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The efficacy of combining paraffin oil with conventional fungicide treatments against grape powdery mildew in Eger

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SUMMARY

We aimed to test the combination of paraffin oil (PFO) with regular fungicide treatment to assess its efficacy against grape powdery mildew (GPM) in a small spraying experiment on two Vitis vinifera L. cultivars (Chardonnay and Kékfrankos) with different susceptibility to Erysiphe necator. The visual symptoms of GPM on leaves and clusters were examined at three phenological states. The harvest yield was characterized by two methods, data were analyzed with one-way ANOVA and Tukey post-hoc test. Regular fungicide treatment (CT) and its combinations with PFO showed better results in both varieties to repress GPM in 2015 relative to sole PFO treatments. Mean values of combined treatments were often lower than CT but did not differ significantly from each other. The same was observed in 2016, despite the higher pressure of GPM, and missed the third survey. No significant differences were detected between treatments in yield. In contrast, the mean cluster weight of CT and combined treatments resulted in (insignificantly) higher values in each variety and year. In summary, the sole PFO showed some disease control capability as reported earlier, but this effect was greatly affected by the given vintage. Combining PFO with CT resulted in increased protection against GPM relative to the solely applied fungicides. However, this effect was not significant in all cases. It also depended on the vintage and cultivar characteristics. The beneficial impact of paraffin oil as an additive to CT may be due to the induction of plant stress responses and/or its ability to support the adherence and absorption of the combined agents.

Keywords: *paraffin oil, combined treatment, disease incidence, symptom severity, Erysiphe necator, Vitis vinifera*

INTRODUCTION

Climate change and anthropogenic effects (national trade, environmental pollution) influence plant health through several direct and indirect mechanisms (Pautasso et al., 2010). The frequency of extreme meteorological events (high temperature, rapid distribution and huge amount of precipitation, windstorms, and increased humidity) directly affects plant health due to the increased abiotic stress and imbalance of natural resources (Rosenzweig et al., 2001; Pautasso et al., 2010, 2012). The indirect effects of these factors to plant health mostly shows up in the promotion of pathogen spread (already present and new ones) and evolution (shorter incubation periods) with decreased resilience of plants against pathogens (Pautasso et al., 2010, 2012). As a result of the above-mentioned factors, the use of pesticides becomes more intensive to support the production of food supplies in appropriate quantity and quality (Miraglia et al., 2009) including in the case of viticulture (Nesler et al., 2015). Intensive sprayings have significant costs and a load on the environment with an increased risk of accumulation in the food chain (Özkara et al., 2016). As a consequence of the increased pesticide use, fungicide resistance is an emerging problem for one of the most important grapevine pathogens, *Erysiphe necator* (Nesler et al., 2015; Vielba-Fernández et al., 2020).

To reduce the negative effect of increased spraying, various oils have been applied for several years to control a wide range of pests and pathogens in different cultivars in integrated and biological agriculture (Ebbon, 2002; Holb, 2005). The petroleum-derived spraying oils (PDSO), such as paraffin oil (or JMS Stylet oil) can be applied in various ways against the grape powdery mildew: wash spraying, rotation partner of conventional fungicides, or adjuvant of other pesticides (Dell et al., 1998; Rae, 2002; Grove et al., 2005; Janousek et al., 2009). However, these horticultural oils are phytotoxic in high concentrations, they inhibit photosynthesis and transpiration (Hodgkinson et al., 2002; Finger et al., 2002). Other negative effects were also reported in some studies: delaying blooming in the case of peach (Sams et al., 2002), delaying sugar accumulation, and reduction in berry and cluster weight by winegrapes (Finger et al., 2002; Nazari et al., 2014). Therefore, the applied dosage and timing should be carefully determined (Martín et al., 2005).

Our first study aimed to test paraffin oil in three different dosages on grapevine against grape powdery mildew (GPM) caused by *E. necator* (Pálfi et al., 2016). This survey was carried out on two *Vitis vinifera* varieties with different susceptibility to this pathogen in the Eger wine region, Kőlyuktető in 2013 and 2014. *V. vinifera* varieties are generally sensitive to GPM infection with individual differences; even with a unique difference in leaf and cluster sensitivity (Doster and Schnathorst, 1985; Gaforio et al., 2011). This depends on cultivar and environmental conditions (Gaforio et al., 2011), such as meteorological characteristics of vintage or the properties and management of the vineyard. The condition of grapevine stocks (age, nutritional supply status) is also important (Szőke, 1996). Chardonnay and Kékfrankos cultivars are both susceptible to GPM (Szőke, 1996). However, there are minor differences between the two varieties (Pálfi et al., 2016). The white variety

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(Chardonnay) has berries with a thin wax layer, and the fruits are more sensitive to fungal infection and rotting as well as its berries can easily split (Bényei and Lőrincz, 2005; Pálfi et al., 2016). In the case of Kékfrankos, the opposite is true: leaves are more susceptible to GPM or downy mildew (caused by *Plasmopara viticola*) infection compared to Chardonnay with slightly dense prostrate hairs on the abaxial leaf surface. The Kékfrankos clusters with thick wax layers are less sensitive to GPM or rotting. The visual symptoms of GPM were investigated only before harvest in this survey (Hajdu, 2003; Bényei and Lőrincz, 2005; Pálfi et al., 2016).

