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The Effect of Paraffin Oil Spraying and Powdery Mildew Infection on Leaf Gas Exchange and Yield of Chardonnay and Kékfrankos (*Vitis vinifera* L.) in Hungary

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Abstract: Various oils can efficiently control a wide range of pests and pathogens on plants. In this study, we tested the effectiveness solely applied paraffin oil (PFO) spraying against *Erysiphe necator*, the causal agent of grape powdery mildew (GPM). Its effects on gas exchange and yield at harvest were also investigated. Experiments were conducted in Eger between 2013 and 2014 with two cultivars (Kékfrankos and Chardonnay) showing differing susceptibility to GPM. Treatments with 2.2 and 3.3 *v*/*v*% PFO spraying inhibited GPM; however, this effect was influenced by the individual resilience of the variety and the infection pressure of the vintage. The PFO treatment caused decreased leaf gas exchange parameters compared to conventional treatment. The berry yield was also lower in PFO-treated parcels, although yield may be affected by numerous factors and requires further investigation. The mineral oils may have a phytotoxic effect on the grapevine through impairment of the photosynthetic performance, although this effect cannot be connected to a particular PFO concentration according to our research results. This negative effect of PFO highly depends on the ampelographic characteristics of the examined cultivars and vintage. In addition, the physical properties of the spraying agent may also play an important role.

Keywords: *Vitis vinifera*; Chardonnay; Kékfrankos (Blaufränkisch); paraffin oil; GPM control; gas exchange; extrinsic WUE; cultivar-specific resilience

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1. Introduction

One of the most considerable challenges of the 21st century is climate change. The increased frequency of extreme meteorological events due to climate change combined with anthropogenic effects (such as international trade and environmental pollution) negatively influence plant health [1,2]. The use of pesticides has intensified to ensure an adequate quantity and quality of food supplies for the growing population, leading to increased costs, environmental load, and risk of chemical contamination in the food chain [3]. Grapevine is one of the most important and most intensively sprayed crops worldwide [4,5]; an average of 35% of produced pesticides are applied in viticulture [6] covering a growing area of an estimated 7.4 mha in 2018 [7]. Therefore, the optimization of pesticide use in viticulture is of particular importance, although this goal is hampered by several factors. The actual quantity and type of applied chemicals depend on the climatic characteristics of the vintage and the relative occurrence of each pathogen and pest that can affect the grapevine [4].

As key fungal pathogens, problem in viticulture are caused by *Erysiphe necator* (grape powdery mildew, GPM) and *Plasmopara viticola* (grape downy mildew, GDM) [5]. *P. viticola* infection can result in 70% yield losses without chemical control [8]. However, it is not easy

to determine yield loss caused by GPM and GDM, owing to several factors [9]. For example, the susceptibility of berries to GPM significantly changes with the host developmental stage; for example, an infection of young berries may result in as much as a 45% decrease in yield [10,11]. These two diseases can influence gas exchange drastically by reducing the photosynthesizing leaf area [12]; the leaf assimilation rate of visibly damaged leaves or leaf parts also decreases in the case of asymptomatic green surfaces [13]. These effects lead to decreased yields and quality, as well as reduced vine size [12]. Nevertheless, asymptomatic leaves may compensate for the photosynthetic deficit, although this phenomenon has not been verified in grapevine [12].

Various fungicides can be applied to control GPM and GDM, and vine canopy management also plays an important role [8,10]. However, the use of fungicide to avoid the potential risk and the harmful effects of these foliar infections can be overwhelming [14]; the occurrence of fungicide resistance has been reported worldwide [15,16]. Therefore, agricultural surveys have focused on the possibility of using environmentally friendly products alone and/or combined with conventional fungicides, such as alternative organic or inorganic agents, resistance inducers, and biostimulants [5,17,18]. Horticultural oil, as natural products, can be applied efficiently to control a wide range of insects and pests on various crops with less harmful effects on the ecosystem than chemical-based pesticides [19,20]. The fungus *E. necator*, as an epidermal parasite [21], seems to be an ideal target of PDSOs (petroleum-derived spray oils), such as JMS Stylet-oil (97.1% paraffinic oil) with multiple means of application: (1) wash spraying, (2) as an additive, (3) sole fungicide treatment, or as rotation partner for commonly used agents [20,22–25]. Furthermore, PDSOs showed pre-and post-lesion curative action against GPM with an antisporulation effect in greenhouse experiments [26]. In addition to alternative sprays, precision viticulture plays an increasingly important role considering the characteristics of the cultured grapevine varieties and terroir [27]. *Vitis vinifera* cultivars are typically sensitive to *E. necator*, with minor differences between leaves and clusters depending on the cultivar and environmental conditions [28,29]. Therefore, it can be particularly relevant to deal with the individual resilience of grape cultivars and forecasts of ontogenic resistance, which may help to determine the optimal frequency and rate of sprayings according to local characteristics [27,30].

The aim of the present study was to test high-purity paraffin oil (PFO) (Ovispray EC, Total Fluids, France) on grapevine against GPM. The active ingredient of Ovispray is 800 g/L PFO with extremely low aromatic and sulfur contents (>99% USR), suggesting a low phytotoxicity [20]. This product is not recommended for use if the ambient temperature is above 32 °C and/or the relative humidity drops under 30%, or in combination with sulfur-containing compounds, as is generally the case for most horticultural oils [20]. When testing the effectiveness of an alternative fungicide, it is required to examine how plants respond to spraying treatment. Fungicides could also impair physiological processes in plants; nevertheless, the related literature data are controversial as they relate to several cultivars and fungicides through examination of a few physiological parameters [31]. For example, fungicides may stimulate photosynthetic activity and enhance carbohydrate availability in grapevine [32], or they may negatively affect photosynthetic function through a decrease in net CO_2 assimilation [33]. PDSOs were also found to be phytotoxic in high concentrations, which may have resulted from the inhibition of photosynthesis and transpiration [34]. JMS Stylet-oil (JSO) may negatively influence gas exchange in grapevine, primarily through stomatic behavior, depending on the volume and frequency of spraying [35]. Another report suggests that oils induce a heating effect of leaf tissues under high air temperatures, which may lead to non-stomatal inhibition of photosynthesis [36]. Reduced berry and cluster weights and delayed sugar accumulation were observed in wine grapes subjected to oil sprayings [35,37]. While maintaining the fungicide efficiency, this phytotoxic effect can be reduced by adjusting the appropriate dose and spray volume with the optimal timing of sprayings [20,38,39].

