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ORIGINAL RESEARCH ARTICLES

Rugulopteryx okamurae extract provides protection against *Plasmopara viticola*

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ABSTRACT

There is currently a need to assess new, sustainable strategies for downy mildew control on grapevines in accordance with the Goals of the 2030 Agenda for Sustainable Development. Seaweed has proven to be one of the marine resources with the highest potential as plant protective agent, representing an environmentally friendly solution for sustainable wine production. Recently, the biostimulant capacity of *Rugulopteryx okamurae*, an invasive seaweed, has been demonstrated. The current work evaluates the ability of a *R. okamurae* extract (RO) to prevent *Plasmopara viticola* damage on two clones of Tempranillo plants.

A greenhouse experiment demonstrated that RO can elicit defense mechanisms and prime grapevines. The response to the seaweed treatment (applied three times at 48-hour intervals) was pronounced at the molecular level, including changes in: i) gene expression (three from PR proteins group and four other genes involved in the flavonoid pathway, lipoxygenase pathway, redox status regulation and cell wall reinforcement), ii) antioxidant enzyme activities (in particular those of superoxide dismutase and gluthatione reductase), and iii) polyphenols, such as total piceid and *E*-resveratrol. Meanwhile, photosynthetic pigments and photochemical parameters were less affected. The two Tempranillo clones (VN40 and RJ43) exhibited differences in their responses, particularly in their antioxidant enzymes induction, but RJ43 showed stronger elicitation in general.

Importantly, plants previously treated with RO, especially RJ43 plants, exhibited a more efficient response to *P. viticola* infection, with grapevine resistance to the disease being enhanced. Twenty-four hours after pathogen inoculation, RO plants showed a higher induction of antioxidant enzymes (particularly glutathione reductase and ascorbate peroxidase, key to protecting cells from damage), as well as higher viniferins concentrations and an enhancement in the downregulation of various SA-related genes, together with acute upregulation of CHI2 and GST1 expression compared to the biostimulation timepoint. Overall, the results indicate that RO is able to elicit a more efficient response from plants to abiotic stress; the visual inspection of incidence and severity of the disease corroborated the findings that RO treatment significantly reduced both disease incidence and severity in RJ43 by 20 % and 25 %, respectively.

INTRODUCTION

Viticulture is a sector with great socio economic importance worldwide, the largest cultivated area being in Europe and the top wine producing countries being Italy, France and Spain (OIV, 2023, https://www.oiv.int/what-we-do/global-report?oiv). However, grapevine development and production are increasingly subject to a biotic (drought, salt, mineral nutrition disturbances, light and temperature) and biotic (wounding, pathogens and herbivores) stress, all of which contribute to the overuse of chemical fertilisers and synthetic pesticides (Samuels *et al.,* 2022). Therefore, there is a need and an increasing demand for more ecologically-sustainable agricultural products, as outlined by the United Nations Sustainable Development Goals (SDGs). Within this framework, the "From Farm to Fork" strategy aims to reduce chemical usage and promote sustainability, one of its objectives being to achieve 25 % of agricultural land under organic production by 2030.

Grapevine plants are among the most frequently treated crops due to their high susceptibility to a number of pathogens, particularly fungi and oomycete. *Plasmopara viticola (P. viticola)* (Berk. & M.A. Curtis; Berl. & De Toni) a biotrophic oomycete, the causal agent of grapevine downy mildew, is one of the most devastating diseases in vineyards worldwide. The incidence of downy mildew has increased notably in recent years in Europe (Koledenkova *et al.,* 2022). While most commercially important European grapevine cultivars (*Vitis vinifera*) are highly susceptible to this pathogen, differences in level of sensitivity to it have been documented (Boso *et al.,* 2014). *P. viticola* severely attacks flower clusters, leaves and young berries, and is therefore controlled with frequent applications of chemical fungicides and copper-based formulations to avoid decreases in yield and quality. Depending on the meteorological conditions, up to twelve fungicide applications per growing season may be necessary to control it. The use of copper can cause environmental problems owing to its accumulation in the soil, with negative side effects, such as decreases in yield and soil microbial diversity (Gessler *et al.,* 2011; Lamichhane *et al.,* 2018). Despite its unfavourable ecotoxicological profile, the use of copper is still tolerated for its unique properties as a wide-spectrum fungicide and bactericide. However, restrictions have been applied by the European Commission on the number of pesticide treatments (Directive 2009/128/EC, http://data.europa.eu/eli/dir/2009/128/oj) and on the maximum allowed quantity per year of copper fungicides (Regulation 2002/473/EC, http://data.europa.eu/eli/reg/2002/473/oj). Moreover, copper, which is still considered the most important antifungal product in organic viticulture, has been added to the list of candidates for substitution (European Commission Implementing Regulation 2018/84, http://data.europa.eu/eli/reg_impl/2018/84/oj), and has been further limited to 4 kg per hectare/year spread over 7 years from February 2019 (European Commission Implementing Regulation 2018/1981, http://data.europa.eu/eli/reg_impl/2018/1981/oj).

The application of environmentally friendly products has become increasingly important in the last few years. Biofungicides, biostimulants and resistance inducers are sustainable biocontrol products that are being currently studied to counteract the overuse of chemical fungicides (Jindo *et al.,* 2022). Among biostimulants, the use of seaweed extracts is increasing. The application of algal extracts has been shown to stimulate defense mechanisms and plants growth (Cappelletti *et al.,* 2016; García-Sánchez *et al.,* 2022; Gutiérrez-Gamboa *et al.,* 2019). In viticulture in particular, foliar applications have been proven to improve grape and wine composition (*i.e*., nitrogen, phenolic and volatile contents (Gutiérrez-Gamboa *et al.,* 2019; Jindo *et al.,* 2022; Rantsiou *et al.,* 2020). However, information regarding the effects of using seaweed extract for the prevention of grapevine fungal diseases is scarce. When used in several foliar treatments on grapevine, a crude extract of the green seaweed *Ulva armoricana*, rich in the polysaccharide ulvan, showed anti-powdery mildew activity (90 % reduction of symptoms) (Jaulneau *et al.,* 2011). In fact, defense mechanism activation related to the jasmonic acid pathway has been related to ulvan (Jaulneau *et al.,* 2010; Jaulneau *et al.,* 2011). The treatment of grapevine plants with laminarin, a polysaccharide from the brown algae *Laminaria digidata*, reduced the infection of *Botrytis cinerea* and *P. viticola* by 55 % and 75 %, respectively (Aziz *et al.,* 2003). Gauthier *et al*. (2014) and Pagliarani *et al*. (2020) have provided insights into the modifications that occur within molecular pathways related to resistance following seaweed extract applications in studies that applied "omics" technologies, microarray analysis and high throughput sequencing, respectively. The defense mechanisms that are activated under pathogen stress vary, depending on the sensitivity of the grapevine genotypes to the disease (Molitor *et al.,* 2018; Nogueira Júnior *et al.,* 2020; Adrian *et al.,* 2024), and thus the elicitation of plant response by plant protection products or biostimulants can vary depending on the plant's genetic background (Godínez-Mendoza *et al.,* 2023).

Recently, an aqueous extract from *Rugulopteryx okamurae (R. okamurae)* - an invasive brown alga that has had a huge negative environmental, social and economic impact on the Andalusian coast (southern Spain) - was found to be a grapevine biostimulant under greenhouse conditions (Zarraonaindia *et al.,* 2023). The extract induced grapevine defense by the expression of immunity genes and secondary metabolites, such as stilbenes, phytohormones and antioxidant enzymes, which are involved in protecting Tempranillo plants from stress and increasing their resistance to it. Additionally, the fungal community composition of the leaves was altered following the treatment, and an increase in the abundance of several beneficial genera known for their antifungal or antagonistic properties occurred in the treated grapevines. However, it remains to be studied whether the elicitation by the extract could indeed provide protection for grapevines against fungi and oomycetes. In addition, while variations to downy mildew resistance have been previously evidenced, even between clones of the same grapevine variety (Boso *et al.,* 2006), a very limited number

of studies have addressed these differences and unraveled the underlying mechanisms. To address these gaps in knowledge, the objective of the present study was to assess the efficacy of a *R. okamurae* aqueous extract in inducing resistance to *P. viticola* in two clones of *Vitis vinifera* L. cv. Tempranillo. The molecular and metabolic responses of the clones together with the changes to plant physiology were investigated after i) *R. okamurae* extract applications, and ii) *P. viticola* inoculation. In addition, the efficiency of the treatments for protecting the grapevines from downy mildew disease was assessed. The differential response of each of the clones to the treatments is discussed here, shedding more light on the influence of the interaction between genotype and elicitor on level of plant protection.

MATERIALS AND METHODS

1. *Rugulopteryx okamurae* extract elaboration and biochemical characterization

The brown macroalgae *R. okamurae* (Div. Phaeophyta, O. Dictyotales) was collected from Algeciras (Cadiz, Spain, 5° 25′ 34.75″ W, 36° 4′ 37.56″ N) in April 2022. After harvesting, the seaweed biomass was washed with desalinised water to remove epiphytes and salt, then drained and frozen at −80 °C, and freeze-dried (Cryodos, Telstar, Spain). To produce the extracts, batches of 50 g of freeze-dried *R. okamurae* were combined with 500 mL of milliQ water and subjected to vigorous shaking in a thermoshaker at 70 ºC for 2 h. The product was centrifuged, the supernatant stored and the solid phase mixed once more with 500 miliQ water and then shaken for 2 h at 70 ºC and centrifuged again. The two liquid phases resulting from the centrifugations were combined, frozen and then lyophilised. The composition of the aqueous extract was characterised. Briefly, ash content, CNS content, macronutrients (Ca, K, Mg, P), micronutrients (Si, Cl, Fe, Mn, Br, Cu, Zn), ashes, total proteins, total lipids and total carbohydrate were analysed according to Zarraonaindia *et al*. (2023) (Table S1).