This study presents the results of the second survey with paraffin oil (PFO) in 2015 and 2016, based on the results of the first experiment. We aimed to test the combination of PFO with regular fungicide treatment to assess its efficacy against grape powdery mildew in a small spraying experiment. The monitoring of GPM was extended with two additional time points to have a more detailed insight into the symptom development of GPM. The determination of the yield parameters was evaluated similarly to the first experiment (Pálfi et al., 2016).

MATERIALS AND METHODS

The sprayings have been carried out in 2015 and 2016 on two grapevine varieties (*Vitis vinifera* L. cv. Chardonnay and Kékfrankos) in the vineyard of Eszterhazy Karoly Catholic University, Kőlyuktető (Eger). *Table 1* shows the arrangement of the experimental area (3 rows) and the description of treatments. Each row contained 10–14 stocks, respectively. The date of sprayings, the applied amounts, and the dosage of regular chemical treatments was based on vintage characteristics (meteorological data and plant protection forecasts) and phenological stages (BBCH) of the grapevine. In 2015, 7 sprayings have been executed and 6 sprayings in 2016, with one herbicide spraying in both years.

The doses of oil treatments were determined on the basis of our earlier spraying experiment (2013–2014). In this survey, the PFO was applied as an alternative against GPM in 1.1 v/v%, 2.2 v/v%, and 3.3 v/v% with negative (no fungicide treatments) and positive (conventional chemical treatments) control. The 1.1 v/v% of PFO did not show an effect in each experimental year (Pálfi et al., 2016). Therefore only 2.2 and 3.3 v/v% of PFO were further investigated in this survey. Using of horticultural oils influences the cluster and berry weight negatively (Finger et al., 2002). We hypothesized that PFO can influence the process of fruit set through pollination droplets. To test this hypothesis, P3CT spraying was also executed with a modification (*Table 1*). The following pesticides with fungicide effect were applied as part of the conventional chemical treatment as regular fungicides: Champion (copper hydroxide), Collis (boscalid, kresoxim-methyl), Cymbal 45 (cymoxanil), Dynali (cyflufenamid, difenoconazole), Folpan 80 (folpet), Karathane Star (metyldinocap), Kumulus S (sulphur), Manzate 75 DF (mancozeb), Pergado F (folpet, mandipropamid), Tebusha 25 (tebuconazole) and Vegesol eReS (copper, sulphur, oil) in 2015. In 2016, Champion, Cymbal 45, Falcon 460 (tebuconazole, triadimenol, spiroxamine), Folpan 80, Kumulus S, Karathane Star, Penncozeb (mancozeb), Rally Q (miclobutanil, quinoxyfen), Tebusha 25, Teldor 500 (fenhexamid) and Vegesol eReS were used. The applied spraying amount was depending on the extent of the canopy. The spraying of combined treatments (P2CT, P3CT, P3CTm) was executed by spraying the PFO dosages first, followed by the actual mixture of the conventional chemical treatment (CT).

Table 1: **Arrangement of experimental rows in examined years (2015–2016) and description of treatments**

1. row [1]	2. row	3. row [1]	Abbreviation and description of treatments
P3	P2	C0	**C0 (absolute control):** no chemical treatments
P3CT	P2CT	CT	**CT (regular control):** chemical treatments based on weather forecasts and vegetation period
C0	P3CTm	P3CTm	**P2:** paraffin oil 2.2 v/v%
CT	P3	P2	**P2CT:** paraffin oil 2.2 v/v% and regular chemical treatment
P2	P3CT	P2CT	**P3:** paraffin oil 3.3 v/v%
P2CT	CT	P3	**P3CT:** paraffin oil 3.3 v/v% and regular chemical treatment
P3CTm	C0	P3CT	**P3CTm:** P3CT treatment, with modification: only CT treatment under blooming and fruit set

[1] The experimental rows were bordered with 1–1 buffer rows, and on June 2–2 rows in the case of rows marked with an asterisk (*).