Our knowledge of the connection between phytotoxicity and the activity of PFO to control fungal diseases suggests an opposition between these two effects on plant physiological state (e.g., photosynthetic activity) and the harvest yield. We hypothesized that the balance between these effects (and the optimal dosage of PFO) is also affected by vintage characteristics (with distinct infection probability) and host characteristics (differing susceptibility of cultivars toward PFO toxicity and infection) in the case of grapevine–*E. necator* interaction under field conditions. The focus of the current study was GPM, owing to the penetration and colonization properties of its causative agent, as well as the applicability of PDSOs against GPM, as previously detailed. To test our hypothesis, a spraying experiment was conducted in 2013 and 2014 on two grapevine varieties (*V. vinifera* L. Chardonnay and Kékfrankos) with differing sensitivity to GPM under field-grown conditions in the Eger wine region (Hungary). We assumed that the efficiency of sole applied PFO against GPM for disease management may be lie between the outcome of unsprayed and conventional treatments. Therefore, to assess the effect of PFO on the host plant, PFO was applied in three dosages in the presence of two control treatments: a negative (untreated) and a positive control treated with conventional pesticides (fungicides and agents against pests). The tested oil product has an inhibitory effect against arthropod pests; thus, non-sprayed and PFO-treated plants were not sprayed with agents specifically targeting these pests. The aim of our study was to reveal a possible relationship between the efficiency of PFO treatments, leaf gas exchange parameters, and yield parameters.

2. Materials and Methods

2.1. Experimental Design and Spraying

Spraying was carried out in 2013 and 2014 in the experimental area of the vineyard of Eszterházy Károly Catholic University, Kőlyuktető, Eger, Hungary. The PFO was tested on two grapevine varieties (*Vitis vinifera* L. cv. Chardonnay and Kékfrankos) with differing susceptibility against GPM. Chardonnay has crunchy berries with a thin wax layer [40,41]. The fruits of this variety are sensitive to fungal infection and rotting because they can easily split, especially in rainy weather. Infection with *E. necator* may also cause berry cracking [10]. Kékfrankos (*syn*. Limberger, Blaufränkisch) berries with thick wax layers are moderately or less susceptible to *E. necator* infection. However, the leaves of this red cultivar are more sensitive to GPM and GDM compared to Chardonnay leaves, which have dense, prostrate hairs on the lower side of the leaf blades [40–44].

The experimental area was on ~0.25 hectares for each variety, with a random block design, including two buffer rows separated from the other parts of the vineyard. Each block contained 20 vine stocks. The vines were 0.7 m apart, with a distance of 2 m between each row. Five different treatments were applied in three replicates (blocks) on both grape cultivars. The negative control (C) was unsprayed (no chemical treatment was applied). The positive control (CT) was treated according to a conventional spraying protocol depending on vintage characteristics, forecasts of infection and pests, as well as the phenological stage of the grape. The PFO was solely applied at the same time as the CT treatment in three different dosages: D1 (1.1 v/v%), D2 (2.2 v/v%), and D3 (3.3 v/v%). The sprayed amount was in the range of 150–450 L/ha depending on the developmental stage of the canopy. The applied PFO treatment dosages were determined based on unofficial results of European (France and Spain) field spraying trials conducted by the manufacturer. The sprayings were carried out with a Stihl SR 430 backpack sprayer in the experimental area. Figure 1 shows the date of sprayings, including the dates of the main phenological stages of grapevine, harvests, and the gas exchange measurements. Table A1 summarizes the pesticides applied in CT treatments. The meteorological data (e.g., the monthly average air temperature and the sum of precipitation) were monitored by an automatic agrometeorological station (Boreas Ltd., Hungary) in each experimental year. These data were compared to the average of the last 50 years. The yearly average air temperature (T), total precipitation (mm), and total effective heat sum (DD°) were also determined in both examined years.

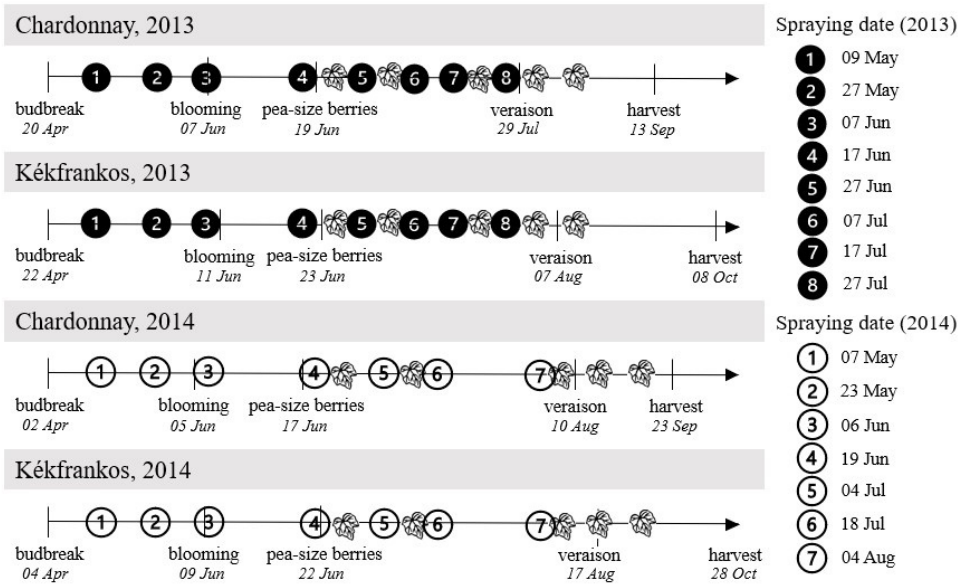

Figure 1. Dates of main phenological stages, harvests, sprayings, and gas exchange measurements (marked with leaf symbols) for both cultivars and years.

2.2. Gas Exchange Measurements, Monitoring of GPM Symptom Intensity, and Yield Parameters

The gas exchange measurements (leaf transpiration rate (E, mol H_2O m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), leaf assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), and intercellular CO_2 concentration (C_i, CO_2 ppm) were performed with a portable Ciras-1 infrared gas-analyzer (PP Systems, Hitchin, UK) equipped with a round-shaped Parkinson's leaf cuvette (2.5 cm^2). The mid-day measurements were carried out for at least 5 time points in a growing season between June and the end of August, depending on the local weather conditions: in cloudless, windless weather between 12:00 and 14:00 h, when photosynthetically active radiance (PAR) was at least 1000 µE [13,35,45–47]. In practice, the gas exchange measurements were executed within 1 h for each variety. There were no remarkable differences between the treatments in terms of leaf temperature during the measurements. Mean PAR values ranged between 1200 and 2000 µE when surveys were carried out. Measurements were conducted on 7–10 plants in each treatment and at each sampling time. Mature, intact leaves with full exposure to sun and dry surfaces were randomly selected from the middle canopy level (approx. 10–11 leaves). The reference CO_2 level of the gas analyzer was set according to the ambient atmospheric CO_2 concentration in both years, ranging from 380 to 400 ppm. The reference H_2O concentration corresponds to 70% of the ambient H_2O, and the airflow through the leaf chamber was 200 cm^3/min. Instantaneous (extrinsic) water-use efficiency (*WUE*, µmol CO_2 mol^{-1} H_2O) was also calculated (A/E) [48].

