application of BD-501. Leaves were immediately frozen with liquid nitrogen after treatment. In Lab, the samples were ground with A11 Basic IKA laboratory mortar.

The analysis of carotenoids (Car) and chlorophylls (Chl-a and Chl-b) content was carried out according to Porra et al. [39] and Yang et al. [40]. The concentrations were expressed as μg/g of fresh weight (fw) of sample. The assay for the polyphenol determination was carried out using the Folin–Ciocalteu colorimetric method as previously described by Bianchi et al. [41], expressing the results as mg of gallic acid equivalents (GAE) per g of fresh weight (fw) of sample.

The extraction of the chitinase (β-*N*-acetyl-hexosaminidase EC 3.2.1.52) was determined according to Botelho et al. [10] and expressing the results as the values in μM/min referring to the enzymatic activity.

Physiological and photochemical analyses

Physiological and photochemical traits of cv. Cesanese d'Affile have been assessed by chlorophyll (Chl), the quantum yield (QY) and stomatal conductance (gs).

The Chl content was used to assess leaf functionality [42], and was estimated using a hand-held MC-100 Chlorophyll Concentration Meter (Apogee Instruments, Inc., Logan, UT, USA), that performs a non-destructive measurement on the leaf without damaging or disrupting it. The meter includes equation with grapevine specific coefficient and display output unit as μmol of Chl per m^2 of leaf surface.

The quantum yield of photosystem II (QY) was recorded by the portable fluorescence meter, FluorPen FP 110 (Photon systems instruments, Czech Republic), which also allowed for the measurement of photosynthetically active radiation (400–700 nm—PAR–PPFD). The values are expressed as mol/mol quanta absorbed and provide us the photochemical efficiency of leaf [42].

Stomatal conductance (gs) was determined using a leaf porometer (SC-1, Decagon Devices, Pullman, WA, USA). Measurement was taken from 12:00 to 14:00 h on basal mature fully exposed leaves. Measurements were done on ten random vines per treatment. From this tested vine four basal mature and full exposed leaves were selected and three measurements for leaf were done, among them two closes to the base of petiole sinus and one on the main tip lobe (tip). The values are expressed as μmol of H_2O/m^2 s. Parameter monitoring was done at ripening of berries (BBCH 89).

Statistical analysis

Statistical analysis was performed by JMP Pro 17.0 software package (SAS Institute, Cary, NC, USA). All the evaluations were performed in quadruplicate, and data are reported as mean values ± standard deviation (SD). One-way ANOVA (CoStat, Cohort 6.0) was performed on data, followed by the Tukey's HSD test at $p \leq 0.05$ of significance.

Results and discussion

Chlorophylls and carotenoids

The values of Chl content expressed in μg/g fw in both years, decreased linearly along the phenological phases, irrespective of the treatment (Table 1).

In some phenological phases, the content significantly differed from each other although no substantial disparities have occurred. In the leaf of BD sample, a high decrease in Chl in BBCH 83 compared to the previous phase, was measured. In the second year the Chl contents were higher than in the previous year (Table 1). Leaf of BD-501 treatment, showed less Chl content during the first two phenological phases but, in the last phenological phase, increased the value, scoring the highest one (Table 1).

By comparing the concentrations of Chl-b between the 2 years, it is possible to observe in Table 1 a decreasing

Table 1 Leaf pigments (carotenoids and chlorophyll) detected during three different phenological phases of berry development, during both years of the study

Treatment	Phenological phases	Car (μg/g fw)	Chl-a (μg/g fw)	Chl-b (μg/g fw)	Total Chl (μg/g fw)	Ratio (% a/b)
2019						
BD-501	BBCH 71	11.91^{bc}	497.90^{b}	211.75^{b}	709.65^{b}	2.35^{bc}
	BBCH 79	15.32^{a}	459.17^{bc}	187.16^{b}	646.32^{b}	2.45^{b}
	BBCH 83	12.87^{b}	403.85^{c}	149.65^{c}	553.50^{c}	2.69^{ab}
BD	BBCH 71	13.05^{b}	617.43^{a}	261.19^{a}	878.62^{a}	2.36^{bc}
	BBCH 79	13.45^{ab}	603.77^{a}	257.71^{a}	861.62^{a}	2.34^{c}
	BBCH 83	10.21^{c}	338.02^{d}	121.59^{d}	459.61^{d}	2.77^{a}
2020						
BD-501	BBCH 71	12.68^{b}	503.43^{b}	227.63^{b}	731.06^{b}	2.21^{ab}
	BBCH 79	15.82^{a}	482.36^{bc}	207.65^{bc}	690.01^{b}	2.32^{a}
	BBCH 83	14.03^{ab}	409.89^{c}	181.56^{c}	591.45^{c}	2.25^{ab}
BD	BBCH 71	13.25^{b}	622.43^{a}	289.13^{a}	911.79^{a}	2.15^{b}
	BBCH 79	13.72^{b}	613.12^{a}	276.65^{a}	890.32^{a}	2.21^{ab}
	BBCH 83	10.18^{c}	331.35^{d}	139.67^{d}	471.03^{d}	2.37^{a}