A modified method of R. W. Emmett (Wicks and Hitch, 2002) was applied to monitor the intensity of GPM infection. The percentage (%) of the infected area was determined by visual estimation concerning the area of the surveyed leaves and clusters. The calculation of GPM frequency was based on the intensity data: if GPM intensity was 0, prevalence is 0 (0=0%), and 1 (1=100%) if the examined objects were infected (Pálfi et al., 2016). The number of samples was 20–20 per replicates (in summary 60 samples/treatments) and were randomly selected. The survey of GPM infection was carried out in three phenological stages of grapevine: pea-sized berries (BBCH 75), veraison (BBCH 79), and ripening (BBCH 89).

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The harvest yield was characterized by the weight of clusters of 3 vine stocks from every experimental block (9 data/treatment). The mean weight of clusters was also determined; 20–30 clusters per treatment were measured (Pálfi et al., 2016). The data were analyzed with GraphPad Prism version 8.0.1 for Windows biostatistical program: one-way ANOVA was carried out with Tukey's multiple comparison test.

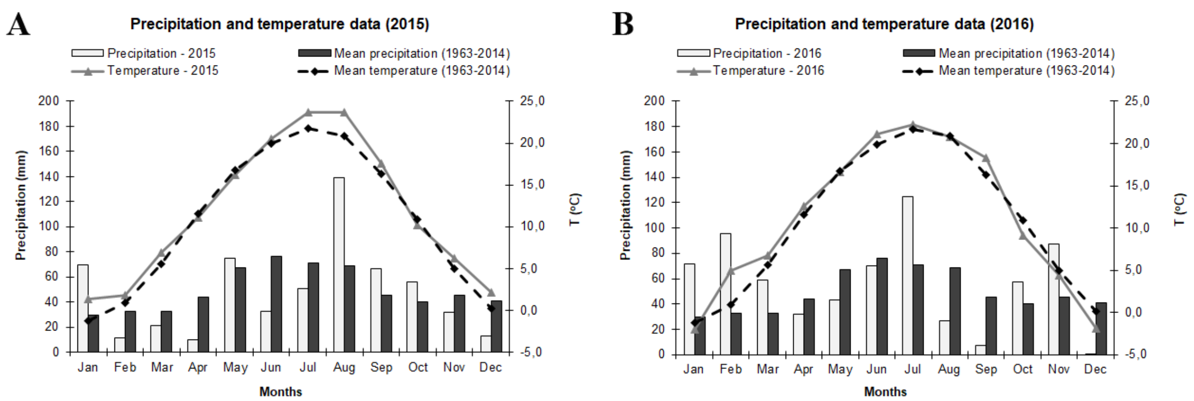
RESULTS AND DISCUSSION

Characteristics of vintage in 2015 and 2016

The mean monthly precipitations and air temperatures of 2015 and 2016 are summarized in *Figure 1*. In 2015, the vintage was warm and dry (especially under vegetation) compared to the mean data for the last 51 years. April and May were relatively cold. The average air temperature was outstandingly high between June-September. The hot June accelerated the blooming and fruit set period and was not favorable for fruit set in the case of Kékfrankos (Nádudvari and Horváth, 2016). The distribution of rainfall was unequal; the quantity was low in the first half of the year, except in May. These conditions were optimal for *E. necator* infection, however, the drastically decreased precipitation in June and July was not favored the spread of this pathogen. The rainy May promoted the grapevine development, the veraison and harvest have happened earlier (Nádudvari and Horváth, 2016). In general, the warm weather and a few wet months caused no serious problems in disease control with controlled sprayings and phytotechnics. The extreme amount of precipitation in August and harvest time (September–October) resulted in favorable conditions for fungi. The mild temperature of autumn and winter promoted the overwintering of pathogens and pests.

In 2016, there were more rain and lower mean air temperature compared to 2015, however it was hotter than the average of the last 51 years (except for the last 3 months). The distribution of precipitation was rhapsodic this year. Powdery mildew was observed early in May (Anonymus, 2017). In July, an extreme quantity of precipitation fell (76% more than the average of the last 51 years). This phenomenon with the high temperature caused elevated air humidity and resulted in optimal conditions for grape pathogen fungi from June. The berries were cracked due to the huge amount of precipitation, especially on Chardonnay. This led to an increased risk of infections by other fungi, e.g. *Botrytis cinerea* (the causal agent of grey mold). The weather in August was optimal for the late infection of *E. necator* and *P. viticola*, and the grey rot also caused a problem before harvest. In summary, this warm and humid vintage was favorable for fungal infection. The rainy weather caused frequent difficulties in the execution of canopy and disease management (sprayings).