The intensity of GPM symptoms was estimated by visual inspection based on a modified method described by R. W. Emmett [49] near harvest with randomly selected 50–50 leaves and clusters in the case of each treatment [50]. The GPM intensity data refers to the percentage coverage of visible GPM symptoms relative to the surface area of each examined plant part [50]. The yield parameters of experimental parcels (blocks) were estimated on 3-3 vine stocks with an average loading. These measurements were carried out at harvest by measuring the cluster weight and counting the number/stocks in the case of each treatment.

2.3. Statistical Analysis

Data (mean with SD) were analyzed with GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com (accessed on 16 October 2018)). In the case of gas exchange parameters and calculated extrinsic *WUE*, a grouped analysis

method (mixed-effect analysis) was applied, owing to the imbalanced nature of the dataset. In this case, the results of the mixed-effect analysis can be interpreted as a repeated-measures (two-way) ANOVA. This method is suitable for examination of which factors (measuring time, treatment, or both) might influence the results of the experiments in the case of gas exchange parameters. Tukey's multiple comparison test was applied to compare all treatments on each measurement date for each parameter, variety, and year. Correlation analysis was carried out between these gas exchange parameters: the correlation between mean g_s and A, E, or C_i datasets, as well as between the mean A and C_i datasets. These correlation examinations were performed separately for all five measurement dates for each treatment, variety, and year. The Pearson correlation coefficients were computed by Prism. One-way ANOVA with Tukey's multiple comparison test was applied in the case of GPM intensity, as well as yield parameters.

3. Results and Discussion

3.1. Climatic Conditions of the Experimental Years (2013–2014)

The climatic conditions of the examined years influenced the characteristics of the vintage, especially in terms of fungal infection pressure and the harvest yield. In 2013, the air temperature was lower (except in January and February) in the first half of the year relative to data from the last 50 years. The mean air temperature was 16.4 °C in May and 20 °C in June, with a high amount of precipitation during the flowering and berry set growth stages (159 and 131 mm, respectively) (Figure 2A). The pattern of the rainfall was irregular: 76% of the yearly precipitation fell in the first part of the year. A sudden increase in temperature from the end of June after a rainy period resulted in high air humidity and favorable conditions for fungal infections. However, precipitation decreased after July, and the air temperature was high (22.8 °C) compared to the mean data (21.7 °C) of the last 50 years. July and August (22.8 °C) were especially hot and dry; 6.5 mm of precipitation fell in July and 27.1 mm in August. These conditions did not lead to severe fungal (particularly GPM) infections in the growing and ripening stages of the grapevine in 2013 [50].

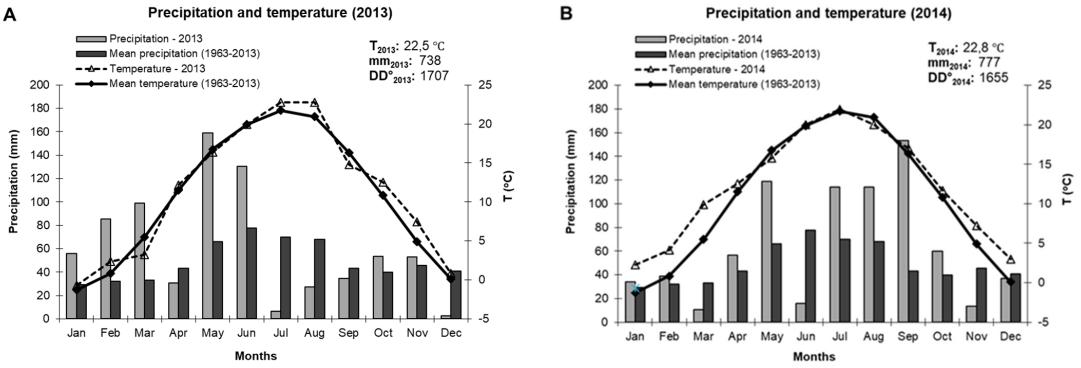

Figure 2. (**A**,**B**) Average air temperature (lines) and the monthly sum of precipitation (columns) in 2013 (**A**) and 2014 (**B**) compared to the data of the last 50 years. The characteristics of the vintage are summarized: average air temperature (T), total precipitation (mm), and total effective heat sum (DD°).

In 2014, generally high mean air temperatures (Figure 2B) were measured, except in May (15.8 °C) and August (20.0 °C), compared to the mean data of the last 50 years (16.8 and 20.9 °C, respectively). This year had an uneven distribution of annual rainfall, with 65% of the yearly precipitation falling between May and September (except in June, with 15.6 mm). April (56.7 mm) and October (60.0 mm) were also rainy. The winter was mild in both years, which generally favored the overwintering of pathogens and pests. The high amount of precipitation negatively affected the blooming and berry set [51], promoting the development of the canopy. The year 2014 was humid due to the high mean air temperature and considerable amount of precipitation. Therefore, the 2014 vintage was strongly hazardous in terms of fungal diseases (e.g., GPM), despite the sprayings [50].

3.2. Effects of PFO Treatments on GPM Infection

GPM infection data for the experimental setup examined in the present study were published in a previous paper [50]; therefore, only the main findings are presented here for a better understanding of the results. In general, the ANOVA results showed that the differences between the GPM intensity values in different treatments were the result of treatments rather than random sampling ($p < 0.05$). In 2013, all treated parcels showed significantly lower ($p < 0.0001$) GPM intensity regardless of the examined plant part or variety compared to the negative control (C). However, lower dosages of paraffin oil (D1 and D2) resulted in about twofold higher values ($p < 0.05$) relative to the very low values of D3 and CT parcels in the case of Chardonnay clusters. The higher sensitivity of Kékfrankos leaves to GPM was manifested in 2013. The mean values of GPM intensity were significantly ($p < 0.0005$) higher in PFO treatments than in the CT treatments, with a small but significant ($p < 0.05$) decrease in the case of D3 relative to D1 and D2. The differing susceptibility of the examined varieties to GPM was expressed in the GPM intensity data of untreated (C) parcels in 2013; the clusters showed about twofold lower mean values in Kékfrankos than in Chardonnay ($p < 0.05$). However, the leaves showed similar GPM intensity in the two varieties. The PFO treatments showed significantly ($p < 0.0001$) higher (four to seven times) GPM intensity values than CT in Chardonnay clusters. Moreover, significant differences ($p < 0.0001$) were observed in GPM intensity between the oil treatments, with a negative correlation to PFO dosage. In the case of Kékfrankos clusters, the lowest values were associated with CT, D3, and D2. The D1 treatment also showed a lower but significant ($p < 0.05$) disease control capability relative to the C treatment [50].

