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Control of olive anthracnose and leaf spot disease by bloom treatments with a pomegranate peel extract

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ABSTRACT

Olive anthracnose (OA) caused by *Colletotrichum* spp. and olive leaf spot (OLS) caused by *Ventuaria oleaginea* are two of the most important diseases affecting olive trees. OA mainly affects mature olive drupes while OLS is typically known as a defoliating pathogen. Field treatments with a pomegranate peel extract (PGE) at 3, 6 or 12 g/l seven days before the expected full bloom prevented the colonization of flowers by *Colletotrichum* spp. and reduced latent infections in small olive drupes over the summer period by 53-100%. The same treatments significantly reduced or completely prevented the latent infections of *V. oleaginea* in olive leaves. Interestingly, PGE at the higher concentration (12 g/l) proved always more effective that Flint Max, a chemical formulate recently registered in Europe on olive. On plants sprayed with PGE, the lower quantity of over-summering inoculum of *Colletotrichum* spp. effectively delayed the disease outbreak in autumn of approximately ten days. In particular, PGE at 12 g/l reduced the incidence of anthracnose by 84.5% as compared to the untreated control. Treatments also reduced the premature defoliation of plants. Similar results to those described for a single PGE treatment made before the full bloom where achieved with two applications made before and after flowering. Overall, the present study highlighted the importance of controlling the establishment of latent infections of both *Colletotrichum* spp. and *V.oleaginea* to effectively protect olive trees. These results together with previous reports suggest the possible use of PGE as a key natural antifungal preparation to control the olive diseases affecting aerial plant organs.

1. Introduction

Olive anthracnose (OA) and olive leaf spot (OLS) or peacock's eye are important olive diseases that can have a significant economic impact by reducing plant productivity and quality of productions (Agosteo and Schena, 2011; Talhinhas et al., 2018). OA is considered the most important fungal disease of olive drupes. On ripe fruits, symptoms appear as circular sunken lesions that, with humid conditions are quickly covered by pink sporal masses erupting from acervuli (Moral et al., 2014). Infected drupes are commonly shriveled and fall prematurely to the ground but in dry conditions may mummify and remain attached to the tree (Cacciola et al., 2012). Rots mainly occur in the field but can also develop after harvest from quiescent infections occurred before harvesting. In both cases the quality of oil is dramatically compromised (Leoni et al., 2018; Peres et al., 2021). Mature fruits are much more sensitive to the disease, but green fruit of susceptible cultivars can also show rot symptoms in wet conditions. Furthermore, the disease can causes chlorosis and necrosis of leaves, defoliation and dieback of twigs and branches (Cacciola et al., 2012).

OA has been associated with several species of *Colletotrichum*, mostly clustering in the *C. acutatum* species complex (Talhinhas et al., 2018, 2011). In Italy, *C. godetiae* (syn. *C. clavatum*) has been historically considered the prevalent species although recent reports have documented a population shift from this species to *C. acutatum sensu stricto* (s.s.) (Faedda et al., 2011; Mosca et al., 2014; Schena et al., 2017). The disease cycle and epidemiology of OA has been extensively studied, but several aspect are still debated within the scientific community also because it can significantly change according to regional factors such as agronomical practices, plant cultivars, climatic conditions and prevalent pathogen populations (Moral et al., 2009; Romero et al., 2021; Talhinhas et al., 2018). *Colletotrichum* fungi can survive on mummified fruits

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and on olive leaves and branches as resting conidia, appressoria or mycelium (Agosteo et al., 2015; Talhinhas et al., 2011). At bloom, the pathogens can infect flowers (calyx, petals, stamens and pistil) leading to blossom blights (Moral et al., 2009; Moreira et al., 2021). Since only 1%–2% of the flowers are able to set fruit in olive, the direct impact on productions of flower rots has been traditionally considered negligible (Moral et al., 2014). However, it has been also reported that environmental conditions favorable to the disease during the flowering time may determine very severe losses (Moreira et al., 2021). In any case, the colonization of flowers can also lead to fruit infections, which commonly remain latent until ripening. The incidence of these infections has not been clearly established but they seem to play an important role by favoring the survival of the pathogens during the summer period and representing an important source of inoculum for epidemics on ripe fruit (Moral et al., 2014, 2009). On ripe drupes conidia germinate and form germ tubes and appressoria from which infection pegs develop to penetrate the cuticle (Cacciola et al., 2012). With mild temperatures, frequent rain events and prolonged high humidity periods the disease severity can increase at a high rate.