2. Experimental design

2.1. Biostimulation and infection

Two clones of *Vitis vinifera* L. cv. Tempranillo plants (VN40 and RJ43) grafted on Richter-110 rootstock were provided by two commercial nurseries (Vitis Navarra and Viveros Villanueva, Navarra, Spain, respectively). The plants (n = 30 per clone) were grown in a containment level 2 greenhouse (NCB2), in 5 L pots containing an enriched nutrient substrate of organic matter (90 %), sphagnum peat (160 g/L), calcium carbonate (7 g/L), NPK fertiliser (1.5 g/L) and trace elements (PotgrondH, Klasmann-Deilmann GmbH, Germany). The plants were grown for 2 months, with a 16 h day/8 h night photoperiod at an average room temperature of 18 ºC and were irrigated to field capacity when necessary.

When the plants had 10 to 12 fully expanded leaves, they were treated with either *R. okamurae* extract (RO, 6 g/L), water (W) or copper (BB, cuprous oxide 4.2 g/L, Nordox 75 WG, NordoxIndustrier, Oslo, Norway) spraying 3 times on leaves once every 48 hours (Figure 1). The soil in the pots was protected from the rinse with filter paper during each application. Ten plants were used per treatment type and clone type. To all the treatments (W, BB, RO) 0,1 % Retenol (Daymsa, Zaragoza, Spain) was added as a coadjutant.

Forty-eight hours after the last treatment, all the plants (n = 60) were inoculated with a suspension of *P. viticola* sporangia by pulverisation on the abaxial side of the leaf with a manual hand sprayer (Figure 1). *P. viticola* isolate was collected in 2022 from a commercial vineyard (Etxano, Biscay, Spain), isolated and regularly multiplicated in the laboratory on detached leaves of *V. vinifera* cv Tempranillo until use. Sporangia concentration was quantified using a Thomahaemato cytometer (GMBH + CO KG, Germany) as described by Llamazares De Miguel *et al.* (2022). The sporangia was dissolved in commercial mineral water, and its concentration was adjusted to 5 x 10^4 sporangia/ml.

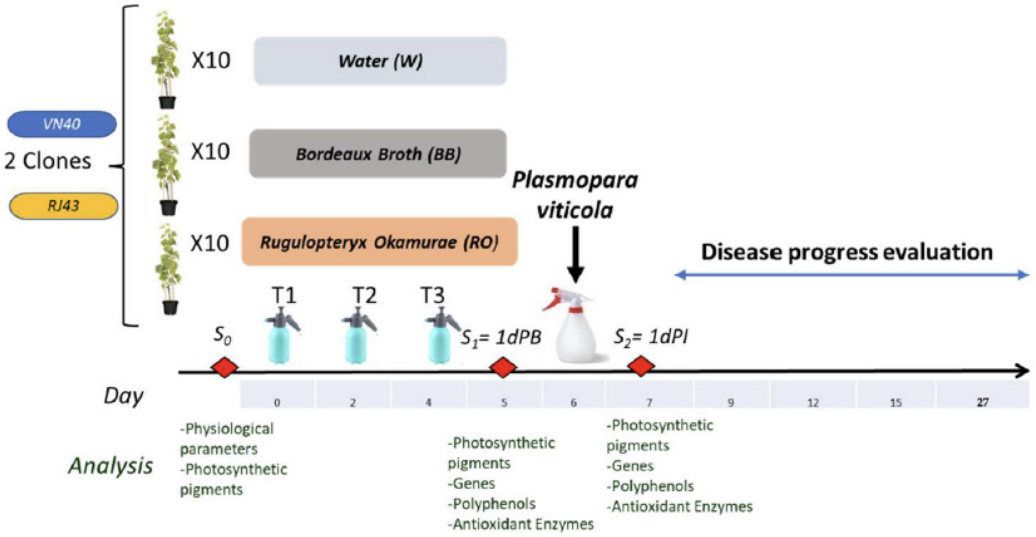


FIGURE 1. Experimental design.

dPB= days post biostimulation, dPI= days post inoculation.

2.2. Sample collection and analysis

Three crucial time-points were studied: S_0 sampling, the initial sampling prior to the application of the treatments (Day 0); S_1 sampling, 24 h after foliar application of the last treatment, referred to as the biostimulation time-point (1 day post-biostimulation, 1dPB); S_2 sampling, 24 h after *P. viticola* inoculation, referred to as the post-inoculation time-point (1dPI) (Figure 1).

To assess the initial physiological state of the plants, the photochemical parameters of the leaves and plant height were determined in S_0 (n = 60 plants). At the biostimulation time-point (S_1, 1dPB), the 5th leaf (starting from the apex) was sampled, and a leaf from two plants was pooled to create five replicate samples per treatment type/clone. Leaves were milled in liquid nitrogen and were then used for gene expression and secondary metabolites analysis (including enzyme activity and polyphenols). The 7th leaf was used to study photochemical parameters in n=10 plants/treatment. In this case, while the same sampling approach was used 1 day after inoculation (S_2, 1dPI), the 4th and 6th leaf of two plants were pooled together for gene, polyphenol and enzyme analysis (n = 5 biological replicates per treatment/clone). In addition, disease development was evaluated by visual observation in all the treated plants (W, BB and RO, n = 60) at three different monitoring times: 3, 10 and 17 days after observing the first symptoms. Incidence and severity rates were measured as a percentage and then transformed into a 0–7 scale similar to the EPPO evaluations for the efficacy evaluation of fungicides: a sporulation of 0 % was given a "0" value, a sporulation lower than 5 % a "1" value, a sporulation lower than 10 % a "2" value, a sporulation lower than 25 % a "3" value, a sporulation lower than 50 % a "4" value, a sporulation lower than 75 % a "5" value, and a sporulation higher than 75 % a "6" value. Those values were transformed into a disease index (DI) using the Townsend-Heuberger formula (1943) to obtain the Incidence Disease Index and the Severity Disease Index, respectively.

3. Gene expression analysis

RNA was extracted with Spectrum™ Plant Total RNA kit (Sigma-Aldrich Co., MI, USA), following the manufacturer's instructions, from water-treated and RO-treated plants leaves at the S_1 and S_2 timepoints. For each biological replicate (n = 5 per treatment/clone), DNA was removed by On-Column DNase I Digestion Set (Sigma-Aldrich Co., MI, USA) during the extraction protocol. First-strand cDNAs were synthesised, starting from around 750 ng of total RNA, using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific Waltham, MA, USA) and following the manufacturer's instructions. cDNA samples were analysed for gene expression using the INRAE BC2 Grape platform (https://bc2grape-inrae.fr/nos_services/neovigen). The expression of genes was monitored by quantitative polymerase chain reaction (qPCR) using an implemented version of the NeoViGen microarray and a Fluidigm® de BioMark HD, following the protocol described by Dufour *et al.* (2016). The array contained five reference genes and a total of 89 targeted genes: 74 from Dufour *et al*. (2016) and 15 from Bodin *et al*. (2020). NADH and RNAse P were the negative and positive control, respectively. A list of the genes is provided in Table S2.

The comparative Ct (2−ΔΔCt) method was used to calculate transcript expression levels, as all the genes showed similar amplification efficiencies (between 90 and 110 %). The gene expression profiles of RO-treated and water-treated (W) plants were compared 24 h after treatment application (S1) and 24 h after *P. viticola* inoculation (S2), respectively. To determine the significance of a differential gene expression, the fold change threshold was fixed at 1.5 or -1.5 (log transformed) and a one-way analysis of variance (ANOVA) was carried out in SPSS 15.0 (SPSS Corporation, Chicago, IL, USA) per gene; the ANOVA values were considered statistically significant when $p < 0.05$.

4. Polyphenol analysis

Polyphenols were extracted from W, BB and RO leaves which had been collected in S1 and S2 following the method described by Krzyzaniak *et al.* (2018) with modifications. Briefly, 50 mg of freeze-dried powdered leaves was extracted using 10 mL of methanol and by shaking overnight at 4ºC. The supernatants were evaporated, redissolved in methanol:water (30:70), and then passed through a cartridge C_{18} (Scharlab, Spain) to clean up the samples. Polyphenols were eluted in methanol:water (90:10), concentrated under vacuum, redissolved in methanol:water (50:50), and finally filtered through a 0.22 µm filter (PTFE Teknokroma, Barcelona, Spain). For compound identification, 20 µL of the samples were analysed using a Waters HPLC system (Milford, MA, USA) equipped with a model 1525 pump, a W2707 injector and a Waters 2996 photodiode detector, and a Mediterranean Sea C18 column (Tecknokroma, Barcelona, Spain) (RP-18, 25 × 0.46 cm; 5 µm particle size), with a precolumn of the same material. Caftaric acids, coutaric acid, *cis*- and *trans*-piceid, and viniferins (ε and δ) were quantified at 320 nm with their respective standards; *trans*-Resveratrol was quantified at 306 nm as *trans*-resveratrol; *cis*-Piceid was quantified as *cis*-piceid with its respective standard generated by UVC treatment on *trans*-piceid. Quercetin 3-glucoside and quercetin-3-glucuronide were quantified at 360 nm as their respective standard. Concentrations were expressed in mg/L.