Figure 1: **Precipitation and temperature data under experimental years compared to the average of the last 51 years***



*Based on measured meteorological data of Boreas Ltd. on Kőlyuktető, Eger. Figure (a) shows data from 2015, and (b) from 2016.

The intensity and frequency of powdery mildew symptoms in 2015–2016

All data of GPM infections from 2015 (frequency and intensity) are summarized in *Table 2* in the case of each variety, treatment, and plant part. A minimal prevalence of GPM was detected during the pea-sized stage (BBCH 75) on Chardonnay leaves; only C0 and P3 treatments showed symptoms. Only C0 showed significantly higher intensity and frequency values on leaves compared to the other treatments. On Kékfrankos, GPM was detected on leaves in every parcel, except in which received P2CT treatment. The C0 and P3CTm treatments showed significantly higher GPM prevalence compared to CT, P2CT, P3, and P3CT treatments. The lowest frequency and intensity percentage were observed in the case of P2CT, P3CT, and CT treatments. In GPM intensity, only C0 differed significantly from the other treatments. In the case of Chardonnay clusters, CT and combined treatments showed significantly lower intensity and prevalence percentage values than P2 (and C0, only in GPM symptom severity). The combined treatments not differed significantly from each other and CT, as well as no difference was observed between P2 and P3. The clusters of Kékfrankos were slightly less infected than the clusters of Chardonnay, in contrast with the

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observations of leaves. In case of Kékfrankos clusters, only untreated C0 showed significant differences (higher values) compared to the other treatments, except P2 in GPM intensity (P2 did not differ from C0). The lowest values were detected in the case of combined and CT treatments, as well as in P3.

The second survey at veraison (BBCH 79) showed significantly lower GPM intensity and frequency on Chardonnay leaves treated with CT or combined treatments compared to the C0- and P2-treated leaves, as well as than P3-treated leaves in GPM prevalence. In general, there were no significant differences between C0 and P2, between CT and combined sprayings; as well as between sole oil (except in GPM frequency, when P2 had a higher mean value than P3) and between the combined treatments. The monitoring of Kékfrankos leaves gave similar statistical results to leaves of Chardonnay, however with higher mean percentage values compared to the white variety. Leaves with CT and the combined treatments were significantly less infected relative to P2, as well as C0. The C0 and sole oil treatments resulted in a significantly higher GPM frequency than the other treatments, as well as P2 showed higher prevalence compared to P3. The clusters of Chardonnay showed similar statistical results to its leaves. In GPM intensity, P2 showed a significantly higher value compared to the other treatments (except C0, which was more infected). P3 treatment differed also significantly higher compared to CT and combined treatments, while P3CTm treatment showed worse results compared to P2CT and P3CT treatments. The prevalence of GPM in Chardonnay clusters was similar to leaves, however, there were no significant differences between C0 and sole oil treatments and P3CTm, which latter was worse than CT. The sole oil treatments also differed significantly from each other, as well as P3CTm and P3. On the clusters of Kékfrankos, significantly higher GPM intensity was detected in the case of C0 and P2 treatments compared to the other treatments (except between P2 and P3). The combined treatments did not differ from each other, as well as from the CT. The prevalence of GPM on clusters showed the same result, however, the P3 treatment had also a significantly higher mean value than P3CT.

Table 2: **Effects of treatments on GPM infection intensity and frequency (%) on leaves and clusters of Chardonnay and Kékfrankos in 2015***