An acceptable control of OA may be achieved combining cultural practices with chemical and biological methods. In olive growing areas where anthracnose is endemic the early fruit harvesting is extremely important in order to avoid secondary infections cycles originating from conidia produced on early rotted drupes. Furthermore, the use of copper-based fungicides is generally considered essential to control the disease. Copper needs to be applied prior to the emergence of symptoms and then repeated according to cultivar susceptibility, the maturity/harvest date and the frequency and intensity of rainfall (Moral et al., 2014).

Recently, several fungicides and natural compounds have been experimentally evaluated as potential alternative control means against OA (Moral et al., 2018). In particular, an alcoholic extract from pomegranate peel proved to be more effective than copper in controlling OA when applied 30 and 15 days before the expected epidemic outbreak (Pangallo et al., 2017a). Another possible strategy to control the disease is the protection of flowers and small fruits in order to reduce the incidence of latent infections and delay the appearance of the disease in autumn (Cacciola et al., 2012). In this context, new commercial formulations have been registered in Europe with the commercial names Flint Max (tebuconazole + tri floxystrobin, Bayer CropScience S.r.l.) and Cabrio Olive (pyraclostrobin, BASF) and can be used only once per year until flowering (Flint Max) or slight after, when drupes reach the 40% of the expected final size (Cabrio Olive).

The OLS is one of the most common and serious diseases in all olive growing areas although its importance and economic impact is strictly related to weather conditions and varietal susceptibility (Issa et al., 2019; Obanor et al., 2011). Infections are much more diffused on leaves but all aerial plant organs including shoots, leaf stalks, fruit pedicles, fruit, and inflorescences can be interested. Severe infections may have a great impact on olive productions since they cause extensive defoliations and reduce plant vigour, number of flower buds and fruit set (Agosteo and Schena, 2011). Infections may occurs in a wide range of temperatures (5–25 °C) providing that abundant rains and prolonged periods of leaf wetness occur (Thomidis et al., 2021). After penetration, the pathogen colonize the outer cuticle layer of the epidermal cell wall and typical symptoms appear on the upper part of the leaf surface, followed by the eruption of conidia. The duration of the incubation period may vary from a minimum of 10 days up to several months according to wheatear conditions after infections. Cold winter temperatures or dry and hot conditions typical of the summer period in the Mediterranean climate prevent the development of the pathogen within leaf tissues and causes long periods of latency (Viruega et al., 2013). As a consequence new infections and sporulation occur during spring and autumn while latent infections enables the over-wintering and over-summering of the pathogen (Issa et al., 2019; Viruega et al., 2013). However, the higher winter temperatures caused by climate changes may enable continue infections of the pathogen and have been associated with very intense attacks observed in southern Italy, in recent years. The chemical control of the disease is mainly based on copper-based fungicides and cannot be avoided in areas with conducive weather conditions and on susceptible cultivars. To be effective, copper treatments must be done during active sporulation and infection periods since copper enter the leaves through wounds caused by erupting conidia, causes the premature drop of infected leaves and prevents new infections. In fact, conidia produced on fallen leaves are of no practical importance for new infections, due to their low dispersal ability (Viruega et al., 2013). Copper-based treatments should be avoided where there is a very high incidence of OLS since they may induce an excessive, early loss of leaves. In such conditions, possible alternatives to copper may include the formulations mentioned above (tebuconazole + trifloxystrobin or pyraclostrobin) or mancozeb, difenoconazole and dodine. Resistance-inducers and biological control agents have been also tested (Tziros et al., 2021).

Overall the management of OA and OLS can be very complex since it may require several chemical treatments and it does not always provide an acceptable level of control for both diseases (Agosteo and Schena, 2011; Moral et al., 2018; Scibetta et al., 2020). Furthermore, registered synthetic fungicides may lose efficacy due to the selection of resistant fungal strains and, more importantly, they may represent a serious risks for human health and the environment due to their toxicity and negative impact on non-target microorganisms (Materatski et al., 2019). In particular, copper is currently the most important chemical to control olive fungal diseases but, due to its toxicity, it has been inserted in the list of substances identified as "candidates for substitution" by the European Commission under Regulation No 1107/2009. Therefore, the increasing legislative restrictions on the use of chemical pesticides and the rise of consumer awareness in food safety and healthy living are prompting the development of safe and environmentally friendly alternative control means.