The values are the mean (± SD) of six leaves from 60 plants subjected to two treatments. Different letters in the same column for the same year indicate significant differences among samples ($p \leq 0.05$)

pattern of the values; in the first phenological phase, the leaves of BD-501 treatment have higher values than the other two phases. Both management systems showed a decreasing trend of the Chl content. However, it can be noted that, in the cluster closure phase, the Chl values of BD are lower than that of BD-501. The leaves of the BD-501 block did not undergo the strong decrease which the leaves of BD are subjected to, in the BBCH 83 phase. This highlights the ability of the 501 application to retain a higher content of pigments over time. In fact, Si foliar applications increase the Chl content, preserving it from non-enzymatic and enzymatic antioxidants either under well water or water deficit conditions in compared to the respective controls (sprayed with distilled water) [43]. In a work made by Malagoli et al. [44] regarding the application of BD-501, the results of the content of Chl-a and Chl-b was higher in plant treated with 501 manure than in plant non-treated.

The sum of both pigments of chlorophyll indicates that there is an induction for greater synthesis of pigments while the ratio indicates if, in some way, the situation of water stress stimulates a greater synthesis of pigments. In fact, in conditions of water and temperature stress, i.e., stress that affects the osmotic potential of the leaf, the pigments undergo a decrease in their value, but the Chl-b decreases more than Chl-a, and the a/b Chl ratio increases. With the increase in solar radiation, one of the ways used by the plant to resist climate change is to induce a lower synthesis of chlorophyll.

It has been demonstrated that adding Si to other plants causes them to accumulate more Chl and other pigments, both in Si accumulators like rice [23, 45] and wheat [46], and in Si non-accumulators like tomato [47, 48]. One of the most significant effects of adding Si to salt-stressed plants in all of those test conditions was the rise in chlorophyll content. However, the rise in Chl brought on by Si fertilization varies by species and cultivar and is not always appreciable [49–51]. Si may have a more direct impact on photosynthesis through improved light distribution across the photosystems and a resulting increase in quantum efficiency [47]; nevertheless, no molecular explanation of this phenomenon has been proposed.

The primary silicon transporters, namely Lsi1 and Lsi6 from the aquaporin family, play a crucial role in the allocation of silicon within root and shoot tissues [26]. In contrast, Lsi2 functions as a putative anion transporter, primarily expressed in the endodermis of roots [23, 24].

Concentrations of chlorophyll (Chl) undergo a reduction, and according to existing literature, the degradation of chlorophyll constitutes a pivotal aspect of nitrogen recycling. This process holds significance in preventing cellular damage. When the degradation of chlorophyll is not adequately regulated, it leads to severe photodamage and triggers cell death [52–54].

By comparing the concentrations of total chlorophyll and carotenoids over time, BD-501 sample shows a lower loss of concentrations as the phenological phase progress. In fact, the BD-501 system remains partially constant until the BBCH 71 stage and after dropping down of about 20% to BBCH 83 phase, while the BD presents a loss of more than 40%. Analyzing the ratio of the sum between the two pigments there are no significant differences between the content of Chl inside the leaves, but only a decrease in the final phase close to veraison (BBCH 83) in which the BD system has the lowest values can be noted (Table 1). Therefore, the BD-501 system maintains the highest values of chlorophylls over time, resulting more efficient in maintaining photosynthetic pigments content.