A one-way analysis of variance (ANOVA) of the mean values was used to test for differences between time-points and treatments. Significant results ($p \leq 0.05$) were then evaluated via the LSD test, using Statistix version 9.0 (Analytical Software, Tallahassee, FL, USA).

5. Analysis of the antioxidant enzymes

The activity of the enzymes superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) was determined as described in Pérez-López *et al*. (2009). In summary, ground leaf tissue (40 mg fresh weight) of 5 replicates per treatment/clone was extracted using enzyme-specific buffers (0.6 mL per sample). The resulting homogenates were subjected to centrifugation at 16,100 g for 25 min. Superoxide dismutase

(SOD) activity was determined via a spectrophotometric assay based on the reduction of ferricytochrome-c, utilising xanthine and xanthine oxidase as sources of superoxide radicals. One unit of SOD was defined as the amount of enzyme required to achieve a 50 % decrease in the ferricytochrome-c reduction rate. For the glutathione reductase (GR) assay, the activity was measured by monitoring the decrease in absorbance at 340 nm, corresponding to the NADPH oxidation dependent on oxidized glutathione (GSSG), at 25 °C. Ascorbate peroxidase (APX) activity was evaluated by tracking the ascorbate (ASA) oxidation at 290 nm. Statistical analysis was carried out using SPSS version 28.0 (IBM Corp, Armonk, NY, USA). The differences between the treatments were assessed via a one-way analysis of variance (ANOVA) on the mean values. When ANOVA indicated significant differences ($p \leq 0.05$), Tukey's post-hoc test was employed to compare the tested treatments.

6. Photochemical parameters

Measurements of the photochemical efficiency of photosystem II (φ_{PSII} *or* Fv/Fm) and the leaf chlorophyll level (SPAD index) were performed in situ at each sampling point (on the 5th leave in S_0 and S_1 and on the 7th in S_2) using a FluorPen FP 100 fluorometer (Photon Systems Instruments, Brno, Czech Republic) and a Konica-Minolta SPAD-502 (n = 60 plants,

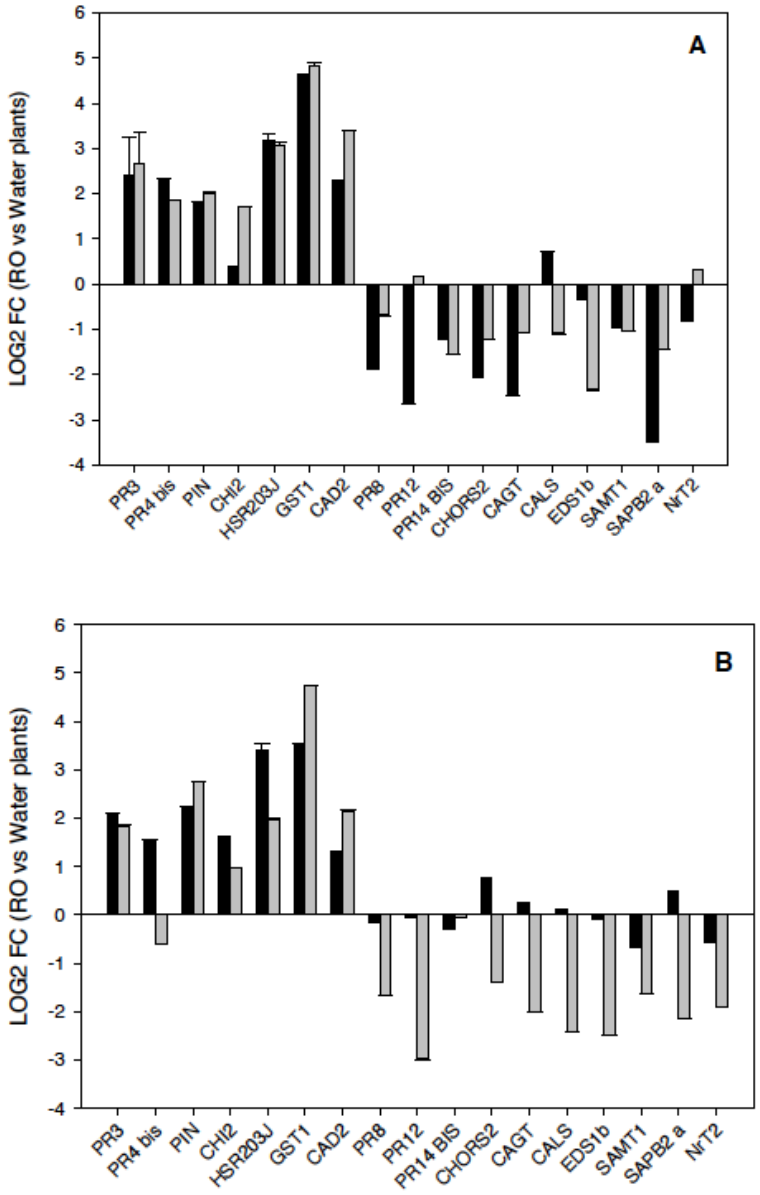


FIGURE 2. Transcript levels of defense-related genes (log transformed) in grapevine leaves induced by applications of *Rugulopteryx okamurae* (S1, 1dPB) in A) and by *Plasmopara viticola* (S2, 1dPI) in B) relative to the water treated samples. Only genes with a log-transformed-fold change greater than 1.5 or less than -1.5 relative to water for any clone/sampling point are shown. Black bars refer to the VN40 clone and grey bars refer to the RJ43 clone. The values represent 5 biological replicates per treatment/clone. The error bars indicate the variation range of the Log2 FC values calculated as follows: ((Log2 FC (RO vs Water plants) x (RO ΔCq variation coefficient (SD/|mean|) + Water plants ΔCq variation coefficient))/ 100.

n = 10 per treatment/clone). Chlorophyll a, chlorophyll b, carotenoids, xantophylls and phaeophytinization quotient chlorophyll a were determined in 0.7 cm diameter leaf discs from the 7th leaf in S1 and S2. Statistical analysis was conducted in SPSS28.0 0 (IBM Corp. Armonk, NY: IBM Corp, USA) by means of an ANOVA and a Tukey´s test.

RESULTS AND DISCUSSION

1. Initial state (S_0): basal differences between VN40 and RJ43 Tempranillo clones

Being the most widespread red variety in Spain, Tempranillo is well known throughout the world. In the present work, the two clones VN40 and RJ43 were assessed in order to gain a broader perspective of our findings. According to the commercial nurseries, the VN40 clone plants exhibit higher production but lower vigour and compactness than the RJ43 clone, which may be key aspects regarding infection development.

During the two-month growing period in the greenhouse prior to biostimulation and pathogen inoculation, a delayed growth of the RJ43 clone was observed. Accordingly, in S_0, the measured development parameters (stem height, number of leaves, number of stems) showed that the RJ43 clone plants (n = 30) were less developed than the VN40 clone plants (n = 30) (Table S2), thereby already indicating differences in vegetative and phenological stages between these clones at this initial time-point.

2. Biostimulation state (S_1, 1dPB): responses to *Rugulopteryx okamurae* extract (RO) application

Grapevine leaves were sampled after three applications of water (W), copper (BB) and seaweed (RO) (S_1, 1dPB, Figure 1) to assess the metabolic response of the plants. At this sampling point, our results are compared with a previous study conducted on Tempranillo VN40 clone, in which two foliar treatments with the same seaweed extract were conducted (Zarraonaindia *et al.,* 2023).

2.1. Gene expression modulation by RO

Differential expression analysis was carried out on the targeted 89 genes (Table S3) following three applications of *R. okamurae* (RO) and Water treatment (W). The results revealed that the extract significantly enhanced the expression of three genes belonging to the PR protein group (PR3, PR4bis and PIN) and four other genes, each involved in a different functional group: CHI2 (only in RJ43), HSR-203J, GST1 and CAD2 genes related to the flavonoid pathway, lipoxygenase pathway, redox status regulation and cell wall reinforcement, respectively (Figure 2A). In addition, the applied extended gene expression analysis using NeoViGen microarray strategy (compared to the 15 targeted genes in Zarraonaindia *et al*. (2023)) allowed down-regulated genes to be identified after seaweed applications, among which PR8, PR12, CHORS2, CAGT, EDS1b and SAPB2a showed the highest inhibition values, with log values (RO versus Water plants) close to -2 (Figure 2A).

The GST1 gene, commonly associated with ascorbate peroxidase (APX) levels (Gullner *et al.,* 2018), exhibited the highest induction values following RO treatment, with a 4.62-fold change in VN40 and a 4.83-fold change in RJ43, consistent with previous findings in Zarraonaindia *et al*. (2023), which showed that RO extract preferentially induced jasmonic acid (JA) related genes, while inhibiting salicylic acid (SA) responsive genes. In particular, PR3, PR4bis and PIN (gene function of PR6), whose expressions are known to be strongly dependent on JA signalling (Ali *et al.,* 2018; Van Loon *et al.,* 2006), were significantly higher in RO-treated leaves. Moreover, the expression of HSR-203J, known to be enhanced by JA signalling (Dufour *et al.,* 2016), was also increased by RO treatments. On the other hand, RO was found to down-regulate PR-8, PR-12, PR-14, EDS1b, SAMPT1 and SAPB2a genes, which are known to be related to SA signalling (Adrian *et al.,* 2012; Bodin *et al.,* 2020; Dufour *et al.,* 2016); this thus corroborates the antagonistic regulation of both JA and SA pathways suggested by other authors (Paolacci *et al.,* 2017).