Recently a pomegranate peel extract (PGE) proved very effective in controlling OA when applied in autumn during the early ascending phase of the disease outbreaks (Pangallo et al., 2017a). The same extract showed a broad range of activity against several different fungal diseases, long persistence of efficacy and both preventive and curative activity (Li Destri Nicosia et al., 2016; Pangallo et al., 2021). It was also demonstrated that PGE possess a strong fungicidal and bactericidal activity and induces resistance in treated tissues (Belgacem et al., 2021, 2019; Pangallo et al., 2017b). Based on these features, aim of the present study was to evaluate the efficacy of PGE against OA and OLS by early treatments made before, or before and after flowering. We hypothesized that bloom PGE treatments can prevent the colonization of olive flowers and reduce the establishment latent fruit infections of *Colletotrichum* spp., which facilitate the survival of the pathogens during the summer period and represent the main source of inoculum for the epidemic development of OA on ripe fruits in autumn. Furthermore, we also hypothesized that the same treatment can reduce the incidence of latent infections of *V. oleaginea*, preventing the survival of pathogen in asymptomatic leaves during the summer period and reducing the quantity of inoculum for new infections.

2. Material and methods

2.1. Compounds

Treatments were made with a pomegranate peel extract (PGE) obtained as described by Pangallo et al. (2017a) and diluted to have

12 (PGE12), 6 (PGE6) or 3 (PGE3) g/L of dry matter. The formulate Flint Max was used as chemical control at the suggested concentration of 20 g/L. Plants sprayed with tap water were used as controls.

2.2. Experiment design and treatments

Experiments were conducted in 2019 in two olive orchards (1 and 2) located in Cosoleto, Reggio Calabria, Italy (GPS coordinate 38.248907, 15.911859 and 38.248142, 15.910550, respectively). Both orchards contained olive trees of cv Ottobratica and were managed following an integrated control scheme. Experiments were performed according to a randomized block design providing a total of 12 plants per treatment (4 replicates of 3 plants each). Plants were selected to be uniform for size of the canopy, vegetative state, and production load.

In orchard 1, plants were sprayed only once, on June 7, approximately 7 days before the expected full bloom. In orchard 2, plants were sprayed twice, on June 7 and on June 21 (7 days after full bloom). All treatments were made with a commercial backpack atomizer (Mod. Serena; Italdifra) using approximately 2 L per plant that enabled the uniform wetting of the whole vegetation without water dropping.

2.3. Latent infections of *Colletotrichum* spp.

The incidence of latent infections of *Colletotrichum* spp. in developing olive fruit was evaluated in both orchards on July 25, August 26, and September 25, 2019. For each biological replicate, 50 drupes were randomly collected in order to have 200 asymptomatic drupes per treatment. Olives were kept in sterile plastic bags and maintained at 4–5 °C for no more than 24 h, before their analysis. Drupes were immersed in a solution of 2% sodium hypochlorite for 5 min, washed twice with sterile distilled water, and dried on sterile blotting paper under a laminar flow hood. Surface sterilized olives were sectioned transversely to have two equal spherical shells that were placed cut surface down in Petri dishes containing potato dextrose agar (PDA, Sigma Aldrich) containing steptomycin (250 mg/L) and ampicillin (250 mg/L) to prevent the growth of bacteria.

Plates were incubated at 24 °C for 3–4 days. Olive shells with growing mycelium resembling that of *Colletotrichum* spp. were recorded to determine the percentage of infected drupes. A representative number of colonies were transferred on new PDA plates to confirm the identity of isolated fungi. To this aim, the ITS1-5.8S-ITS2 region and a fragment of the b-tubulin 2 gene (TUB2) between exons 2 and 6 were sequenced as described by Faedda et al. (2011) and phylogenetically analyzed along with related species of the *C. acutatum* s.l. complex as described by Schena et al. (2014).

2.4. Incidence of olive anthracnose

The incidence of rotted olives was evaluated in both orchards during the disease outbreak on November 14, 2019. Per each treatment, 400 olives (100 per each replicate) were randomly collected from the canopy and visually analyzed to determine the presence of anthracnose symptoms. Analyses were extended to a field control represented by olive trees that did not receive any treatment. Results were expressed as percentage of rotted drupes.