Carotenoids allow the absorption of photons from wavelengths other than chlorophyll pigments and protect them from photo-oxidation [55]. Moreover, they play a relevant role in the synthesis of the stress-related plant hormones since they are being precursors of phytohormones such as ABA and strigolactones [56, 57]. Analyzing the first year, in BBCH 71 the concentrations show similar values and in the following phase they undergo an increase in both treatments (Table 1). The leaf of BD-501 treated undergo a stronger increase in comparison to the BD-treated leaf in BBCH 79 and a subsequent decrease in BBCH 83 occurs (Table 1). During the second year an analogous behavior is observed. In BBCH 71, leaf treated with BD-501 has the lowest value in comparison with leaf treated with BD (Table 1). In BBCH 79 the carotenoids in leaves of both treatments are subjected to an increase, but in the following phase they present lower statistically values, since there is no univocal connection between the data but only this increase during cluster closure. Leaf treated with BD-501 presents the higher value in the last phase in both years.

Polyphenols

Polyphenol content of leaves showed increasing values starting from the BBCH 71 phase (Table 2) in both years, and an analogous behavior is measured. There is no substantial difference between the 2 years analyzed, witnessing that the synthesis pathway of these molecules was not subject to variation between the 2 years of analysis.

At the first analyzed phase, a high value of these compounds is detectable in the leaf treated with preparation BD-501 compared to the BD. The last phase (BBCH 83) is characterized by an increase in the polyphenols amount in the leaf treated with BD-501, which has the highest value (Table 2).

The biodynamic system 501 seems to favor and stimulate the production of polyphenols, in fact according to the literature biochemical/molecular mechanisms are induced or increased in their activity by Si allowing the plant to improve resistance to biotic stress and include defensive compounds such as phenolics, phytoalexins and momilactones [58]. In fact, silica nanoparticles were shown to protect wheat seedlings against UV-B stress by stimulating the antioxidant defense system [59].

Fawe et al. [60] detected and identified flavonoids and phenolic acids that were specifically and strongly induced in a pattern typical of phytoalexins, following the Si treatment. Thus, Si was hypothesized to play an active role in disease resistance by stimulating defense mechanisms [61].

It is known that higher plant tolerance to abiotic stresses is related to increased synthesis of polyphenols, such as phenolic acids and flavonoids [62].

Malagoli et al. [44] affirm that the application of preparation 501 may trigger the biosynthesis of antioxidants beneficial to enhance stress tolerance in grapevine plants, although the different variations in the leaf metabolite levels recorded in the plants may be attributable to the site effect of the vineyards.

Table 2 Polyphenols and enzymatic activity (chitinase) measured during three different phenological phases of berry development, during both years of the study

Treatment	Phenological phases	Enzymatic activity (μM/min)	Polyphenols (mg GAE/g fw)
2019			
BD-501	BBCH 71	0.60^{c}	6.92^{b}
	BBCH 79	0.57^{c}	4.65^{c}
	BBCH 83	1.20^{a}	12.36^{a}
BD	BBCH 71	0.65^{bc}	2.85^{d}
	BBCH 79	0.76^{bc}	6.10^{bc}
	BBCH 83	0.90^{b}	11.50^{a}
2020			
BD-501	BBCH 71	0.83^{c}	5.45^{b}
	BBCH 79	0.97^{bc}	6.82^{b}
	BBCH 83	1.25^{a}	12.01^{a}
BD	BBCH 71	0.85^{c}	3.16^{c}
	BBCH 79	0.99^{bc}	6.45^{b}
	BBCH 83	1.10^{ab}	10.71^{a}

The values are the mean (± SD) of six leaves from 60 plants subjected to two treatments. Different letters in the same column for the same year indicate significant differences among samples ($p \leq 0.05$)

Enzymatic activity

The chitinase activity overall increased during the phenological phases and followed an increasing linear trend (Table 2). In the first year during BBCH 71 average values are very similar between the two samples; in BBCH 83 phase there was an increase in the leaves of both treatments and those treated with preparation BD-501 had the highest value. In the second year BD and BD-501 treated-leaves showed higher activity in comparison with the previous year and there is a linear increasing trend from the beginning. Leaf of BD-501 treatment presented the highest value during the ripening stage, BBCH 83.