In 2014, the pressure of GPM infection was high, especially in clusters of both varieties. The leaves of Kékfrankos could not be evaluated as a result of weather conditions and subsequent harvest. A significant twofold difference ($p < 0.05$) was observed only between the GPM intensity of C and CT in the case of Chardonnay leaves, with a lower value for the latter condition. These numbers of PFO treatments fell between the C and CT values. In the case of Chardonnay clusters, CT was significantly ($p < 0.0001$) less infected than parcels with other treatments. The C treatment showed more than 90% GPM intensity, and the same parameter fell between the values of C and CT in the case of PFO treatments. The untreated (C) plants also showed a similarly high GPM intensity in Kékfrankos clusters. The CT treatment resulted in significantly lower ($p < 0.0001$) intensity relative to C and PFO treatments [50].

In summary, PFO showed varying efficiency in the examined years. The highest dosage of PFO (D3) exhibited a similar effectiveness against GPM to CT in 2013, with low GPM risk. This effect was observed especially in the case of less sensitive plant parts of the given variety, notably Chardonnay leaves and Kékfrankos clusters. The oil treatments were not as effective in 2014, owing to the high GPM pressure. The possible dosage-dependent differences between PFO treatments and the individual susceptibility of plant parts to GPM were less manifested this year. The D1 treatment was inefficient in reducing GPM infection in both years [50].

3.3. Effects of PFO Treatments on Gas Exchange Parameters

According to the mixed-effect analysis, the measuring times (as row factor) and the treatments (as column factor) had a significant ($p < 0.0001$) influence on the mean value of main gas exchange parameters (g_s, A, and E) in the case of each variety and year. The results are summarized in Figure 3A–F and Figure 4A–F. Stomatal conductance (g_s) had a considerable effect on leaf assimilation (A) and leaf transpiration rate (E) [10]. However, many environmental and in planta factors can affect stomatal closure, as well as photosynthetic performance, such as photosynthetic limitation and non-stomatal causes [52], as previously described in the case of field-grown grapevine under water deficit conditions [53]. In our study, the gas exchange values showed differences according to the vintages and varieties, as well as the efficacy of PFO treatments. In general, the mean g_s and A values were recorded in a higher range in both varieties in 2013 compared to 2014 (Figures 3 and 4),

indicating a higher impact of treatments on these values in the previous year with lower disease pressure [50]. In both experimental years and cultivars, the mean g_s, A, and E values of PFO-treated plants were between C and CT; however, they fell closer to the C treatment, with the highest values of D3 in 2014 (Figure 4). These results suggest that PFO might have a negligible negative effect—or at least lower than GPM—on the photosynthetic performance of grapevine leaves but also highlight the limitation of its applicability in the case of vintages with high disease pressure.

Kékfrankos leaves showed a dose-dependent positive response to PFO treatments in 2014 (Figure 4D–F), in contrast to Chardonnay (Figure 4A–C). This difference might be due to the lower susceptibility of the latter cultivar [40–44]. Moreover, Chardonnay leaves have dense prostrate hair on the abaxial surface of the leaf blades [36,37], which may prevent the PFO from reaching the leaf epidermis and possibly triggering defense responses in the grapevine.

The data of g_s-E correlation analysis (Table 1) also support the negative effect of GPM infection rather than PFO on E through a change in g_s [13]. In 2013, the control treatments (C and CT) showed no significant correlation in the g_s-E relation in each cultivar, whereas this correlation was significant ($p < 0.05$ or 0.005) in 2014 for C parcels, indicating higher disease pressure [50].

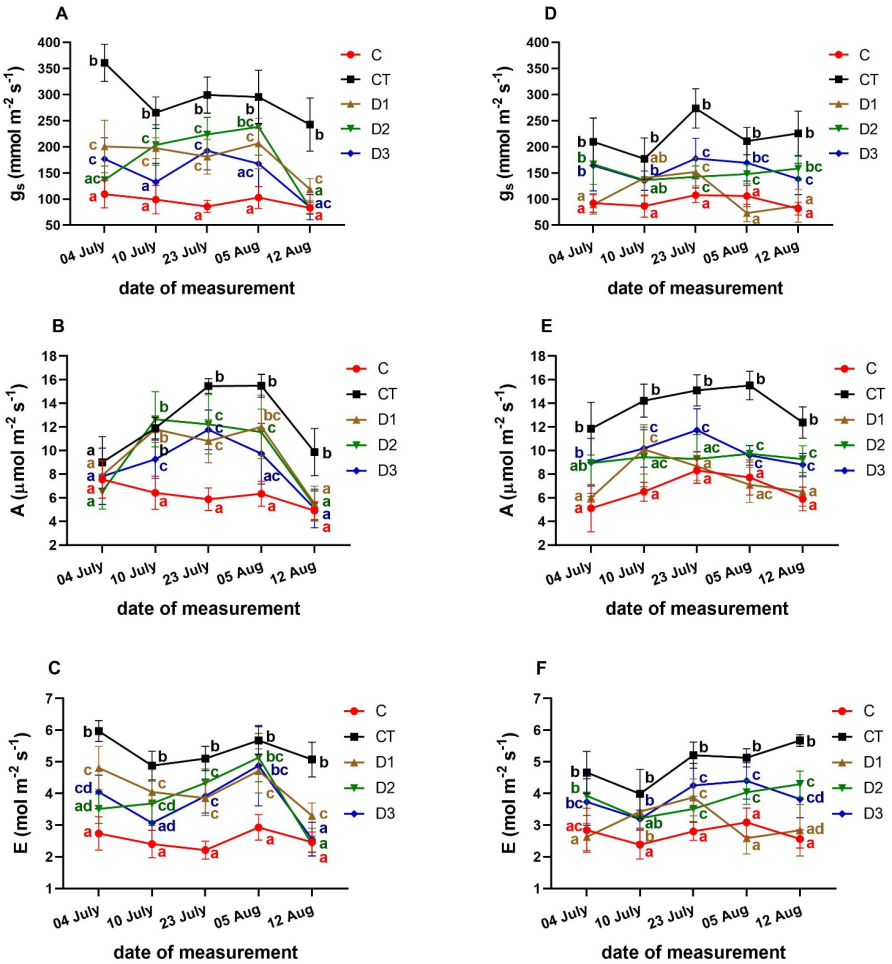

Figure 3. (**A**–**F**) Effects of treatments on gas exchange parameters on Chardonnay (**A**–**C**) and Kékfrankos (**D**–**F**) varieties in 2013: g_s (mmol m^{-2} s^{-1})—stomatal conductance (**A**,**D**), A (µmol CO_2 m^{-2} s^{-1})—assimilation rate (**B**,**E**), and E (mol H_2O m^{-2} s^{-1})—transpiration rate (**C**,**F**) during the growing seasons. Each symbol represents the mean value ± standard deviation (SD). Significance groups of each measurement date are marked with letters ($p < 0.05$). The color of the letters corresponds to the color of the treatments.