2.5. Latent infections of *Venturia oleaginea*

The incidence of latent infections of *V. oleaginea* was determined on July 7, 2019 (one month after the pre-bloom treatment). Per each biological replicate 50 leaves were randomly collected in order to have 200 asymptomatic leaves per treatment. Leaves were immersed for 3 min in a 5% solution of NaOH pre-heated at 50 °C, dried on blotting paper, and visually inspected for small roundish black spots revealing latent infections of *V. oleaginea* (Loprieno and Tenerini, 1959).

2.6. Olive defoliation

The impact of treatments on defoliation was evaluated by collecting fallen olive leaves during the summer season. To this aim commercial harvesting nets were positioned under the canopy on June 23 and collected on September 30, 2019. Fallen dry leaves were separated from weeds and weighed.

2.7. Statistical analyses

Data analysis was performed using R statistical software 3.5 (R Core Team, 2020). Data were fit to a linear model using the lm() function in the package lme4 (Bates et al., 2015) specifying the formula ~orchard * Treatment_type * Sampling_date (latent infections of *Colletotrichum* spp.) or ~orchard * Treatment_type (incidence of olive anthracnose, latent infections of *V. oleaginea*, and olive defoliation). The package emmeans was used to infer pairwise contrasts for each dependent variable (corrected using False Discovery Rate, FDR).

3. Results

3.1. Latent infections of *Colletotrichum* spp.

The analysis of data revealed a significant impact of all investigated parameters (orchard, sampling date, and treatment type) on the incidence of latent infections of *Colletotrichum* spp. (Table 1). In particular, regardless of treatment type and orchard the incidence of infections significantly ($P < 0.001$) increased from July to September (Fig. 1). As an example, in control drupes sprayed with tap water, the incidence of the pathogen ranged from a minimum of 42.0% in July to a maximum of 67.5% in September (Fig. 1). The sequencing of ITS1-5.8S-ITS2 region and TUB2 gene enabled the identification of *Colletotrichum* spp. isolates obtained from asymptomatic drupes. In all assessment times both *C. acutatum sensu stricto* and *C. godetiae* where identified with a clear prevalence of the first species, which represented around 90% of isolates (data not shown).

As regard to the treatment type, the incidence of latent infections was significantly ($P < 0.001$) reduced by PGE at the three different concentrations and by Flint Max (chemical control), both when plants were sprayed only once before the expected full bloom (orchard 1) and twice, before and after full bloom (orchard 2). In the orchard 1, a single treatment with PGE at 3, 6 or 12 g/L completely inhibited the establishment of latent infections during the first assessment time (July 25, 2019); the extract was significantly more effective than Flint Max which reduced infections by 67.4% (Fig. 1 A). In the second assessment time (August 26, 2019)

Table 1
Results of data analysis from fitting a linear model of latent infections of *Colletotrichum* spp. using orchard (orchard 1 and 2), treatment type (water, Flint Max, and PGE at three different concentrations), sampling date (July 25, August 26, and September 25, 2019), and their interactions as fixed factors.

Factors	df	Latent infections F	P
Orchard (O)	1	7.87	0.006
Treatment type (T)	4	476.39	< 0.001
Date (D)	2	230.26	< 0.001
O × T	4	0.65	0.62
O × D	2	2.87	0.06
T × D	8	9.73	< 0.001
O × T × D	8	2.91	0.006