Both clones showed similar upregulated and downregulated gene profiles after *R. okamurae* treatments. However, overall, VN40 plants showed a more consistent downregulation of five genes, three of which were related to SA pathway ones (PR8, PR12 and SAPB2a), a nitrate transporter (NrT2) and a glycosyltransferase (CAGT). By contrast, in five cases (PR3, PIN, CHI2, GST1 and CAD2) upregulation was more pronounced in the RJ43 clone (Figure 2A). CAD2 expression, involved in the biosynthesis of monolignols (main component of lignin), was significantly upregulated by RO, especially in RJ43 plants. Lignans play an important role in plant defense and, indeed, one of the characteristic responses of plants to injury and disease is the reinforcement of cell walls (Ninkuu *et al.,* 2022). Therefore, RO treated plants, and particularly the RJ43 clone, might have higher protection responses than untreated plants in the event of a pathogen infection.

2.2. Induction of phenolic compounds by RO

The concentrations of polyphenols in the leaves of Tempranillo VN40 plants increased after treatment with *R. okamurae* (Figure 3A). The concentrations of caftaric acid and coutaric acids were significantly higher in RO- and BB-treated plants than in the control (water). Consistent with this, at this sampling point, significant increases in stilbenes (total piceid and *E*-resveratrol) were also observed in RJ43 plants relative to the control ones (Figure 3B). Viniferins, on the other hand, were detected under the detection limits. The same stilbenes as those found in Zarraonaindia *et al.* (2023) were identified, albeit in higher concentrations, which is probably due to the additional third RO treatment applied in the present experiment.

In general, polyphenols reached higher concentrations in VN40 leaves than in RJ43 without any significant differences. However, stilbenes, key compounds for responding to biotic stresses, reached similar concentrations in RO leaves for both clones, which suggests that biostimulation with regards to these

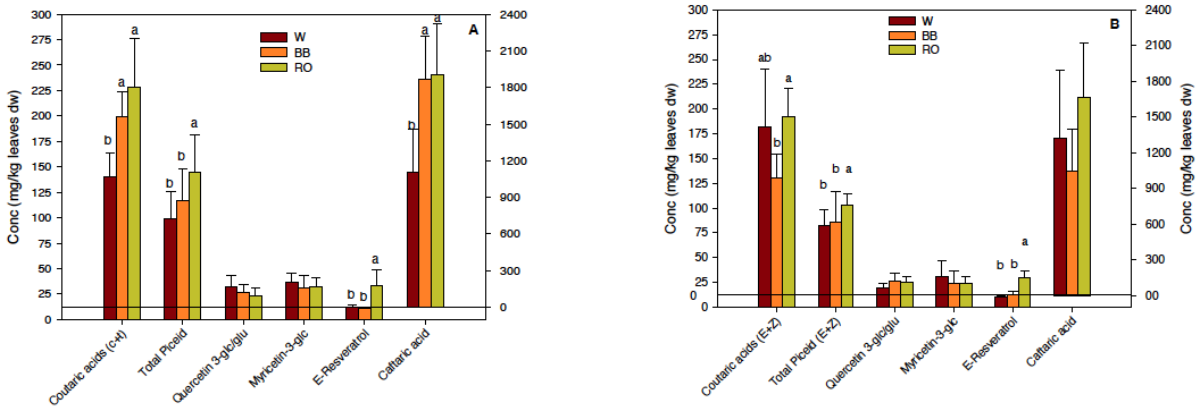


FIGURE 3. Polyphenol content in grapevine leaves after treatment (S_1, 1dpB) of A) VN40 clone plants, and B) RJ43 clone plants. Different letters above the bars indicate significant differences between treatments for each compound (ANOVA, $p < 0.05$).

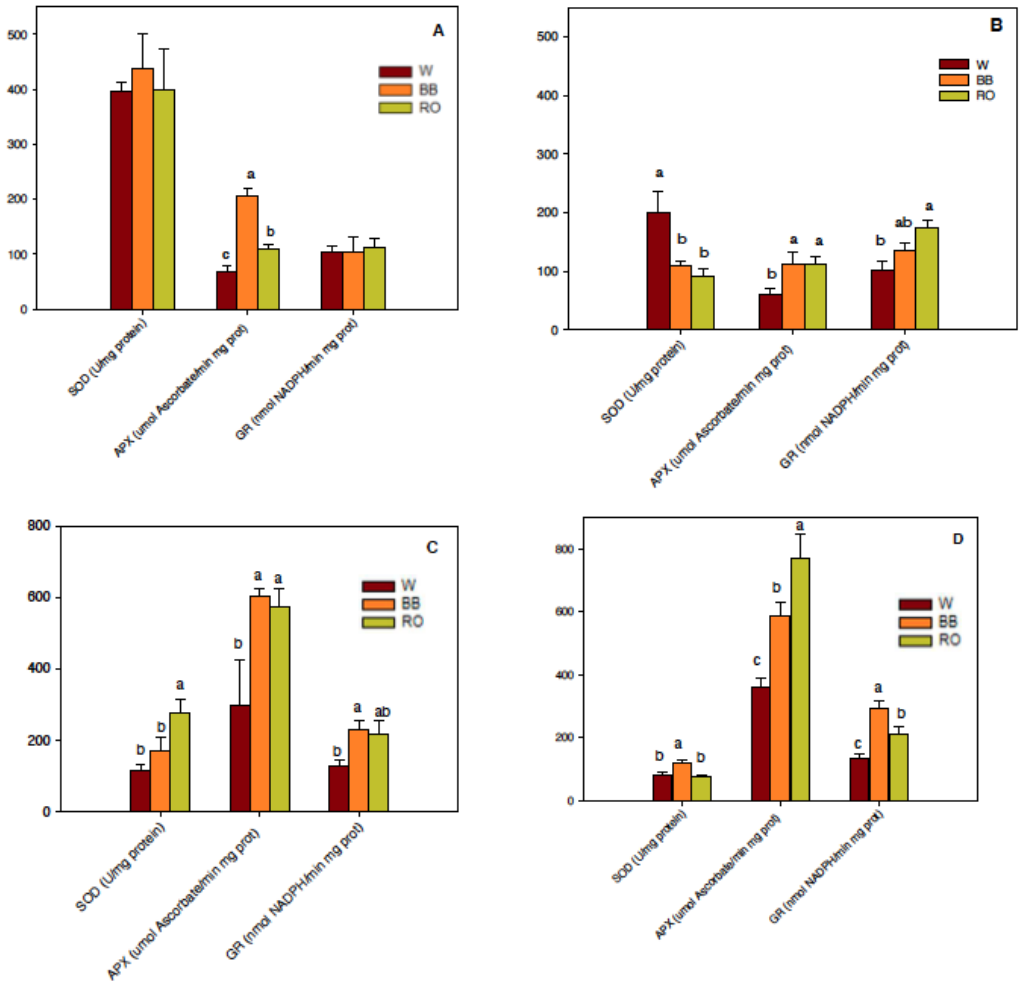


FIGURE 4. Antioxidant enzymes in treated leaves at different sampling times: A) and B) after 24 hours of treatment applications (S1, 1dPB) for VN40 and RJ43 respectively, C) and D) after 24 hours of *Plasmopara viticola* inoculation (S2, 1dPI) for VN40 and RJ43 respectively. Different letters above the bars denote significant differences between treatments (ANOVA, $p < 0.05$) within each compound.

compounds is similar in these clones. Flavonols (quercetin 3-glucoside + glucuronide and myricetin 3-glucoside) were not affected by the treatments, which corraborates our previous results in Zarraonaindia *et al*. (2023). An increase in flavonoids has previously been observed after seaweed treatment in the berries of Tempranillo blanco plants (Cataldo *et al.,* 2022), but, to our knowledge, such an increase has not been associated with leaves. In general, the results regarding phenolic compounds indicate that both clones are equally prepared for a possible infection.

2.3. Activity modulation of the antioxidant enzyme system by RO

SOD, APX and GR enzymes have been described as being related to defense mechanisms in plants. Generally, biostimulants derived from algae are known to induce the antioxidant system (García-Sánchez *et al.,* 2022). However, in VN40 plants, SOD and GR activities did not show any significant differences between the three different treatments (W, BB and RO). By contrast, APX activity significantly increased for RO- and BB-treated plants (1.6 and 2.9 times higher than that of water respectively; Figure 4A), indicating that both treatments were able to reduce H_2O_2. This could be related to a H_2O_2 signaling mediated by the biostimulation, preparing VN40 for any potential stress. At the end of signalling, H_2O_2 must be metabolised, for which APX is needed. This is an interesting strategy, since H_2O_2 is a reactive oxygen species which can damage plant cells (Hasanuzzaman & Fujita, 2022). Importantly, the already high intrinsic SOD values found in the VN40 plants indicate that this clone relies heavily on H_2O_2 signalling and regulation in its basal state (under no biotic or abiotic stress). According to Pérez-López *et al*. (2015), high SOD activity could be considered an advantage as it helps plants protect themselves against potential oxidative damage. Therefore, overall, this high basal SOD could be the reason why the antioxidant system response to biostimulation was less pronounced in VN40 plants. Unfortunately, no reference was found in the literature to the effect of biostimulants on *Vitis vinifera* antioxidant enzymes. However, by comparing our results with those obtained under non-stress conditions, it can be noted that the SOD values observed in our study are higher than those reported for SOD in non-stressed Tempranillo (Salazar-Parra *et al.,* 2012) and Öküzgözü (Özden *et al.,* 2009).