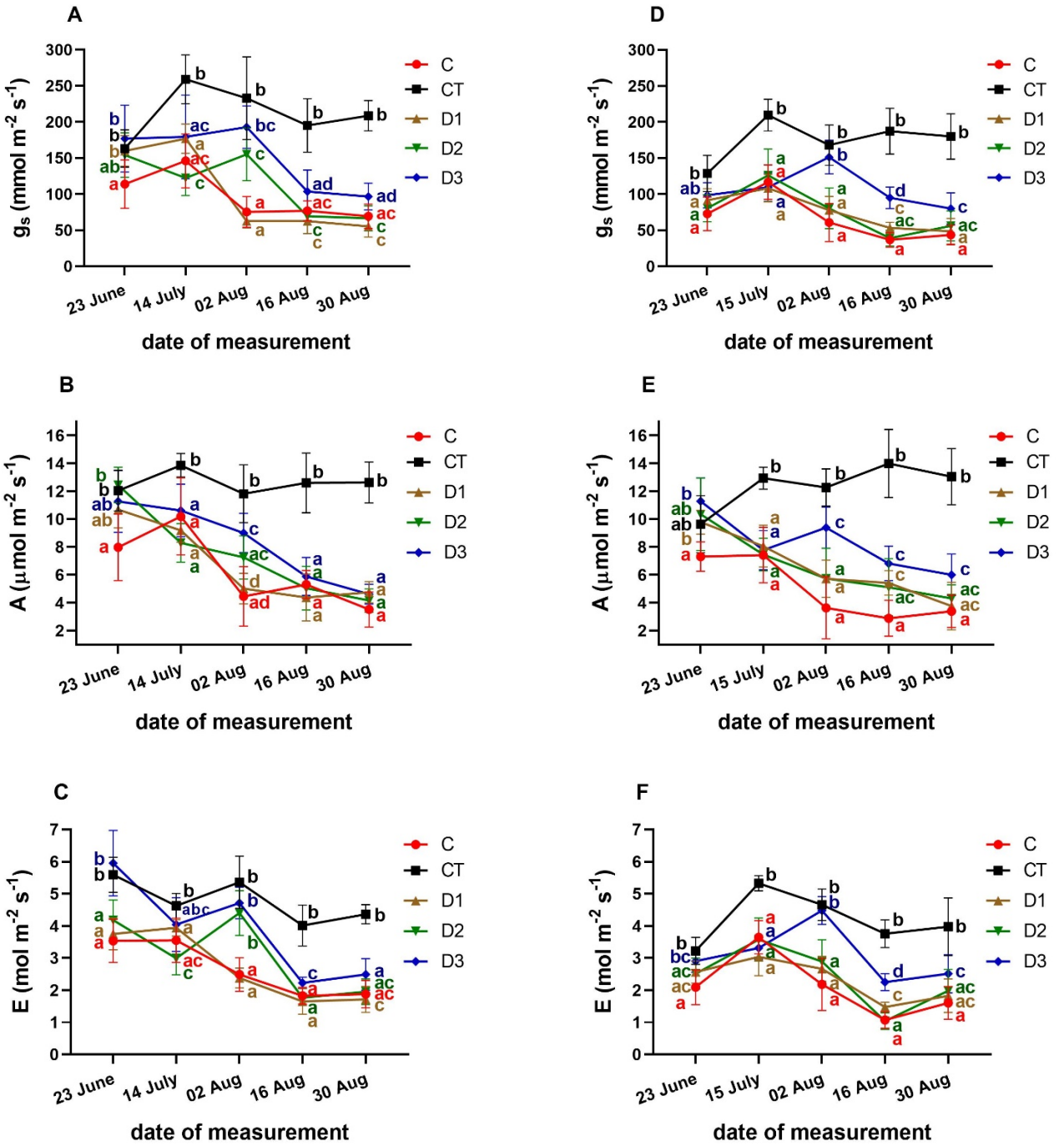

Figure 4. (**A**–**F**) Effects of treatments on gas exchange parameters on Chardonnay (**A**–**C**) and Kékfrankos (**D**–**F**) varieties in 2014: g_s (mmol m^{-2} s^{-1})—stomatal conductance (**A**,**D**), A (µmol CO_2 m^{-2} s^{-1})—assimilation rate (**B**,**E**), and E (mol H_2O m^{-2} s^{-1})—transpiration rate (**C**,**F**) during the growing seasons. Each symbol represents the mean value ± standard deviation (SD). Significance groups of each measurement date are marked with letters ($p < 0.05$). The color of the letters corresponds to the color of the treatments.

Lower PFO dosages (D1, D2) failed to prevent damage to the photosynthetic system, as indicated by the significant ($p < 0.05$) g_s-E correlations in both years and cultivars. This phenomenon was more distinctive in 2014 than in 2013. The g_s-E correlation was absent in D3 treatments, except for Kékfrankos in 2014. The effectiveness of D3 against GPM may have played greater role than its negative effects on the gas exchange on grapevine leaves.

Kékfrankos leaves did not show a positive correlation between g_s and A, regardless of treatment or vintage (Table 1). In contrast, a significantly positive correlation was detected in Chardonnay leaves in the case of C and D2 treatments ($p < 0.05$) in 2013, as well as in the case of D1 and D3 ($p < 0.05$) in 2014. These results indicate the negative effect of GPM infection on gas exchange and highlight the differences between Chardonnay and Kékfrankos leaves. Baudoin et al. [46] reported that JSO coverage and retention ability on the lower surface of hypostomatous grapevine leaves [54] determine the extent of its negative effects on photosynthesis and, presumably, its ability to trigger defense responses [55]. This phenomenon may explain the greater effect of GPM infection on g_s-A correlations on Chardonnay leaves in PFO-treated parcels, despite their lower susceptibility to GPM infection. Our results indicate a possible minimally phytotoxic effect of PFO on the grapevine, in contrast to the results reported in a previous study suggesting a maximum recommended application dosage [35]. However, the phytotoxic effects of oils are also influenced by their physical properties. Low-viscosity oils can penetrate into the leaf and may cause intense photosynthetic inhibition, whereas more viscous oils remain on the leaf surface [12,56]. The PFO formulation used in this study was distributed in small, separate spots on the leaf surface and did not diffuse deeper than the first layer of epidermal cells, suggesting a very limited direct effect on the leaves [55].

Table 1. Results of the correlation analysis between mean stomatal conductance (g_s) and leaf assimilation rate (A) data or leaf transpiration rate (E) data in the case of each treatment, variety, and year, with 5-5 measurement dates/year. Asterisks mark the significance of differences (* $p < 0.05$, ** $p < 0.005$), ns: not significant.