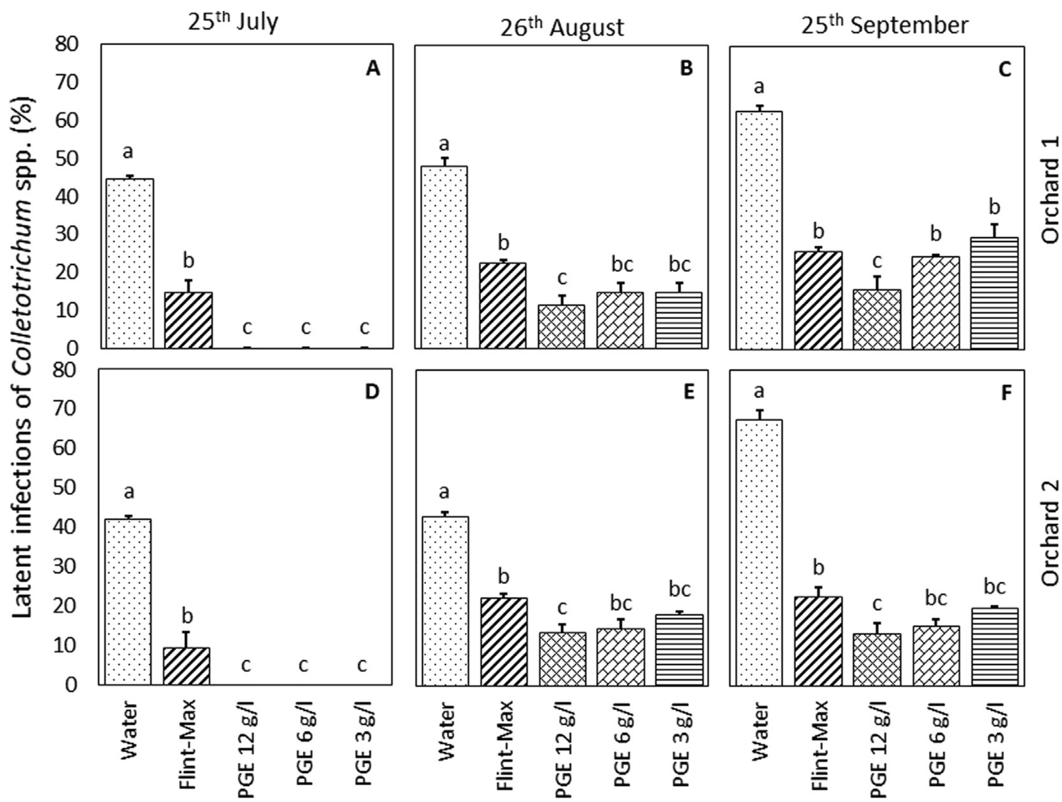

Fig. 1. Incidence of latent infections of *Colletrotrichum* spp. in developing olive fruit analyzed on July 25, August 26, and September 25, 2019. Olive trees have been sprayed with tap water (control), Flint Max (chemical control) or PGE at three different concentrations approximately 7 days before the expected full bloom (orchard 1) or 7 days before and 7 days after the full bloom (orchard 2).

the incidence of latent infections was reduced by 76.0% (PGE12), 68.7% (PGE6 and PGE3), and 53.1% (Flint Max) and PGE12 proved significantly more than Flint Max (Fig. 1B). Similar levels of reduction were achieved in the third assessment (September 25, 2019) although an overall increase of the incidence of latent infections was observed (Fig. 1C). Very similar results to those described for orchard 1 were achieved in the orchard 2 in terms of both efficacy of treatments and development of the disease over time (Fig. 1D, 1E, 1F).

3.2. Incidence of olive anthracnose

On control plants, a very high incidence of OA was recorded during the disease outbreak on November 14, 2019. In fact, 80.3 and 85.1% (orchard 1), and 80.0 and 96.6% (orchard 2) of the analyzed olive drupes showed typical symptoms of anthracnose on untreated olive trees and on plants sprayed with tap water, respectively (Fig. 2).

On November 14, all treatments significantly ($P < 0.001$) reduced the incidence of the disease in both orchards (Table 2, Fig. 1). PGE 12 reduced rotted drupes by 84.5 and 87.0% in orchard 1 and 2, respectively and was always significantly more effective than Flint Max and PGE3 (Fig. 2). No differences were observed among PGE6, PGE3 and Flint Max in the orchard 1 (Fig. 2A), while PGE6 proved more effective than PGE3 and Flint Max in the orchard 2 (Fig. 2B).

The incidence of infected olives after November 14 progressively increased on all plants including those treated with PGE and Flint Max. In about ten days, the percentage of rotted drupes on Flint Max-treated trees was similar to that observed on control plants on November 14 (data not shown).