By contrast, all the enzymes studied in RJ43 plants were affected by the RO treatment. SOD activity decreased 2.2- and 1.8-fold for RO and BB treatments relative to the control plants, respectively. Meanwhile, for the RO and BB treatments, there was an approximate 1.9-fold increase in APX and 1.7- and 1.3-fold increase in GR (Figure 4B). The increase in GR in RJ43 plants allows them to regenerate the important antioxidant molecule glutathione and increase the ability of plants to prepare for an oncoming stress (Noctor & Foyer, 1998). It is important to note that RJ43 seems to rely on APX rather than SOD when reducing the accumulation of H_2O_2.

Thus, our results indicate that each clone modulates antioxidant enzymes differently after RO biostimulation, with the RJ43 clone modulation benefiting more, since GR is activated to a greater extent than in the VN40 clone. However, it should be noted that elicitation may have been lower in the VN40 plants due to their already high basal SOD activity.

2.4. Modifications to photosynthetic pigments and photochemical parameters by RO

While the composition of photosynthetic pigments in RJ43 clone plants were not affected by treatments, VN40 clone plants decreased both chlorophyll a and b, as well as carotenes and xanthophylls, which is in line with the SPAD results (Table 1). Conversely, maximum photochemical efficiency (φ_{PSII}) decreased in the RJ43 plants, while the VN40 clone maintained it at levels similar to those of the control samples (W), despite significantly decreasing the greenness (SPAD) (Table 1). Previous studies have shown that the application of seaweeds in general, and the brown macroalgae *Ascophyllum nodosum* in particular, results in increased chlorophyll content in the leaves of different plants (*e.g*., tomato, dwarf French bean, wheat, barley and maize); this is likely due to the inhibition of chlorophyll degradation, which is caused partly by compounds, such as betaines, present in the seaweed extracts (Sangha *et al.,* 2014). The *R. okamurae* extract used here does not contain betaine lipids, as was revealed by biochemical characterisation conducted by Córdoba-Granados *et al.* (2024), and as would be expected for an aqueous extract, which might justify the differences found with the aforementioned publication.

In general, our results revealed no effect, or just slight negative effects (depending on the clone) of the RO treatment on photosynthetic pigments and the photochemical parameters associated with them. However, it is worth mentioning that the VN40 plants were at a more advanced phenological stage than the RJ43 plants, which may also have contributed to the reduction in photosynthetic pigments observed in VN40. Nonetheless, the reduction was slight, and the values were still within the established range representing good photosynthesis activity in grapevine leaves (Casanova-Gascon *et al.,* 2018).

3. Post inoculation state (S_2, 1dPI): responses to *Plasmopara viticola*

Grapevine resistance to downy mildew has a large genotypic component and defense reactions occur with variable promptness and magnitude, timing and intensity. Generally, it is assumed that highly susceptible grapevines show a weak defense response to *P. viticola* infection (Perazzolli *et al.,* 2012), and therefore they rely heavily on external compounds to help withstand disease. Copper-based products directly target the pathogen, making them the most common control measure. By contrast, it is less common for seaweed to be directly toxic to fungi, their protective efficiency coming rather from their ability to elicit defense mechanisms in the plant. In this section, the effect of *P. viticola* at the molecular and metabolic levels is evaluated in Tempranillo clones (RJ43 and VN40) in order to assess the effectiveness of *R. okamurae* as an elicitor against downy mildew.

3.1. Gene expression modulation after *Plasmopara viticola* inoculation

The expression levels of most RO-modulated genes either remained stable or decreased at S_2 (1dPI) compared to S_1 (1dPB), a pattern that could be due to Tempranillo's inherent sensitivity to *P. viticola*. Tempranillo's response to infection can usually be expected to be moderate, although *P. viticola* is known to be able to modulate JA pathway-related genes in certain grapevine varieties (Figueiredo *et al.,* 2015; Guerreiro *et al.,* 2016). In this study, in the RJ43 clone, six out of the seven RO-induced genes, particularly the PR4bis gene in the JA pathway, showed lower expression values after inoculation (Figure 2B; Table 2). Interestingly, while the expression levels also decreased in RO-treated VN40 plants, CHI2 expression markedly increased (332 %-fold change from S_2 to S_1), suggesting that this gene was primed by the seaweed application.

Remarkably, while the inhibition of SA-related genes was not maintained after infection in the VN40 plants, their downregulation was strengthened in the RJ43 clone (Figure 2B). Gene expression downregulation was particularly marked in PR12 and NrT2, as well as in PR8 and CALS, in RJ43 after *P. viticola* inoculation; this suggests that the extract was able to prime the plants, and therefore the inhibition of the expression of these genes became stronger when the grapevines were further subjected to biotic stress. Several stimuli have been shown to prime plants for an improved response to future stress, thereby triggering earlier, faster and/or stronger responses (Hilker *et al.,* 2016; Mauch-Mani *et al.,* 2017), among which seaweed extract scan be highlighted (Islam *et al.,* 2021). It should be noted that elicitors may also be potentially used in defense priming strategies by downregulating certain genes (Landi *et al.,* 2017), as is the case of susceptibility genes (S-genes) (Liu *et al.,* 2022). However, to our knowledge, no previous evidence of downregulation associated with the priming of plant defense response genes has been described in the literature to date; therefore, the downregulation observed for PR12, NrT2, PR8 and CALS in the previously biostimulated plants requires further validation.

3.2. Concentrations of phenolic compounds after *Plasmopara viticola* inoculation

Total polyphenol concentration increased in all plants after infection; however, no clear trend was observed between treatments or clones (data not shown). Only the RJ43 clone plants exhibited an increase in total piceid, this enrichment being more acute in the plants previously treated with seaweed (1.15-fold for W, 1.31-fold for BB and 1.84-fold for RO). However, piceids, in their glucoside form, are known to be inactive against *P. viticola* (Gabaston *et al.,* 2017). Other stilbenes have been directly correlated with resistance to fungal disease, and differences in the content and accumulation of stilbenes between susceptible and resistant genotypes is known to be key to overcoming infections (Burdziej *et al.,* 2021; Paolocci *et al.,* 2014; Vrhovsek *et al.,* 2012). The biosynthesis of complex stilbenes in grapevine leaves is typically observed during infection around six days after inoculation (Vrhovsek *et al.,* 2012; Burdziej *et al.,* 2021), which may explain the low concentrations and differences observed in our early sampling; *i.e.*, just 24 hours after infection. Despite this early sampling, ε-viniferin and ω-viniferin were detected in both clone plants in all the treatments (W, BB, RO), ε-viniferin concentration being significantly higher in the RO samples of RJ43 plants (Table S4). Susceptible genotypes are thought to accumulate resveratrol and piceid during infection, while other stilbenes, such as ε- and δ-viniferins, have been found to be mostly produced in resistant genotypes (Boso *et al.,* 2012; Paolocci *et al.,* 2014). Therefore, the increase in viniferins observed in the present study suggests that, despite Tempranillo being considered a susceptible variety, the clones studied here were exhibiting a certain level of resistance to infection. Further samplings at later time-points would provide valuable insights into the extent of this resistance.

3.3. Antioxidant system activity modulation after *Plasmopara viticola* infection

In both clones, SOD activity decreased with respect to the S_1 sampling point, especially in plants that had not been previously biostimulated, indicating that the oomycete was likely downregulating SOD activity in order to inhibit the plants' defense mechanisms (Nascimento *et al.,* 2019). Interestingly, in the VN40 clone, plants previously elicited by RO showed an increase of 138 %, 93 % and 73 % for SOD, APX and GR respectively in their leaves, relative to the water-treated plants. In the RJ43 clone, however, RO-biostimulated plants did not alter SOD in response to *P. viticola* inoculation, but APX and GR activities increased by 112 % and 57 % relative to the water samples. The significant induction of APX correlated with the pronounced overexpression of the GST1 gene (Figure 2A) in the RJ43 clone, suggesting that the ROS scavenging and detoxification mechanisms triggered by RO application in S_1 were further enhanced following *P. viticola* infection, thus helping to protect the cells from the combined signal induced by both the seaweed and the pathogen.

Overall, the results indicate that RO biostimulated the plant´s antioxidant metabolism, which was activated after pathogen inoculation, maintaining most of the enzymes at high values in both clones. This indicates that both clones potentially reduce the development capacity of the oomycete. The high SOD values in VN40 could promote some resistance, as high H_2O_2 levels are toxic for fungi and prevent the development of the pathogen. In fact, reactive oxygen species play an important role in pathogen resistance by directly strengthening host cell walls via the cross-linking of glycoproteins, lipid peroxidation and the activation of ROS-signalling networks, leading to the establishment of a resistance response (Figueiredo *et al.,* 2017). However, as mentioned previously, H_20_2 could also be toxic for plants (Hasanuzzaman & Fujita, 2022; Mittler *et al.,* 2004); therefore, we hypothesise that to counteract it, VN40 regulated the high H_20_2 levels with a higher activation of APX. These strategies seem to allow the delicate equilibrium between ROS production and scavenging to be maintained (Mittler *et al.,* 2004).

Thus, we can conclude that *R. okamurae* seaweed treatment was able to prime both clones of the *Vitis vinifera* plants, allowing the plants to respond more efficiently when the infection occurred than the plants that had not been previously biostimulated by *R. okamurae*.

3.4. Modifications to photosynthetic pigments and photochemical parameters by *Plasmopara viticola*

Few significant differences were found in terms of pigments and photochemical parameters between treatments after infection (S_2). The VN40 clone plants treated with RO did not show any significant differences when compared to the water treated plants (Table S5). With regards to the RJ43 clone plants, no differences in terms of pigments, SPAD or photosynthetic efficiency were found between treatments; this demonstrates that any negative effect observed at the biostimulation sampling point (S_1) (Table 3) was transitory and quickly reverted.