Chitinase is a hydrolytic enzyme capable of breaking the glycosidic bonds present in chitin, a homopolysaccharide present in many living beings. The extractable chitinases

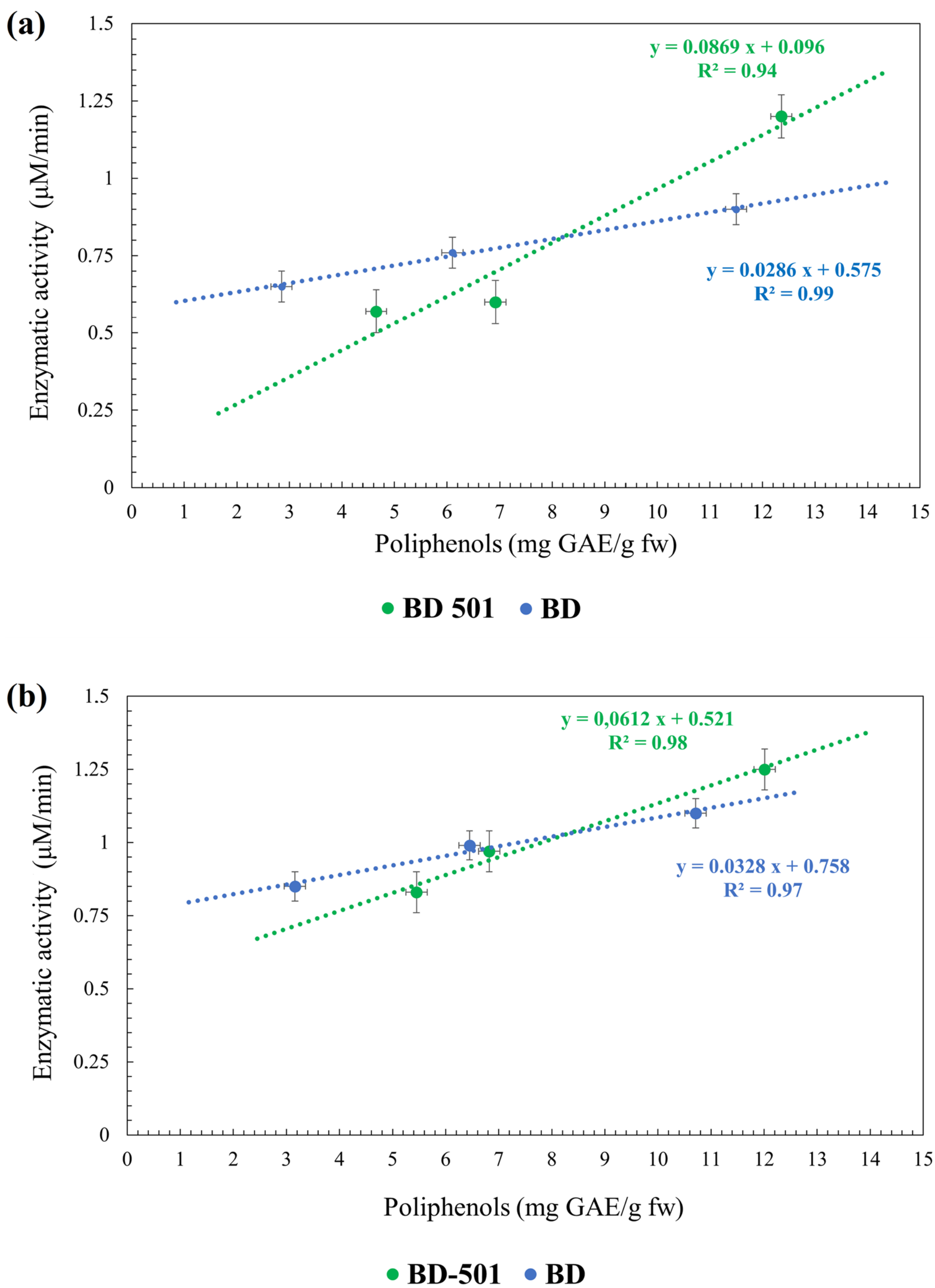


Fig. 1 Regression analysis between polyphenols and enzymatic activity (chitinase) carried on leaves sampled with *R*.^{2} and slope values: year 2019 (**a**); year 2020 (**b**)

from *V. vinifera* can be classified into six classes in relation to structure and homology between the various isoforms [63–65]. Chitinase is involved in the defensive processes of the plant from pathogenic fungi and hydrolyzes the main fibrillar polysaccharide of the fungal wall. The breakdown products of chitin are released and converted into chitosan, which is a molecule that plays a key role in resistance mechanisms. In fact, it is an elicitor, promoter of the activity of lipoxygenase (LOX), an enzyme that plays an important role in hypersensitive responses, of ammonium