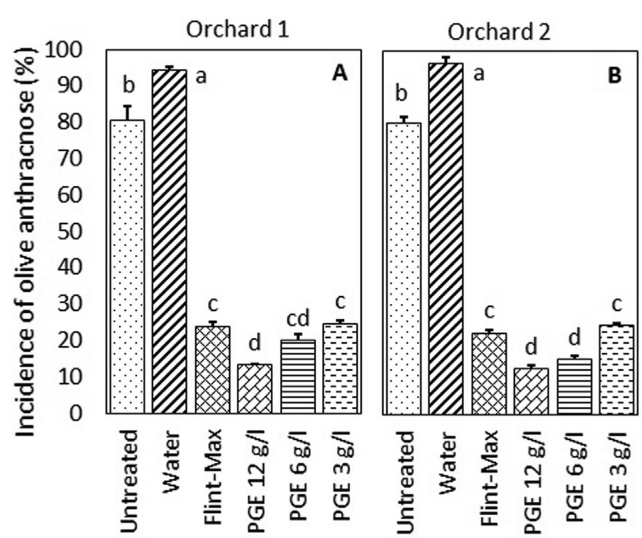

Fig. 2. Incidence of olive drupes showing typical symptoms of olive anthracnose during the outbreak of the disease on November 14, 2019. Olive trees have been sprayed with tap water (control), Flint Max (chemical control) or PGE at 3 different concentrations, approximately 7 days before the expected full bloom (orchard 1) or 7 days before and 7 days after the full bloom (orchard 2). Olive trees that did not receive any treatment (Untreated) were used as additional control.

3.3. Latent infections of Venturia oleaginea

Overall, a low incidence of latent infections of *V. oleaginea* was detected in the investigated fields. In fact, only 2.1 (orchard 1) and 2.3% (orchard 2) of the asymptomatic leaves collected from trees sprayed with tap water during flowering (control) showed

Table 2
Results of data analysis from fitting a linear model of incidence of olive anthracnose, latent infections of *V. oleaginea*, and olive defoliation using orchard (orchard 1 and 2), treatment type (water, Flint Max, and PGE at three different concentrations) and their interactions as fixed factors.

Factors	df	Olive anthracnose		*V. oleaginea*		Defoliation	
		F	P	F	P	F	P
Orchard (O)	1	0.18	0.67	0.68	0.41	2.09	0.15
Treatment type (T)	4	2858.39	< 0.001	48.06	< 0.001	2.92	0.03
O × T	4	0.44	0.77	0.79	0.53	1.62	0.19

the typical black spots of the Loprieno and Tenerini's method. However, treatments made with Flint Max and PGE at the three different concentrations significantly (P < 0.001) reduced or completely prevented infections (Table 2, Fig. 3). No significant difference were observed among different treatments.

3.4. Olive defoliation

According to the quantity of fallen leaves, the treatment type had a significant impact on the defoliation of plants (P = 0.03) (Table 2). In both orchards, approximately, 65% less leaves were collected from plants sprayed with PGE12 as compared to the control plants (Fig. 4). A similar trend was recorded for plants sprayed with Flint Max in orchard 1, but this result was not confirmed in orchard 2. Importantly, means of single treatments were never separated due to the marginal significance (P = 0.03) and the high-level variability within each treatment (Table 2; Fig. 4).

4. Discussion

We evaluated the efficacy of bloom treatments with a pomegranate peel extract to control OA and OLS. A commercial formulation (Flint Max) was used as chemical control. The idea of protecting flowers and young fruits to prevent blossom blight and reduce the establishment of latent infections of *Colletotrichum* spp. is not a novelty (Cacciola et al., 2012). This strategy has been earlier proposed in Australia but it is increasingly used in other geographic areas such as the Mediterranean basin as also demonstrated by the increasing application of Flint Max and Cabrio Olive (see introduction section). In the present study, we demonstrated that treatments with Flint Max have a strong impact on the incidence of latent infections of *Colletotrichum* spp., reduce the quantity of over-summering inoculum and delay the epidemic outbreak of the disease in autumn. More importantly, we demonstrated that similar or even better results, depending on the concentration used, can be achieved with a natural antifungal preparation such as PGE.

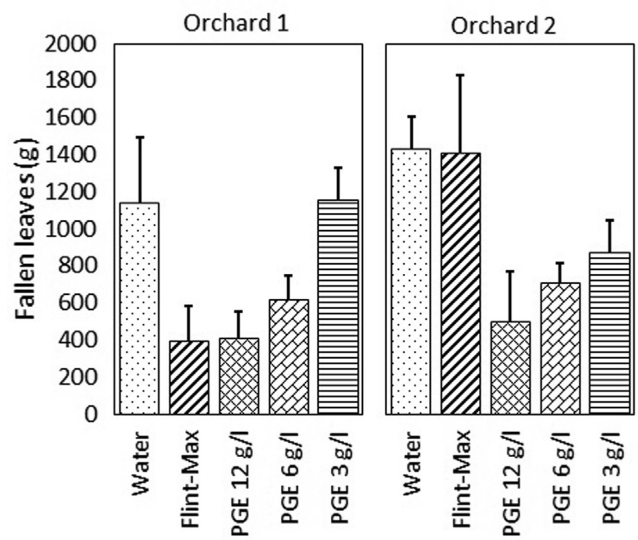

Fig. 4. Quantity of fallen olive leaves collected with commercial harvesting nets from June 23 to September 30, 2019. Olive plants have been sprayed with tap water (control), Flint Max (chemical control) or PGE at three different concentrations approximately 7 days before the expected full bloom (orchard 1) or 7 before and 7 days after the full bloom (orchard 2).