P. viticola has been shown to affect the photosynthesis of susceptible grapevine cultivars by reducing the diffusion of CO_2 in leaf mesophyll (Jermini *et al.,* 2020) and by inhibiting the enzymes involved in chlorophyll synthesis (Nogueira Júnior *et al.,* 2020). However, in the present study, the sampling was performed one day after pathogen inoculation, and therefore the above effects had not yet been observed.

4. Evaluation of the protection conferred by *Rugulopteryx okamurae* extract against *Plasmopara viticola*

The protective efficiency of the copper-based treatment (BB) was higher than that of RO and occurred earlier in the period of infection: by days 3 and 10 for the incidence of clone VN40 and RJ43, respectively, and by day 10 for the severity of both clones (Figure 5). Moreover, a different level of protection was observed depending on the clone, which was more evident by day 17. While in VN40 the incidence and severity index were not significantly different between RO and W samples, in RO-treated RJ43 plants a disease reduction was observed, with 20 % incidence reduction and 25% severity reduction, both of which were significant. All these results align with the findings observed for this clone during both the biostimulation (S_1) and post-inoculation stages (S_2), which had already indicated that RO treatment elicited a higher response in RJ43 clone plants. It is widely known that clones show different susceptibility to fungal diseases (Molitor *et al.,* 2018), as is the case for varieties (Boso *et al.,* 2023). In fact, targeted clone selection represents an integral tool in the control strategy in Integrated Pest Management contributing to pesticide reduction in viticulture.

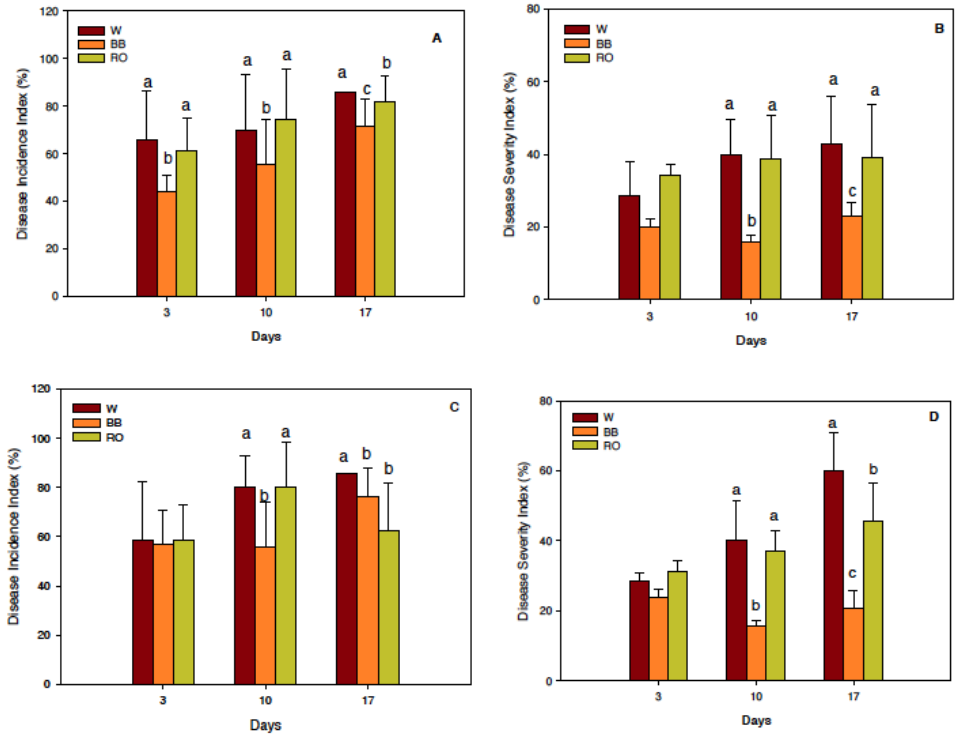


FIGURE 5. Downey mildew incidence and severity indexes on two Tempranillo clones leaves: A) and B) for VN40; C) and D) for RJ43, respectively. Different letters above the bars denote significant differences between treatments ($p < 0.05$) at each inspected day.

Because most research has been conducted *in-vitro* (*e.g.*, on leaf discs), few scientific studies report the efficacy of agricultural products at the whole-plant level under controlled conditions (reviewed by: Adrian *et al.,* 2024; Taillis *et al.,* 2022). Furthermore, while various commercial plant protection products are known for their ability to inhibit fungal infections in grapevine (Llamazares De Miguel *et al.,* 2022), little is known about such ability in seaweed-based products. The *R. okamurae* aqueous extract applied here contained alginate, fucoidan

and laminarin (Córdoba-Granados *et al.,* 2024) that could in part be responsible for the elicitation and disease suppression observed, as polysaccharides have been previously demonstrated to have such activity. For instance, a crude extract of *Ulva armoricana* green seaweed, rich in the polysaccharide ulvan, showed anti-powdery mildew activity in the leaf disc and in the plant after several grapevine foliar treatments (Jaulneau *et al.,* 2011). The treatment of grapevine plants with laminarin, a polysaccharide from the brown algae *Laminaria digidata*, was found to reduce the infection of *Botrytis cinerea* and *P. viticola* by 55 % and 75 %, respectively (Aziz *et al.,* 2003). Therefore, the present work represents a stepping-stone towards understanding the capacity of seaweed to protect plants from disease in agriculture, and natural defense elicitation by *R. okamurae* extract was demonstrated. However, further studies are needed to determine the relationship between the conferred rate of protection by *R. okamurae* and *P. viticola* disease progression, as the protection induced by natural elicitors could indeed be affected by the time interval between elicitation and pathogen attack, or the number of elicitation treatments given, among other factors.

CONCLUSION

Rugulopteryx okamurae extract (RO) was able to induce defense reactions in grapevine plants at both molecular and metabolic levels. Importantly, plants that had been previously treated with the seaweed responded more efficiently to *Plasmopara viticola* infection, leading to a reduction in the disease incidence and severity indexes. This suggests that *Rugulopteryx okamurae* elicitation was effective in priming plants, ultimately protecting them from the disease. The defense elicitation and extent of protection were however clone-dependent, being more effective in the tempranillo RJ43 clone plants.

Overall, the results of this study contribute to the future development of sustainable products based on seaweed formulations, which could be used in environmentally friendly protection strategies in viticulture. Despite RO-mediated disease suppression being less effective than with the copper-based treatment, the seaweed aqueous extract still showed promise as a sustainable biocontrol candidate. Its use in combination with other conventional products would be advisable for more effective disease control, and it could contribute to reducing the reliance on and dosage of copper in viticulture, a growing necessity and a hot topic in the agriculture sector. Furthermore, the results of this study present an opportunity to valorise the invasive *R. okamurae* seaweed by integrating it into a circular system. Further studies are required to characterise the bioactive compounds in the extract and to elucidate the precise mechanisms behind elicitation and disease suppression, given the complexity of the involved factors. Importantly, the differences in efficacy observed, even between clones, underscore the need to explore grapevine genomics and epigenomics further, extending research beyond grapevine variety.

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REFERENCES

Adrian, M., Corio-Costet, M. F., Calonnec, A., Cluzet, S., Poinssot, B., Trouvelot, S., Wiedemann-Merdinoglu & Viaud, M. (2024). Grapevine defence mechanisms when challenged by pathogenic fungi and oomycetes. *Grapevine: From Origin to the Vineyard*, 110, 101-195. https://doi.org/10.1016/bs.abr.2024.02.013

Adrian, M., Trouvelot, S., Gamm, M., Poinssot, B., Héloir, M.-C., & Daire, X. (2012). Activation of Grapevine Defense Mechanisms: Theoretical and Applied Approaches. In J. M. Mérillon & K. G. Ramawat (Eds.), *Plant Defence: Biological Contro*l, 12, 313–331. https://doi.org/10.1007/978-94-007-1933-0_13

Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., Tyagi, A., Islam, S. T., Mushtaq, M., Yadav, P., Rawat, S., & Grover, A. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiological Research*, 212–213, 29–37. https://doi.org/10.1016/j.micres.2018.04.008

Aziz, A., Poinssot, B., Daire, X., Adrian, M., Bézier, A., Lambert, B., Joubert, J.-M., & Pugin, A. (2003). Laminarin Elicits Defense Responses in Grapevine and Induces Protection Against Botrytis cinerea and *Plasmopara viticola*. *Molecular Plant-Microbe Interactions®*, 16(12), 1118–1128. https://doi.org/10.1094/MPMI.2003.16.12.1118

Bodin, E., Bellée, A., Dufour, M.-C., André, O., & Corio-Costet, M.-F. (2020). Grapevine Stimulation: A Multidisciplinary Approach to Investigate the Effects of Biostimulants and a Plant Defense Stimulator. *Journal of Agricultural and Food Chemistry*, 68(51), 15085–15096. https://doi.org/10.1021/acs.jafc.0c05849

Boso, S., Alonso-Villaverde, V., Gago, P., Santiago, J. L., & Martínez, M. C. (2014). Susceptibility to downy mildew (*Plasmopara viticola*) of different Vitis varieties. *CropProtection*, 63, 26–35. https://doi.org/10.1016/j.cropro.2014.04.018