Chardonnay 2013	g_s **vs.** A					g_s **vs.** E				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	0.921	−0.114	0.787	0.940	0.821	0.730	0.833	0.870	0.930	0.806
p value	0.026	0.854	0.114	0.017	0.088	0.161	0.079	0.055	0.020	0.099
Significance	*	ns	ns	*	ns	ns	ns	ns	*	ns
Kékfrankos 2013	g_s **vs.** A					g_s **vs.** E				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	0.793	0.208	0.834	−0.651	0.467	0.775	0.677	0.970	0.761	0.807
p value	0.109	0.737	0.079	0.234	0.428	0.123	0.209	0.006	0.135	0.090
Significance	ns	ns	ns	ns	ns	ns	ns	**	ns	ns
Chardonnay 2014	g_s **vs.** A					g_s **vs.** E				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	0.987	0.606	0.955	0.815	0.909	0.899	−0.210	0.969	0.985	0.865
p value	0.001	0.278	0.011	0.092	0.032	0.038	0.734	0.006	0.002	0.058
Significance	**	ns	*	ns	*	*	ns	**	**	ns
Kékfrankos 2014	g_s **vs.** A					g_s **vs.** E				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	0.859	0.864	0.833	0.498	0.442	0.979	0.760	0.929	0.957	0.948
p value	0.062	0.058	0.080	0.393	0.455	0.003	0.135	0.022	0.010	0.014
Significance	ns	ns	ns	ns	ns	**	ns	*	*	*

The increased C_i values (CO_2 ppm) may indicate photosynthetic limitation due to the change in mesophilic activity, as previously reported in wilt fungi-infected tomatoes [57]. This parameter was also measured on the examined grapevines in the present study (Figure A1). However, there were no remarkable differences between the treatments in mean terms of C_i values in any year or cultivar. There was also no correlation between C_i and A (Table A2) or between C_i and gs (Table A2) values. These data are in agreement with the results reported in study by Nail and Howell [12].

The transpiration of grapevine leaves was mostly unaffected by GPM; however, the infected leaves showed less water-use efficiency *WUE* (μmol CO_2 mol^{-1}) according to the results reported by Lakso et al. [58]. The *WUE* parameter—as an additional indicator of plant health—was also calculated in the present study. According to mixed-effect analysis, the measuring times (as row factor) and the treatments (as column factor) had a significant

($p < 0.0001$) influence on the mean extrinsic *WUE* values in both varieties and years, except for Chardonnay in 2013. Relevant differences between the examined varieties or treatments could not be observed in the extrinsic *WUE* values in 2013 (Figure 5A,C). The effect of cultivars and treatments was expressed in 2014 (Figure 5B,D). Kékfrankos plants showed somewhat higher extrinsic *WUE* values relative to Chardonnay and seemed to be affected at a higher level by the PFO treatments (Figure 5D) in 2014.

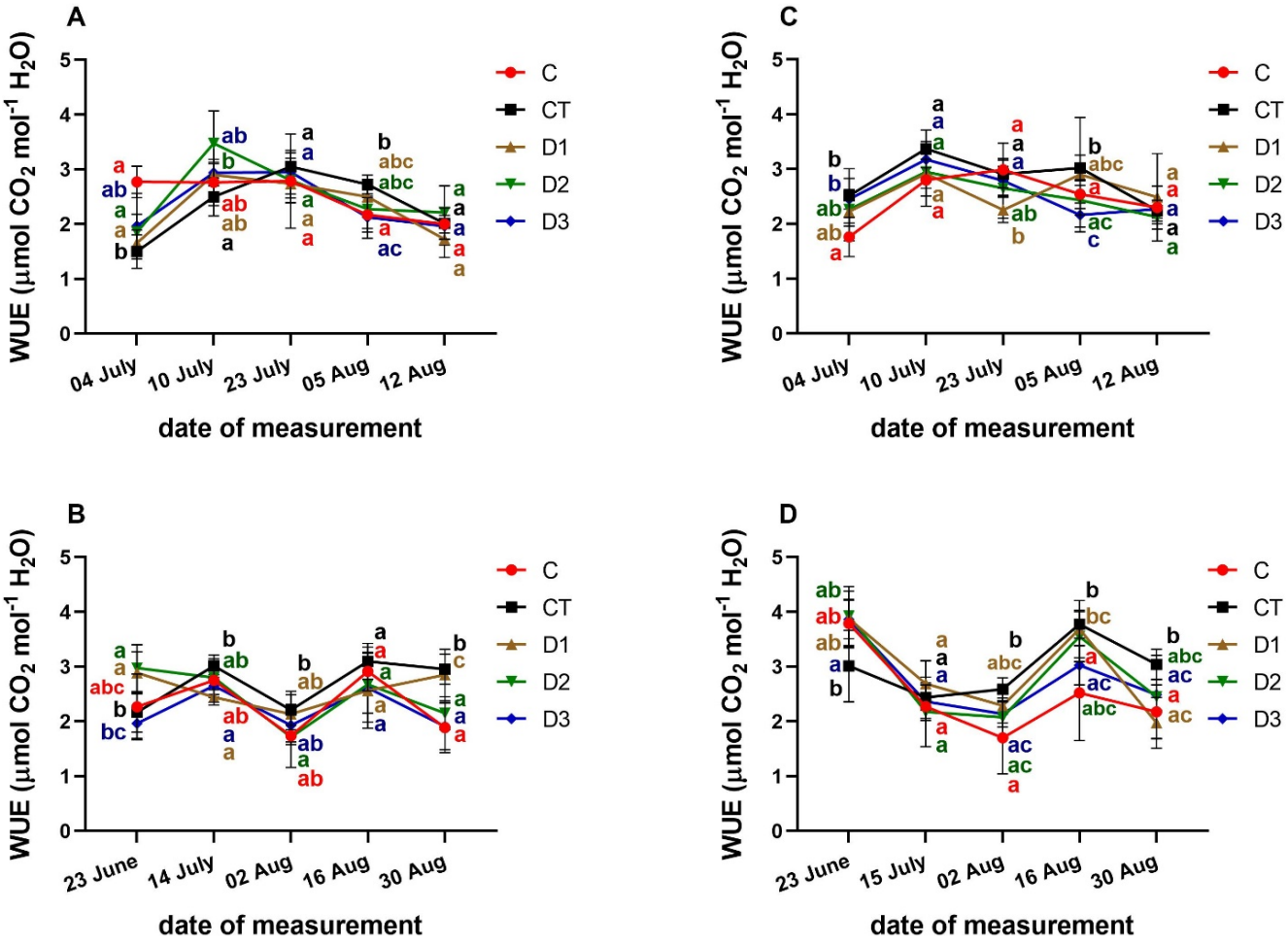

Figure 5. (**A**–**D**) Effects of treatments on extrinsic water-use efficiency (*WUE*) in Chardonnay (**A**,**B**) and Kékfrankos (**C**,**D**) varieties in 2013 (**A**,**C**) and 2014 (**B**,**D**). Extrinsic *WUE* was calculated in the case of each measurement: *A* (assimilation rate)/*E* (transpiration rate). Each symbol represents the mean value ± standard deviation (SD). Significance groups of each measurement date are marked with letters ($p < 0.05$). The color of the letters corresponds to the color of the treatments.