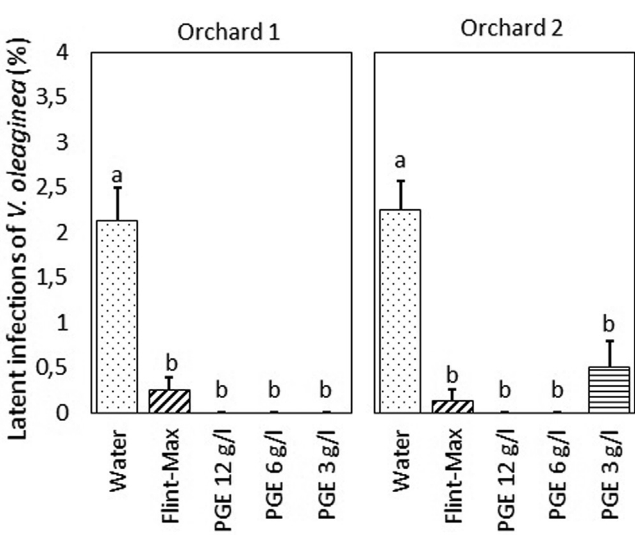

Fig. 3. Incidence of latent infections of *Venturia oleaginea* in olive leaves analyzed on July 7, 2019. Olive trees have been sprayed with tap water (control), Flint Max (chemical control) or PGE at three different concentrations approximately 7 days before the expected full bloom (orchard 1) or 7 before and 7 days after the full bloom (orchard 2). Latent infections were evaluated by dipping asymptomatic leaves in a solution of NaOH according to Loprieno and Tenerini (1959).

Our results also highlighted the great importance of infections on flowers and young fruit as inoculum sources of anthracnose epidemics in the investigated area. Previous studies conducted in Portugal and Spain suggested that infections of flowers and young fruits might not be relevant to explain the final disease outcome, which is mainly determined by weather conditions in autumn (Talhinhas et al., 2011). As opposed, in Uruguay abundant rains and long periods of high humidity during the flowering have been reported to cause severe blossom blight and high yield losses (Moreira et al., 2021). In the present study we did not reveal a high incidence of blossom blight but the incidence of latent infections was very high since more than 40% of the drupes were found to be infected in July and this incidence, further increased in August and September (Fig. 1). In the study by Talhinhas et al. (2011) the percentage of fruits harboring latent infections was not determined but in a previous study conducted in Spain a much lower (<1.5%) incidence of infected fruits was reported (Moral et al., 2009). The very high incidence of latent infections assessed in the present study is likely a consequence of the investigated area, the Gioa Tauro plain, Southern Italy, which is characterized by very conducive environmental conditions and by the prevalence of the very susceptible cv. Ottobratica (Cacciola et al., 2012). In the same area, previous investigations conducted in 2014 using a qPCR method confirmed a high concentration of DNA of *C. acutatum s.s.*

and *C. godetiae* in asymptomatic leaves and fruitlets (Schena et al., 2017). It should also be considered that although we did not evaluate the incidence of *Colletotrichum* spp. on leaves and branches, bloom treatments with Flint Max and PGE most likely reduced the incidence of the pathogen on these organs, which also serve as reservoir of inoculum (Talhinhas et al., 2011). In fact, the active ingredients of Flint Max have both a mesostemic and a systemic action. Similarly, the curative activity of PGE and other pomegranate peel extracts in several host/pathogen combinations has been already reported (Belgacem et al., 2021).