Boso, S., Alonso-Villaverde, V., Martínez, M.-C., & Kassemeyer, H.-H. (2012). Quantification of Stilbenes in Vitis Genotypes with Different Levels of Resistance to *Plasmopara viticola* Infection. *American Journal of Enology and Viticulture*, 63(3), 419–423. https://doi.org/10.5344/ajev.2012.11127

Boso, S., Gago, P., Santiago, J.-L., Muñoz-Organero, G., Cabello, F., Puertas, B., Puig, A., Domingo, C., Valdés, M. E., Moreno, D., Diaz-Losada, E., Cibriain, J. F., Dañobeitia-Artabe, O., Rubio-Cano, J.A., Martínez-Gascueña, J., Mena-Morales, A., Chirivella, C., Usón, J.J., & Martínez, M.C. (2023). Variation in Susceptibility to Downy Mildew Infection in Spanish Minority Vine Varieties. *Plants*, 12(14), 2638. https://doi.org/10.3390/plants12142638

Boso, S., Martínez, M. C., Unger, S., & Kassemeyer, H. (2006). Evaluation of foliar resistance to downy mildew in different cv. Albariño clones. *Vitis*, 45(1), 23. https://doi.org/10.5073/vitis.2006.45.23-27

Burdziej, A., Bellée, A., Bodin, E., Valls Fonayet, J., Magnin, N., Szakiel, A., Richard, T., Cluzet, S., & Corio-Costet, M.-F. (2021). Three Types of Elicitors Induce Grapevine Resistance against Downy Mildew via Common and Specific Immune Responses. *Journal of Agricultural and Food Chemistry*, 69(6), 1781–1795. https://doi.org/10.1021/acs.jafc.0c06103

Cappelletti, M., Perazzolli, M., Antonielli, L., Nesler, A., Torboli, E., Bianchedi, P. L., Pindo, M., Puopolo, G., & Pertot, I. (2016). Leaf Treatments with a Protein-Based Resistance Inducer Partially Modify Phyllosphere Microbial Communities of Grapevine. *Frontiers in Plant Science*, 7. https://doi.org/10.3389/fpls.2016.01053

Casanova-Gascón, J., Martín-Ramos, P., Martí-Dalmau, C., & Badía-Villas, D. (2018). Nutrients Assimilation and Chlorophyll Contents for Different Grapevine Varieties in Calcareous Soils in the Somontano DO (Spain). Beverages, 4, 90. doi:10.3390/beverages4040090

Cataldo, E., Fucile, M., & Mattii, G. B. (2022). Biostimulants in Viticulture: A Sustainable Approach against Biotic and Abiotic Stresses. *Plants*, 11(2), 162. doi: 10.3390/plants11020162

Córdoba-Granados, J. J., Jiménez-Hierro, M. J., Zuasti, E., Ochoa-Hueso, R., Puertas, B., Zarraonaindia, I., Hachero-Cruzado, I. & Cantos-Villar, E. (2024). Biochemical characterization and potential valorization of the invasive seaweed *Rugulopteryx okamurae. Journal of Applied Phycology*, 1-11. https://doi.org/10.21203/rs.3.rs-4831629/v1

Dufour, M.-C., Magnin, N., Dumas, B., Vergnes, S., & Corio-Costet, M.F. (2016). High-throughput gene-expression quantification of grapevine defense responses in the field using microfluidic dynamic arrays. *BMC Genomics*, 17(1), 957. https://doi.org/10.1186/s12864-016-3304-z

EPPO. (2001). EPPO standards—Guidelines for the efficacy evaluation of plant protection products: P1/31(3) *Plasmopara viticola*. Bulletin OEPP/EPPO, 31, 313–317.

Figueiredo, A., Martins, J., Sebastiana, M., Guerreiro, A., Silva, A., Matos, A. R., Monteiro, F., Pais, M. S., Roepstorff, P., & Coelho, A. V. (2017). Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to *Plasmopara viticola. Journal of Proteomics,* 152, 48–57. https://doi.org/10.1016/j.jprot.2016.10.012

Figueiredo, A., Monteiro, F., & Sebastiana, M. (2015). First clues on a jasmonic acid role in grapevine resistance against the biotrophic fungus *Plasmopara viticola*. *European Journal of Plant Pathology*, 142(3), 645–652. https://doi.org/10.1007/s10658-015-0634-7

Gabaston, J., Richard, T., Biais, B., Waffo-Teguo, P., Pedrot, E., Jourdes, M., Corio-Costet, M.-F., & Mérillon, J.-M. (2017). Stilbenes from common spruce (*Picea abies*) bark as natural antifungal agent against downy mildew (*Plasmopara viticola)*. *Industrial Crops and Products*, 103, 267-273. http://dx.doi.org/10.1016/j.indcrop.2017.04.009

García-Sánchez, F., Simón-Grao, S., Navarro-Pérez, V., & Alfosea-Simón, M. (2022). Review: Scientific advances in biostimulation reported in the 5th Biostimulant World Congress 8(7), 665.

Gauthier, A., Trouvelot, S., Kelloniemi, J., Frettinger, P., Wendehenne, D., Daire, X., Joubert, J.-M., Ferrarini, A., Delledonne, M., Flors, V., & Poinssot, B. (2014). The Sulfated Laminarin Triggers a Stress Transcriptome before Priming the SA- and ROS-Dependent Defenses during Grapevine's Induced Resistance against *Plasmopara viticola*. *PLoS ONE*, 9(2), e88145. https://doi.org/10.1371/journal.pone.0088145

Gessler, C., Pertot, I., & Perazzolli, M. (2011). *Plasmopara viticola*: a review of knowledge on Downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea*, 50(1), 3–44. http://www.jstor.org/stable/26458675

Godínez-Mendoza, P. L., Rico-Chávez, A. K., Ferrusquía-Jimenez, N. I., Carbajal-Valenzuela, I. A., Villagómez-Aranda, A. L., Torres-Pacheco, I., & Guevara-González, R. G. (2023). Plant hormesis: Revising of the concepts of biostimulation, elicitation and their application in a sustainable agricultural production. *Science of The Total Environment*, 894, 164883. https://doi.org/10.1016/j.scitotenv.2023.164883

Guerreiro, A., Figueiredo, J., Sousa Silva, M., & Figueiredo, A. (2016). Linking Jasmonic Acid to Grapevine Resistance against the Biotrophic Oomycete *Plasmopara viticola*. *Frontiers in Plant Science,* 7. https://doi.org/10.3389/fpls.2016.00565

Gullner, G., Komives, T., Király, L., & Schröder, P. (2018). Glutathione S-Transferase Enzymes in Plant-Pathogen Interactions. *Frontiers in Plant Science*, 9, 1836. https://doi.org/10.3389/fpls.2018.01836

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: Effects on prevention of grapevine diseases. *Journal of the Science of Food and Agriculture*, 99(3), 1001–1009. https://doi.org/10.1002/jsfa.9353

Hasanuzzaman, M., & Fujita, M. (2022). Plant Oxidative Stress: Biology, Physiology and Mitigation. *Plants*, 11(9), 1185. https://doi.org/10.3390/plants11091185

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hincha, D. K., Kunze, R., Muelle-Roeber, B., Rillig, M. C., Rolff, J., Romeis, T., Schmülling, T., Steppuhn, A., Van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E., & Kopka, J. (2016). Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews*, 91(4), 1118–1133. https://doi.org/10.1111/brv.12215

Islam, M. T., Arioli, T., & Cahill, D. M. (2021). Seaweed Extract-Stimulated Priming in *Arabidopsis Thaliana* and *Solanum Lycopersicum*. *Plants*, 10(11), 2476. https://doi.org/10.3390/plants10112476

Jaulneau, V., Lafitte, C., Corio-Costet, M.-F., Stadnik, M. J., Salamagne, S., Briand, X., Esquerré-Tugayé, M.-T., & Dumas, B. (2011). An *Ulva armoricana* extract protects plants against three Powdery mildew pathogens. *European Journal of Plant Pathology*, 131(3), 393–401. https://doi.org/10.1007/s10658-011-9816-0

Jaulneau, V., Lafitte, C., Jacquet, C., Fournier, S., Salamagne, S., Briand, X., Esquerré-Tugayé, M.-T., & Dumas, B. (2010). Ulvan, a Sulfated Polysaccharide from Green Algae, Activates Plant Immunity through the Jasmonic Acid Signaling Pathway. *Journal of Biomedicine and Biotechnology*, 2010, 1–11. https://doi.org/10.1155/2010/525291

Jermini, M., Blaise, P., & Gessler, C. (2020). Quantitative effect of leaf damage caused by Downy mildew (*Plasmopara viticola*) on growth and yield quality of grapevine "Merlot" (*Vitis vinifera*). *Vitis*, 49 (2). https://doi.org/10.5073/vitis.2010.49.77-85

Jindo, K., Goron, T. L., Pizarro-Tobías, P., Sánchez-Monedero, M. Á., Audette, Y., Deolu-Ajayi, A. O., Van Der Werf, A., Goitom Teklu, M., Shenker, M., Pombo Sudré, C., Busato, J. G., Ochoa-Hueso, R., Nocentini, M., Rippen, J., Aroca, R., Mesa, S., Delgado, M. J., & Tortosa, G. (2022). Application of biostimulant products and biological control agents in sustainable viticulture: A review. *Frontiers in Plant Science*, 13, 932311. https://doi.org/10.3389/fpls.2022.932311

Koledenkova, K., Esmaeel, Q., Jacquard, C., Nowak, J., Clément, C., & Ait Barka, E. (2022). *Plasmopara viticola* the Causal Agent of Downy Mildew of Grapevine: From Its Taxonomy to Disease Management. *Frontiers in Microbiology*, 13, 889472. https://doi.org/10.3389/fmicb.2022.889472