Table 3 Values of non-destructive analyses [chlorophyll (Chl), quantum yield (QY) and stomatal conductance (gs)] detected directly on leaves in the field during the phenological phase of berry development immediately before the harvesting (BBCH 89)

Treatment	Phenological phases	Chl (μmol/m^2)	QY (mol/mol quanta)	gs (μmol H_20/m^2 s)
2019				
BD-501	BBCH 89	275.51^a	0.36^a	489.58^a
BD	BBCH 89	237.39^b	0.40^a	499.21^a
2020				
BD-501	BBCH 89	291.22^a	0.37^a	459.01^a
BD	BBCH 89	263.82^b	0.38^a	417.61^a

The values are the mean (± SD) of six leaves from 60 plants subjected to two treatments. Different letters in the same column for the same year indicate significant differences among samples ($p \leq 0.05$)

phenylalanine (PAL), an enzyme that intervenes in the metabolic pathways of synthesis of polyphenol and among them precursors of phytoalexins and salicylic acid, and of the chitinase itself [66].

The plants of the vineyard treated with the preparation BD-501 show a strong increase in the enzymatic activity in the final stage (BBCH 83) in both years (Table 2). This effect and its association with the biodynamic treatment would support the thesis, according to which the preparation BD-501 is able to stimulate elements of the plant defense mechanisms [3]. The treatment might in fact have favored the plants on which it was distributed, stimulating a greater capacity to absorb light and therefore an advancement in ripening compared to control. We discovered in the literature that Si lowers oxidative damage by an average of 30% under both salinity and drought stress. This is probably due to an increase in antioxidant enzyme activity, which was elevated by an average of 50% under salt stress and 20% more frequently under drought stress [67].

Ma et al. [25] demonstrated that the Si treatment was linked to increased expression of oxidative stress genes. Mechanistic models, however, are still fully unknown to us; post transcriptional or transcriptional pathways may be used to boost enzyme activity. Both pathways would need cytosolic molecular interactions [31].

Consequently, several studies have linked Si with elevated shoot and root activities of antioxidants, both enzymatic (e.g., superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase) and non-enzymatic (e.g., ascorbate, glutathione, phenolic compounds, etc.), as well as changes in the concentrations of common markers of oxidative stress, including malondialdehyde, hydrogen peroxide (H_2O_2) and proline, under various abiotic stresses [68, 69].

From this it can be deduced that the BD-501 sample presents an increase in chitinase activity over time, in the different phenological phases, which could favor resistance to fungal diseases, especially in the final phases of grape ripening. An important correlation between enzymatic activity and polyphenols content is present along the two experimental years (Fig. 1a, b).

Physiological and photochemical determinations

The photosynthetic efficiency (QY) and stomatal conductance (gs) at berry ripening time (BBCH89) did not show significant differences, scoring analogous average values in both years (Table 3). Similarly, the concertation of foliar chlorophyll for the two managements under investigation, did not show significant differences between them during the 2 years of study.

Conclusions

The results obtained from this research work have shown that the application of preparation BD-501 seems to have effects on the plant physiology during vegetative growth phases in both years of study. Among the analyzed foliar components, we observed that the quantity of polyphenols linearly increased, reaching the higher concentration at the early veraison phase, simultaneously with the decrease of chlorophylls quantity. The chitinase activity increased as well and the values detected in leaves treated with BD-501 manure might be attributed to the promotive induction of silicon.

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Author contributions
PS: data curation; formal analysis; investigation; methodology; visualization; writing—review and editing. LB: formal analysis; investigation. BC: formal analysis; investigation. RM: data curation; roles/writing—original draft; writing—review and editing. AB: data curation; formal analysis; investigation; methodology; visualization; roles/writing—original draft; writing—review and editing. EB: formal analysis; investigation; methodology; writing—review and editing. FM: conceptualization; resources; supervision; validation; visualization; roles/writing—original draft; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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