2015	**Chardonnay**				**Kékfrankos**			
1. survey (BBCH 75)	***frequency of GPM (%)***		***intensity of GPM (%)***		***frequency of GPM (%)***		***intensity of GPM (%)***	
	leaves	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*
C0	28.3±45.4	26.7±44.6	2.2±4.9	2.0±4.5	28.3±45.4	36.7±48.6	2.2±4.9	1.4±3.2
CT	0.0±0.0	15.0±36.0	0.0±0.0	0.5±1.7	3.3±18.1	15.0±36.0	0.1±0.7	0.4±1.0
P2	0.0± 0.0	21.7±41.5	0.0±0.0	2.2±6.2	13.3±34.3	16.7±37.6	0.7±2.5	0.7±2.2
P2CT	0.0±0.0	1.7±12.9	0.0±0.0	0.0±0.1	0.0±0.0	8.3±27.9	0.0±0.0	0.1±0.7
P3	1.7±12.9	16.7±37.6	0.0±0.3	0.5±1.2	10.0±30.3	8.3±27.9	0.1±0.5	0.2±1.0
P3CT	0.0±0.0	1.7±12.9	0.0±0.0	0.0±0.1	1.7±12.9	10.0±30.3	0.0±0.1	0.1±0.2
P3CTm	0.0±0.0	3.3±18.1	0.0 ±0.0	0.1±0.7	28.3±45.4	3.3±18.1	0.8±2.2	0.1±0.5
2. survey (BBCH 79)	***frequency of GPM (%)***		***intensity of GPM (%)***		***frequency of GPM (%)***		***intensity of GPM (%)***	
	leaves	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*
C0	85.0±36.0	98.3±12.9	36.1±31.3	55.7±32.8	98.3±12.9	85.0±36.0	46.2±34.3	8.2±8.6
CT	10.0±30.3	61.7±49.0	0.3±1.3	6.8±10.4	5.0±22.0	20.0±40.3	0.1±0.4	0.4±1.0
P2	75.0±43.7	98.3±12.9	15.7±23.6	42.0±30.7	65.0±48.1	50.0±50.4	11.2±19.1	2.6±4.0
P2CT	16.7±37.6	53.3±50.3	0.7±2.2	2.5± 3.2	3.3±18.1	16.7±37.6	0.1±0.4	0.4±1.1
P3	41.7± 49.7	95.0±22.0	6.1±15.0	26.8±28.0	43.3±50.0	30.0±46.2	4.7±11.9	0.9±2.0
P3CT	1.7±12.9	46.7±50.3	0.0±0.1	2.4± 3.7	1.7±12.9	5.0±22.0	0.0±0.3	0.1±0.4
P3CTm	18.3±39.0	85.0±36.0	0.4±1.2	15.2±18.8	1.7±12.9	16.7±37.6	0.0±0.3	0.6±1.8
3. survey (BBCH 89)	***frequency of GPM (%)***		***intensity of GPM (%)***		***frequency of GPM (%)***		***intensity of GPM (%)***	
	leaves	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*
C0	96.7±18.1	100.0±0.0	64.7±31.6	39.6±37.3	96.7±18.1	71.7±45.4	60.4±34.6	5.9±10.6
CT	85.0±36.0	75.0±43.7	26.1±22.1	9.5±19.3	100.0±0.0	25.0±43.7	41.9±28.4	1.1±2.3
P2	98.3±12.9	98.3±12.9	67.6±26.6	26.5±22.1	100.0±0.0	70.0±46.2	77.5±18.7	2.5±2.4
P2CT	76.7±42.7	53.3±50.3	13.4±16.6	2.8±4.0	86.7±34.3	28.3±45.4	34.8±31.5	0.7±1.2
P3	100.0±0.0	98.3±12.9	64.9±26.3	14.0±16.6	100.0±0.0	51.7±50.4	77.2±16.6	2.0±2.7
P3CT	73.3±44.6	56.7±50.0	17.5±24.1	1.9±2.5	80.0±40.3	31.7±46.9	40.6±33.9	1.4±3.0
P3CTm	80.0±40.3	78.3±41.5	25.3±29.4	10.3±15.0	88.3± 2.4	43.3± 0.0	32.0±31.1	1.2±1.6

*Notes: Columns summarize mean GPM symptom intensity and frequency on leaves and clusters with standard deviation. The significant properties between treatments are detailed in the text. Data were compared between different treatments in the case of each plant variety, survey, and plant part.

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Based on monitoring under ripening (BBCH 89), the intensity and the frequency of GPM were further increased until harvest in the case of both varieties. On Chardonnay leaves, CT and combined treatments resulted in a lower GPM intensity than C0 and the sole oil treatments. The P2 and P3 were significantly more infected than CT and combined treatments. No significant differences were detected between CT and combined sprayings; as well as between the single oil treatments, and the combined treatments did also not differ from each other. These observations also characteristic to the GPM intensity of Kékfrankos leaves. However, the sole oil treatments were resulted in significantly lower infection than C0 in this case, in contrast to the white variety. The prevalence of GPM on Chardonnay leaves showed similar results, however, CT did not differ from any treatments, and the combined treatments had significantly lower values than C0, P2, and P3 (except P3CTm: not differed from C0). The GPM frequency on Kékfrankos leaves showed higher values relative to Chardonnay. Significant differences were observed between the high C0 and lowest P3CT values, while CT, P2, and P3 treatments resulted in increased values compared to the combined treatments (except P3CTm). The GPM symptom severity on Chardonnay clusters showed similar results to its leaves: C0 was significantly more infected than the other treatments, and P2 had a higher percentage value compared to CT, P3, and the combined treatments. P3 was significantly higher than P2CT and P3CT, which two were the most efficient treatments. The clusters of Kékfrankos expressed lower PM intensity than Chardonnay, but only C0 showed significantly higher values compared to other treatments. The best results (lowest mean values) were observed in parcels treated with CT and combined treatments. The frequency of GPM on clusters was significantly lower in Chardonnay parcels, which were treated with CT and combined treatments compared to the C0 and sole oil treatments. Interestingly, P3CTm showed a significantly higher mean value than the other combined treatments, as well as in P2CT and P3CT parcels less GPM was detected compared to CT. However, this characteristic was significant only in the case of P2CT. In Kékfrankos, C0 and P2 showed significantly higher prevalence values on clusters compared to CT and combined treatments, and P3 was higher than CT. Similarly to veraison, the results of this monitoring represent well the difference between grapevine varieties and examined plant parts in resilience to GPM: Chardonnay clusters were more often and severely infected than in the red variety, while the opposite phenomenon was observed in the case of the leaves.