3.4. Yield Parameters of Spraying Experiment at Harvest

In general, the ANOVA results showed that the differences in mean cluster number values between treatments were the result of treatment rather than random sampling in the case of both varieties and years ($p < 0.0001$, Figure 6). The mean cluster weights for PFO-treated plants were significantly lower ($p < 0.05$) than in CT parcels but also higher relative to C parcels in both years and cultivars. However, this latter difference was significant ($p < 0.05$) only in the case of D3 treatment on Kékfrankos in 2013 (Figure 6B). Whereas PFO was less efficient in preventing yield losses, it did not exert negative effects. In contrast with cluster weight, no significant differences or remarkable tendencies were detected between the treatments in the case of average cluster numbers (Figure 6C,D) in any year or cultivar, suggesting that PFO had no negative effect on fruit set.

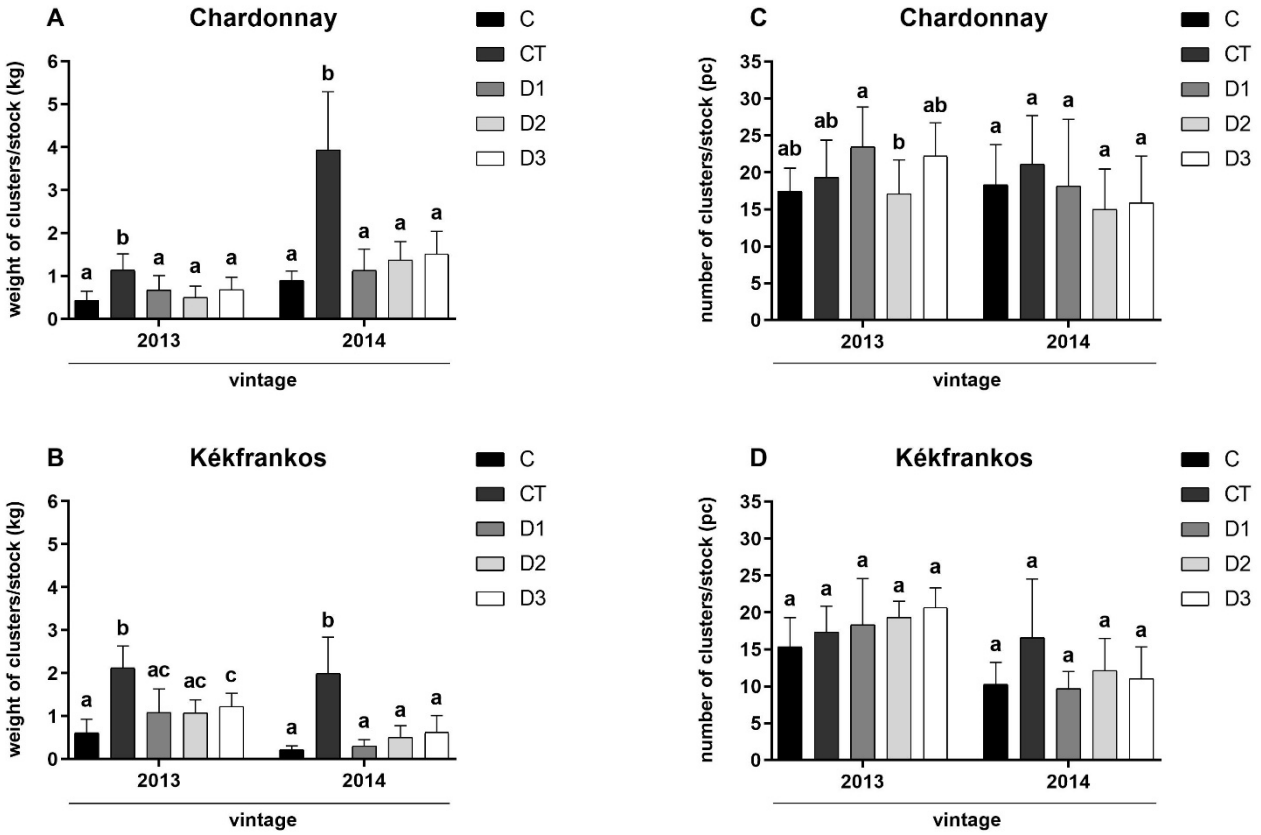

Figure 6. (**A**–**D**) Weight of clusters/vine stock (**A**,**B**) and the number of clusters/vine stock (**C**,**D**) of Chardonnay (**A**,**C**) and Kékfrankos (**B**,**D**) cultivars in 2013 and 2014. The colored bars of treatments indicate untreated (C) or treated with 1.1, 2.2, or 3.3 v/v% PFO (D1, D2, and D3, respectively). Columns represent the average data of nine stocks, and error bars show the standard deviation (SD). Significance groups between vintages are labeled by letters ($p < 0.05$).

4. Conclusions

The results presented in this study suggest that PFO is capable of protecting grapevine from the detrimental effects of GPM on host physiology and yield, although its efficacy is limited and depends on several conditions. Although it seems that the use of PFO positively affects the physiological state (according to leaf photosynthetic parameters) of grapevines in a vintage with particular climate conditions (with low disease pressure), this positive effect is reduces in the case of vintages under high GPM pressure and affects the yields at a lower rate relative to photosynthetic parameters. This vintage-dependent phenomenon was expressed at a higher level in the case of a grapevine cultivar with more susceptible leaves to GPM infection (Kékfrankos) relative to a variety with lower foliar sensitivity to GPM (Chardonnay). This latter finding suggests that PFO dosages should be fitted not just to the vintage but also to cultivar characteristics. Because the experiment was conducted under field conditions, it was not possible to directly measure the possible phytotoxic effects of PFO. However, the lack of PFO dose-dependent decrease in photosynthetic parameters (regardless of cultivar or vintage) suggests that PFO—or at least the formulation used in this study—has no relevant negative effect on grapevine physiology, at least relative to GPM infection.

Author Contributions: Conceptualization, X.P. and Z.Z.; methodology, X.P. and Z.Z.; formal analysis, X.P.; investigation, X.P., S.V. and Z.K.; data curation, X.P.; writing—original draft preparation, X.P.; writing—review and editing, X.P., S.V., Z.K., J.K. and Z.Z.; visualization, X.P.; supervision, J.K. and Z.Z.; funding acquisition, Z.Z. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Pesticides applied in the CT treatment with active ingredients in 2013 and 2014.