Regardless of the treatment type, a significant increasing trend was revealed for the incidence of latent infections of *Colletotrichum* spp. from July 25 to September 25, 2019. In the first assessment time, PGE was significantly more effective that Flint Max since the pathogen was always below the detection limit on plants sprayed with the extract. In the following samplings the incidence of infections slightly increased for all treatments although PGE at the dose of 12 g/l proved always more effective than Flint Max. The progressive increase of the incidence of latent infections during the summer season seem to confirm previous reports indicating that new infections can occur at all developing stages of olive drupes (Moral et al., 2009). However, since the investigated area is characterized by the typical dry and hot summer conditions of the Mediterranean climate, it is also possible that most latent infections originated in spring from flowers infections and their detection was progressively easier over the season just because the fungus slowly colonized infected olive drupes internally (Moral et al., 2009). In fact, the reported presence of internal brown tissues around the pit of infected olives during the summer period, confirms a progressive colonization of internal tissues before the appearance of external rots in autumn (Cacciola et al., 2012).

The primary role of flowers for the establishment of latent infections matches with the observed high efficacy of PGE treatments observed during the disease outbreak, in autumn. In fact, both, the extract at the three tested concentrations and the chemical control significantly reduced the incidence of the disease, being PGE at the dose of 12 g/l the most effective since reduced rots by 84.5 and 87.0% with one or two treatments, respectively. This level of efficacy was achieved at the peak of the disease outbreak, when almost all drupes were rotted on control plants. Soon after, most rotted olives fallen to the ground. A similar incidence of disease was also achieved on plants sprayed with PGE, but with a delay of approximately 10 days, probably because the lower quantity of over summering inoculum determined the need for more secondary cycles (Cacciola et al., 2012). From a practical point of view, a delay of 10 delays is extremely important because it gives more time to the farmers for harvesting before the development of rots and the fall of olive drupes. Furthermore, large-scale treatments might provide even better results since secondary infection cycles should not be triggered by the inoculum produced on neighboring untreated plants.

In a previous study, PGE provided a high level of protection against OA when applied on ripe olives just before the outbreak of the disease (Pangallo et al., 2017a). Future investigation are worth to evaluate the combination of spring and autumns PGE treatments and determine possible additive or even synergic effects in delaying thepeak of the disease outbreak and/or in reducing the incidence of the disease. Surprisingly, similar levels of efficacy where achieved with a single treatment before bloom (orchard 1) as compared to two applications before and after bloom (orchard 2). Although each experiment was conducted in a single orchard, the consistence of this result with all treatments (Flint Max and PGE at the three tested concentrations) represents a good evidence that a second treatment soon after flowering may be avoided with important practical implications on costs.

Although the timing of bloom treatments was planned focusing attention on the control OA a very positive effect was also revealed for OLS. In fact, both PGE at the three tested concentrations and Flint Max significantly reduced the incidence of latent infections; in most treated samples, the pathogen was not detected. Our results confirm once again a broad range of activity of PGE and its ability to control already established infections (Belgacem et al., 2021). The possible control of latent infections is extremely important because asymptomatic infections enable the over-summering and over-wintering of *V. oleaginea* and determine the gravity of the disease during conducive periods in spring and autumn (Thomidis et al., 2021; Viruega et al., 2013).

In the present study, the reduced incidence of latent infections of *V. oleaginea* did not determine a clear positive effect on olive defoliation. In fact, although treatments altogether had a significant impact by reducing the quantity of fallen leaves, single treatments were not differentiated because of a high level of variability within each treatment. The complex etiology of olive leaf defoliation that can be caused by several fungal pathogens as well as abiotic factors (Sanzani et al., 2012) might have contributed to the high variability observed within each treatment. Furthermore, it is important to point out that new infections of *V. oleaginea* mainly occur between the end of the winter and the beginning of the spring period. Consequently, when we made treatments (bloom period) symptomatic infected leaves were already fallen or where in and advanced phase of the disease. Traditionally, the growth phase with vegetation at about two-thirds of the final expected development is considered an ideal period to control OLS (Agosteo and Schena, 2011). Furthermore, we recently highlighted that treatments to control OLS should be anticipated because unlikely previously thought, *V. oleaginea* can infect leaves from the very early growth stages (Scibetta et al., 2020).

5. Conclusions

In conclusion, we demonstrated a high efficacy of bloom PGE treatments against OA and OLS. These findings, together with previous reports on the use of autumn PGE treatments to control anthracnose and its broad range of activity against several different fungal pathogens suggests the possible use of PGE as a key alternative preparation to develop sustainable control methods against olive diseases (Belgacem et al., 2021; Pangallo et al., 2017a). In this context, future investigations should focus on the general protection of olive trees rather than on specific disease in order to define the best schedule for treatments as well as the possible integration with other control means.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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