Table 3: **Effects of treatments on GPM infection intensity and frequency (%) on leaves and clusters of Chardonnay and Kékfrankos in 2016***

2016	Chardonnay				Kékfrankos			
1. survey	*frequency of GPM (%)*		*intensity of GPM (%)*		*frequency of GPM (%)*		*intensity of GPM (%)*	
(BBCH 75)	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*
C0	56.7±50.0	76.7±42.7	5.1±12.8	5.2±5.8	71.7±45.4	91.7±27.9	10.9±16.9	13.3±16.1
CT	25.0±43.7	71.7±45.4	0.7±1.7	5.5±13.1	30.0±46.2	75.0±43.7	0.7±1.5	4.9±6.2
P2	60.0±49.4	93.3±25.2	2.9±3.5	11.7±17.8	58.3±49.7	86.7±34.3	5.3±10.7	8.3±12.0
P2CT	13.3±34.3	76.7±42.7	0.3±0.7	4.0±4.7	16.7±37.6	60.0±49.4	0.5±1.3	2.8±3.4
P3	51.7±50.4	80.0±40.3	2.3±4.9	13.5±22.6	56.7±50.0	83.3±37.6	3.3±5.6	7.3±10.9
P3CT	15.0±36.0	75.0±43.7	0.3±1.0	3.8±6.0	15.0±36.0	71.7±45.4	0.3±0.7	2.8±2.7
P3CTm	23.3±42.7	83.3±37.6	1.2±4.7	6.0±7.4	18.3±39.0	58.3±49.7	0.4±1.0	2.3±2.5
2. survey	*frequency of GPM (%)*		*intensity of GPM (%)*		*frequency of GPM (%)*		*intensity of GPM (%)*	
(BBCH 79)	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*	*leaves*	*clusters*
C0	100.0±0.0	100.0±0.0	67.5±29.7	81.3±23.1	98.3±12.9	96.7±18.1	63.3±31.4	39.6±34.5
CT	75.0±43.7	100.0±0.0	14.3±19.5	54.8±28.1	61.7±49.0	88.3±32.4	5.2±6.2	10.0±10.8
P2	96.7±18.1	100.0±0.0	57.9±23.6	90.4±10.4	96.7±18.1	100.0±0.0	43.7±30.8	29.1±26.6
P2CT	70.0±46.2	100.0±0.0	9.0±14.3	50.5±25.8	26.7±44.6	90.3±30.3	2.1±5.5	4.8±4.2
P3	95.0±22.0	100.0±0.0	36.8±28.9	78.8±23.5	95.0±22.0	100.0±0.0	42.7±29.8	29.3±27.9
P3CT	58.3±49.7	100.0±0.0	12.0±19.6	60.4±27.7	35.0±48.1	95.0±22.0	4.0±7.4	6.0±5.0
P3CTm	71.7±45.4	100.0±0.0	12.6±18.2	60.9±23.9	43.3±50.0	86.7±34.3	3.2±5.5	7.0±13.4
3. survey (BBCH 89)	**Absent data:** the monitoring was not implemented							

*Notes: Columns summarize mean GPM symptom intensity and frequency on leaves and clusters with standard deviation. The significant properties between treatments are detailed in the text. Data were compared between different treatments in the case of each plant variety, survey, and plant part.

In summary, the difference in GPM susceptibility of examined varieties was clearly shown based on the results of 3 monitoring in 2015. In general, CT and combined treatments showed the best results to repress GPM on both varieties, and the combined treatments had often lower infection values than CT. However,

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they differed not significantly from CT and each other with some exceptions. These were observed in the GPM frequency of Kékfrankos leaves and Chardonnay clusters under ripening when P2CT and P3CT (only in the case of Kékfrankos) were significantly less infected than CT. P3CTm showed occasionally higher values than CT, P2CT, and P3CT treatments. This characteristic was significant mostly on Chardonnay clusters under veraison: differed from CT, P2CT, and P3CT in GPM frequency; and only from P2CT in intensity; as well as under ripening: differed from P2CT and P3CT. This phenomenon was also observed in Kékfrankos leaves, P3CTm had a significantly higher GPM prevalence percentage than CT, P2CT, and P3CT under BBCH 75 stage. This difference between P3CTm and the above-mentioned treatments may be due to the modified spraying; these parcels only received CT treatment. Therefore, the beneficial impact of combining PFO with the CT treatment could not be realized in the case of this treatment.