2013	*2014*
Fungicides	
Collis SC (boscalid, kresoxim-methyl) Falcon 460 EC (tebuconazole, triadimenol, spiroxamine) Folpan 80 WDG (folpet) Kumulus S WG (sulphur) Kocide 2000 (WG) Manzate 75 DF WG (mancozeb) Tanos 50 DP (cymoxanil, famoxadone)	Champion WG (copper hydroxide) Curzate F SC (cymoxanil, folpet) Dynali DC (cyflufenamid, difenoconazole) Falcon 460 EC (tebuconazole, triadimenol, spiroxamine) Folpan 80 WDG (folpet) Karathane Star EC (metyldinocap), Kocide 2000 (WG) Kumulus S WG (sulphur) Manzate 75 DF WG (mancozeb) Talendo EC (proquinazid)
Other pesticides	
Actara 25 WG (thiamethoxam) Pyranica 20 WP (tebufenpyrad) Pyrinex 25 CS (chlorpyrifos) Nonit SL (dioctyl sodium sulfosuccinate)	Actara 25 WG (thiamethoxam) Pyranica 20 WP (tebufenpyrad) Pyrinex 25 CS (chlorpyrifos) Nonit SL (dioctyl sodium sulfosuccinate) Spur LC (trisiloxane; modified with polyether + Pluronic L62)

Table A2. Results of the correlation analysis between mean stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) data, as well as mean leaf assimilation rate (A) and C_i data in the case of each treatment, variety, and year, with 5-5 measurement dates/year. The "ns" defined as not significant difference.

Chardonnay 2013	g_s **vs.** C_i					A **vs.** C_i				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	0.470	0.716	0.293	−0.173	0.656	0.533	−0.746	−0.328	−0.441	0.102
p value	0.425	0.173	0.632	0.780	0.229	0.359	0.148	0.590	0.457	0.871
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Kékfrankos 2013	g_s **vs.** C_i					A **vs.** C_i				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	0.055	0.307	0.607	0.803	0.777	−0.467	−0.446	0.147	−0.698	−0.183
p value	0.930	0.616	0.277	0.102	0.122	0.428	0.452	0.814	0.190	0.768
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Chardonnay 2014	g_s **vs.** C_i					A **vs.** C_i				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	−0.687	0.817	−0.200	−0.427	−0.568	−0.783	0.408	−0.469	−0.856	−0.841
p value	0.200	0.091	0.747	0.473	0.318	0.117	0.496	0.426	0.064	0.074
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Kékfrankos 2014	g_s **vs.** C_i					A **vs.** C_i				
	C	**CT**	**D1**	**D2**	**D3**	**C**	**CT**	**D1**	**D2**	**D3**
Pearson r	−0.179	0.468	−0.473	0.074	0.058	−0.618	0.130	−0.841	−0.728	−0.858
p value	0.773	0.407	0.421	0.906	0.927	0.267	0.836	0.074	0.163	0.063
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

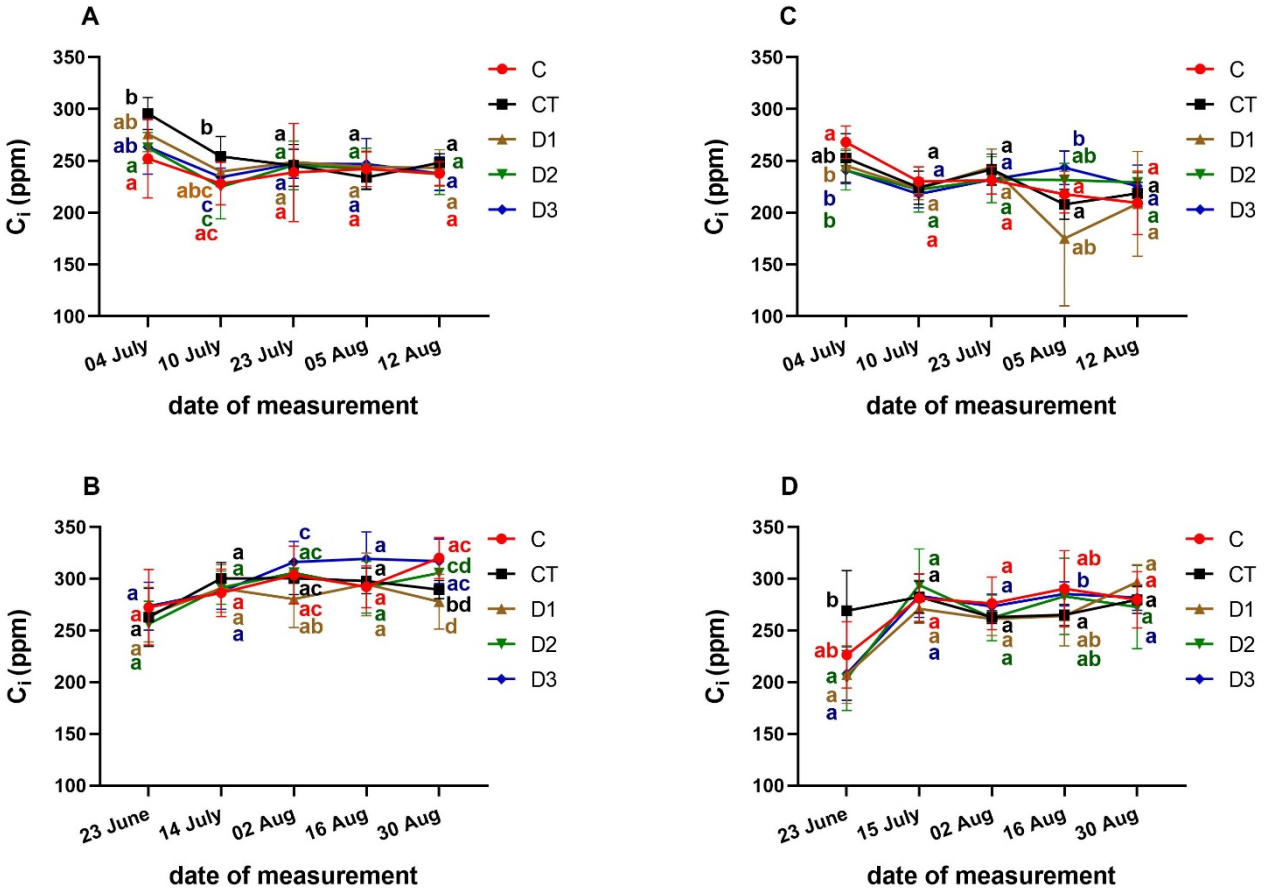

Figure A1. (**A**–**D**) Effects of treatments on intercellular CO_2 concentration (C_i; CO_2 ppm) on Chardonnay (**A**,**B**) and Kékfrankos (**C**,**D**) varieties in 2013 (**A**,**C**) and 2014 (**B**,**D**). Each symbol represents the mean value ± standard deviation (SD). Significance groups of each measurement date are marked with letters ($p < 0.05$). The color of letters corresponds to the color of the treatments.

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