Krzyzaniak, Y., Trouvelot, S., Negrel, J., Cluzet, S., Valls, J., Richard, T., Bougaud, A., Jacquens, L., Klinguer, A., Chiltz, A., Adrian, M., & Héloir, M.-C. (2018). A Plant Extract Acts Both as a Resistance Inducer and an Oomycide Against Grapevine Downy Mildew. *Frontiers in Plant Science*, 9, 1085. https://doi.org/10.3389/fpls.2018.01085

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., & Aubertot, J.-N. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture. A review. *Agronomy for Sustainable Development*, 38(3), 28. https://doi.org/10.1007/s13593-018-0503-9

Landi, L., De Miccolis Angelini, R. M., Pollastro, S., Feliziani, E., Faretra, F., & Romanazzi, G. (2017). Global Transcriptome Analysis and Identification of Differentially Expressed Genes in Strawberry after Preharvest Application of Benzothiadiazole and Chitosan. *Frontiers in Plant Science*, 8. https://doi.org/10.3389/fpls.2017.00235

Liu, X., Bai, Y., & Zhang, Z. (2022). Priming defense by transiently suppressing plant susceptibility genes in ornamental crops: A novel strategy for post-harvest diseases management. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.1025165

Llamazares De Miguel, D., Mena-Petite, A., & Díez-Navajas, A. M. (2022). Toxicity and Preventive Activity of Chitosan, Equisetum arvense, Lecithin and Salix Cortex against *Plasmopara viticola*, the Causal Agent of Downy Mildew in Grapevine. *Agronomy*, 12(12), 3139. https://doi.org/10.3390/agronomy12123139

Mauch-Mani, B., Baccelli, I., Luna, E., & Flors, V. (2017). Defense Priming: An Adaptive Part of Induced Resistance. *Annual Review of Plant Biology*, 68(1), 485–512. https://doi.org/10.1146/annurev-arplant-042916-041132

Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science*, 9(10), 490–498. https://doi.org/10.1016/j.tplants.2004.08.009

Molitor, D., Biewers, B., Junglen, M., Schultz, M., Clementi, P., Permesang, G., Regnery, D., Porten, M., Herzog, K., Hoffmann, L., Beyer, M., & Berkelmann-Löhnertz, B. (2018). Multi-annual comparisons demonstrate differences in the bunch rot susceptibility of nine *Vitis vinifera* L. "Riesling" clones. *Vitis*, 57 (1), 17-25. https://doi.org/10.5073/VITIS.2018.57.17-25

Nascimento, R., Maia, M., Ferreira, A. E. N., Silva, A. B., Freire, A. P., Cordeiro, C., Silva, M. S., & Figueiredo, A. (2019). Early stage metabolic events associated with the establishment of *Vitis vinifera – Plasmopara viticola* compatible interaction. *Plant Physiology and Biochemistry*, 137, 1–13. https://doi.org/10.1016/j.plaphy.2019.01.026

Ninkuu, V., Yan, J., Fu, Z., Yang, T., Ziemah, J., Ullrich, M. S., Kuhnert, N., & Zeng, H. (2022). Lignin and Its Pathway-Associated Phytoalexins Modulate Plant Defense against Fungi. *Journal of Fungi*, 9(1), 52. https://doi.org/10.3390/jof9010052

Noctor, G., & Foyer, C. H. (1998). Ascorbate and Gluthatione: Keeping Active Oxygen Under Control. *Annual Review of Plant Physiology and Plant Molecular Biology*, 49(1), 249–279. https://doi.org/10.1146/annurev.arplant.49.1.249

Nogueira Júnior, A. F., Tränkner, M., Ribeiro, R. V., Von Tiedemann, A., & Amorim, L. (2020). Photosynthetic Cost Associated with Induced Defense to *Plasmopara viticola* in Grapevine. *Frontiers in Plant Science*, 11, 235. https://doi.org/10.3389/fpls.2020.00235

Özden, M., Demirel, U., & Kahraman, A. (2009). Effects of proline on antioxidant system in leaves of grapevine (Vitis vinifera L.) exposed to oxidative stress by H2O2. Scientia Horticulturae, 119(2), 163-168. https://doi.org/10.1016/j.scienta.2008.07.031

Pagliarani, C., Moine, A., Chitarra, W., Meloni, G. R., Abbà, S., Nerva, L., Pugliese, M., Gullino, M. L., & Gambino, G. (2020). The Molecular Priming of Defense Responses is Differently Regulated in Grapevine Genotypes Following Elicitor Application against Powdery Mildew. *International Journal of Molecular Sciences*, 21(18), 6776. https://doi.org/10.3390/ijms21186776

Paolacci, A. R., Catarcione, G., Ederli, L., Zadra, C., Pasqualini, S., Badiani, M., Musetti, R., Santi, S., & Ciaffi, M. (2017). Jasmonate-mediated defence responses, unlike salicylate-mediated responses, are involved in the recovery of grapevine from bois noir disease. *BMC Plant Biology*, 17(1), 118. https://doi.org/10.1186/s12870-017-1069-4

Paolocci, M., Muganu, M., Alonso-Villaverde Iglesias, V., & Gindro, K. (2014). Leaf morphological characteristics and stilbene production differently affect Downy mildew resistance of *Vitis vinifera* varieties grown in Italy. *Vitis* 53 (3), 155–161. http://hdl.handle.net/10261/114410

Perazzolli, M., Moretto, M., Fontana, P., Ferrarini, A., Velasco, R., Moser, C., Delledonne, M., & Pertot, I. (2012). Downy mildew resistance induced by Trichoderma harzianum T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. *BMC Genomics*, 13(1), 660. https://doi.org/10.1186/1471-2164-13-660

Pérez-López, U., Miranda-Apodaca, J., Muñoz-Rueda, A., & Mena-Petite, A. (2015). Interacting effects of high light and elevated CO2 on the nutraceutical quality of two differently pigmented Lactuca sativa cultivars (Blonde of Paris Batavia and Oak Leaf). *Scientia Horticulturae*, 191, 38–48. https://doi.org/10.1016/j.scienta.2015.04.030

Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Muñoz-Rueda, A., Navari-Izzo, F., & Mena-Petite, A. (2009). The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO2. *Physiologia Plantarum*, 135(1), 29-42. https://doi.org/10.1111/j.1399-3054.2008.01174.x

Rantsiou, K., Giacosa, S., Pugliese, M., Englezos, V., Ferrocino, I., Río Segade, S., Monchiero, M., Gribaudo, I., Gambino, G., Gullino, M. L., & Rolle, L. (2020). Impact of Chemical and Alternative Fungicides Applied to Grapevine cv Nebbiolo on Microbial Ecology and Chemical-Physical Grape Characteristics at Harvest. *Frontiers in Plant Science*, 11, 700. https://doi.org/10.3389/fpls.2020.00700

Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J. J., & Morales, F. (2012). Climate change (elevated CO2, elevated temperature and moderate drought) triggers the antioxidant enzymes' response of grapevine cv. Tempranillo, avoiding oxidative damage. Physiologia plantarum, 144(2), 99-110. https://doi.org/10.1111/j.1399-3054.2011.01524.x

Samuels, L. J., Setati, M. E., & Blancquaert, E. H. (2022). Towards a Better Understanding of the Potential Benefits of Seaweed Based Biostimulants in *Vitis vinifera* L. Cultivars. *Plants*, 11(3), 348. https://doi.org/10.3390/plants11030348

Sangha, J. S., Kelloway, S., Critchley, A. T., & Prithiviraj, B. (2014). Seaweeds (Macroalgae) and Their Extracts as Contributors of Plant Productivity and Quality. *Advances in Botanical Research*, 71, 189–219. https://doi.org/10.1016/B978-0-12-408062-1.00007-X

Taillis, D., Pébarthé-Courrouilh, A., Lepeltier, É., Petit, E., Palos-Pinto, A., Gabaston, J., Mérillon, J. M., Richard, T., & Cluzet, S. (2022). A grapevine by-product extract enriched in oligomerised stilbenes to control downy mildews: Focus on its modes of action towards *Plasmopara viticola*. Oeno One, 56(3), 55-68. https://doi.org/10.20870/oeno-one.2022.56.3.4911

Van Loon, L. C., Rep, M., & Pieterse, C. M. J. (2006). Significance of Inducible Defense-related Proteins in Infected Plants. *Annual Review of Phytopathology*, 44(1), 135–162. https://doi.org/10.1146/annurev.phyto.44.070505.143425

Vrhovsek, U., Malacarne, G., Masuero, D., Zulini, L., Guella, G., Stefanini, M., Velasco, R., & Mattivi, F. (2012). Profiling and accurate quantification of trans-resveratrol, trans-piceid, trans-pterostilbene and 11 viniferins induced by *Plasmopara viticola* in partially resistant grapevine leaves: Stilbenoid viniferins in infected grapevine leaves. *Australian Journal of Grape and Wine Research*, 18(1), 11–19. https://doi.org/10.1111/j.1755-0238.2011.00163.x

Zarraonaindia, I., Cretazzo, E., Mena-Petite, A., Díez-Navajas, A. M., Pérez-López, U., Lacuesta, M., Pérez-Álvarez, E. P., Puertas, B., Fernández-Diaz, C., Bertazzon, N., & Cantos-Villar, E. (2023). Holistic understanding of the response of grapevines to foliar application of seaweed extracts. *Frontiers in Plant Science*, 14, 1119854. https://doi.org/10.3389/fpls.2023.1119854