All data of GPM infections from 2016 (frequency and intensity) are summarized in *Table 3* in the case of each variety, treatment, and plant part. GPM symptoms were detected remarkably more often and with more intense severity in both varieties during the surveys compared to 2015. On Chardonnay leaves, C0 was significantly more infected compared to CT and combined treatments and not differed from P2 and P3 under BBCH 75 stage. C0, P2, and P3 showed significantly higher frequency values than CT, P2CT, P3CT, and P3CTm in the case of each variety. In Kékfrankos leaves, C0 and P2 showed significantly higher GPM intensity values compared to the other treatments with some exceptions, e.g. P2 was less infected than C0. The sole oil treatments not differed significantly from each other on leaves, as well as the combined treatments in the case of each variety. Similarly to the observations of 2015, the leaves of the red variety were more infected compared to Chardonnay, and the opposite was realized in the case clusters. On Chardonnay clusters, only a tendency can be observed without significant differences in the GPM frequency: P3CT and P2CT treatments had the lowest values, followed by the P3CTm and CT. P3 treatment showed the highest GPM intensity, it differed significantly from the other treatments (except P2). Significant differences were also observed between P2 and combined treatments (except P3CTm). The controls had not differed from each other in GPM intensity in Chardonnay. The intensity of GPM on Kékfrankos clusters in C0-treated blocks was significantly higher than in other treatments (except P2). P2 showed a significantly higher percentage value than the combined treatments and P3 was significantly higher than P3CTm. (However, P2CT and P3CT had not much higher mean values compared to P3CTm.) The prevalence of GPM showed some significant differences between treatments in Kékfrankos: P2CT and P3CTm treatments had significantly lower frequency values than C0 and sole oil treatments, while they did not differ from CT.

The statistical analysis of GPM symptoms on Chardonnay leaves showed similar results under veraison than under pea-sized stage. C0 and sole oil treatments did not differ significantly from each other and showed higher frequency percentage values than CT and the combined treatments. The GPM intensity was similar to prevalence with a difference: P3 was less infected than C0 and P2, however, had also more severity of GPM compared to the combined treatments. In Kékfrankos, significantly higher GPM frequency was detected in C0- and sole oil-treated leaves than in parcels of combined treatments, as well as C0 differed from CT with higher percentage values. Interestingly, the leaves in P2CT and P3CT parcels were less infected than in CT. These results suggest the beneficial effects of PFO as an additive to conventional fungicides, which phenomenon was also detected in Kékfrankos leaves and Chardonnay clusters under ripening in 2015. The GPM intensity on Kékfrankos leaves was significantly lower in the case of CT and combined sprays compared to the other treatments. The higher susceptibility of clusters of Chardonnay to GPM infection was also observed during this survey. The frequency of GPM was 100% on clusters in the case of each treatment. The prevalence values were also high in Kékfrankos due to the characteristics of this vintage, with one significant relationship: sole oil treatments were less infected than P3CTm. The GPM intensity on clusters was significantly lower in parcels of each variety treated with CT and with its oil combinations than in the case of C0, P2, and P3 treatments. The sole oil treatments not differed significantly from each other on each plant part, as well as the combined treatments in the case of each variety.

The pressure of GPM (and other fungal infections) was severe due to the humid and warm vintage of 2016, as well as other parameters that affected also negatively the survey. The rainy weather encumbered the disease and canopy management of the experimental area. These circumstances resulted in a strong fungal infection, therefore the last spraying was only CT in each experimental row and the last GPM monitoring was not executed. Due to these properties of the survey and the difficulties of this year, the evaluation of the spraying experiment would be incorrect under ripening. In summary, the difference between varieties was also manifested in 2016. Interestingly, the Kéfrankos leaves were slightly less infected under veraison compared to Chardonnay. This phenomenon could be due to the late GPM infection when the mature Kékfrankos leaves were probably less susceptible, or symptoms could be developed later than the monitoring was executed during verasion. The beneficial impact of combining PFO with CT could be also manifested in the case of P2CT and P3CT treatment compared to simple CT. In general, CT and combined treatments showed the best results to repress GPM in both varieties. The P2 and P3 had a negligible impact on disease development this year.

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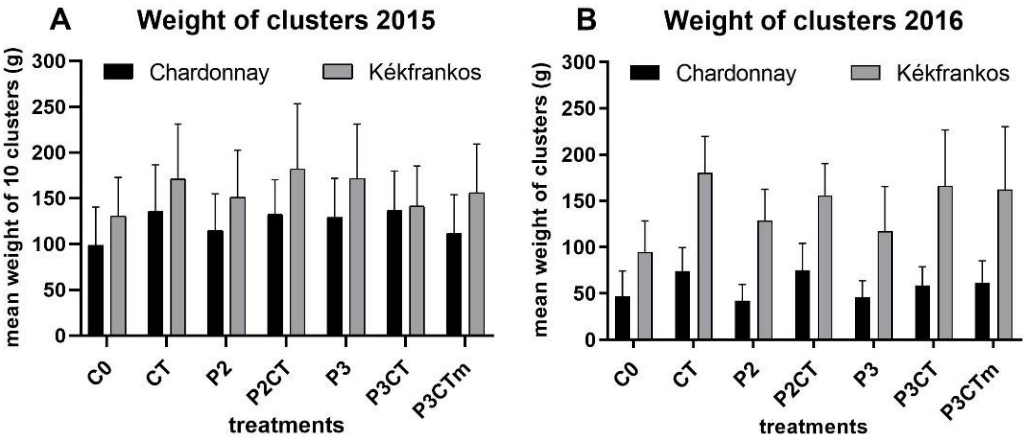
Harvest yield and mean cluster weight in 2015 and 2016

No significant differences were detected in yield between the treatments for either variety and year (data not shown), however, the impact of different treatments was observed in cluster weight.

In 2015, CT, P2CT, and P3CT treatments resulted in significantly higher values of cluster weight (*Figure 2a*) compared to C0 with the lowest values measured in Chardonnay. Treatment P3CTm was near equal with P2 with lower values than the other treatments (except C0). The weight of Kékfrankos clusters showed a similar tendency to Chardonnay, however a significant difference was detected only between C0 and P2CT. Mean cluster weight in 2016 (*Figure 2b*) showed similar results to 2015 in both varieties. In CT- and P2CT-treated blocks significantly higher values were measured compared to C0, P2, and P3 treatments in Chardonnay. The P3CT and P3CTm showed also bigger clusters than P2 and P3, but this difference was not significant. The differences between treatments were more obvious than in 2015 in the case of the red variety The mean cluster weight of CT and combined treatments showed significantly higher values than C0 and D3 treatments in Kékfrankos. Clusters in CT and P3CT blocks were also significantly heavier compared to P2.

Figure 2: **Mean cluster weight of Chardonnay and Kékfrankos with different treatments in 2015 and 2016**



Notes*:* Columns represent the average cluster weight of 20–30 stocks in 2015 (a) and 2016 (b). Error bars show standard deviations (SD). The varieties were not compared with each other under statistical analysis, only the treatments. The significant properties between treatments are detailed in the text.

In summary, no significant differences were detected between the treatments in each variety and year in yield. The experimental area was small and according to the sampling method, the assumed and occurrent differences between treatments could not be observed. The mean cluster weight showed occasionally significant differences between treatments in both years. In general, the CT- and P2CT-treated (as well as occasionally the P3CT-treated) grapevines showed higher yield and bigger clusters based on the characteristics of the harvest in both years. No differences were detected between the P3CT and P3CTm in terms of yield and cluster weight. Therefore, the putatively negative effect of paraffin oil on fruit set was not supported and clear evidence to the contrary was not found in this small plot experiment.

CONCLUSIONS

The efficacy of 2 and 3 v/v% paraffin oil in combination with regular fungicide treatment was tested against grape powdery mildew in a spraying experiment in field conditions. The solely applied oil showed some disease control capability, similar to our previous survey in 2013–2014 (Pálfi et al., 2016). However, this effect was greatly affected by the examined variety and/or plant part and vintage. The combination of paraffin oil with conventional fungicides resulted in increased protection against GPM relative to the fungicides on their own. However, this effect was not significant in every case, it depended on the above-mentioned variables of the experiment. The previously defined variance in susceptibility of leaves and clusters to GPM on examined grapevines (Chardonnay and Kékfrankos) was manifested based on the results in both years.

This beneficial impact of paraffin oil as an additive to regular antifungal agents may be due to two effects or their combination: 1) Paraffin oil in itself has a positive effect on plant immunity due to the induction of stress responses, which leads to decreased susceptibility of grapevine to GPM (Pálfi et al., 2021). 2) Paraffin oil can support the adherence and absorption of the combined fungicide agents (Rae, 2002).

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