Effect of photosensitisers on growth and morphology of *Phytophthora citrophthora* coupled with leaf bioassays in pear seedlings

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Abstract: The phytopathogenic oomycetes of the genus *Phytophthora* cause devastating economic losses worldwide. Naphthodianthrone compounds, present in plant extracts of buckwheat and Saint John's wort act as photosensitiser agents and exhibit antimicrobial activity against a number of pathogens. In this study, we investigated the potential inhibitory effects of fagopyrin and hypericin on *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian 1906, the main causal agent of rot diseases in deciduous trees. Fagopyrin had the highest inhibitory effect in the colony growth at a concentration of 2% of a stock solution (3 mg/mL), inducing clubbed hyphae with round tips. Notably, hypericin also inhibited the radial colony growth and increased the hyphal branching at the subapical region, while also promoting the formation of enlarged cells with irregular shapes growing collectively as biofilm-like structures. In terms of the mycelial dry weight, although both photosensitisers had considerable inhibitory effects, the fagopyrin treatment was most effective. Leaf bioassays showed that under dark conditions the photosensitiser pre-treated zoospores formed a dense, but aberrant, mycelial growth with penetration defects. In contrast, when the zoospore production was performed under light conditions, the zoospores failed to cause necrotic lesions and penetration events implying that their virulence was impaired. These findings shed light on the biological effects of fagopyrin and hypericin in the regulation of the mycelial growth, morphology and pathogenicity of *P. citrophthora*.

Keywords: crop protection; hyphal morphology; oomycetes; phytochemicals; plant-microbe interactions

The oomycete species of the genus *Phytophthora*, which are grouped into the kingdom Chromista are the causal agents for destructive damage to crops worldwide (Martin et al. 2014; Yang et al. 2017). The potential exploitation of bioactive compounds towards the integrated management of these phytopathogenic species is of high importance for crop protection. Among the *Phytophthora* species infecting *Rosaceae* species, *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian 1906, is a soil-borne pathogen causing root rot, trunk gummosis, leaf and shoot blight to pears and other deciduous crops (Pane et al. 2009). This pathogen completes its life cycle in the soil, where chlamydospores germinate and develop hyphae and sporangia, which initiate the asexual reproductive cycle (Elena & Paplomatas 1999; Cacciola & Lio 2008). *P. citrophthora* is a heterothallic species and produces papillated sporangia, often irregular and asymmetrically shaped, whereas the pathogen is characterised by the absence of

sexual structures in culture conditions (Bush et al. 2006). According to a genus-wide phylogeny of *Phytophthora* (Blair et al. 2008), this species belongs to Clade 2 along with *Phytophthora capsici* Leonian 1922, *Phytophthora citricola* Sawada 1927 and *Phytophthora plurivora* Jung & Burgess 2009.

Photosensitiser molecules have been proposed as alternative antifungal agents for the control of plant pathogens (Huang et al. 2018). They are light absorbing molecules that induce chemical changes and photosensitisation within the cells. Natural pigments extracted from various plant tissues may act as photosensitisers through their light-dependent excitation (Villacorta et al. 2017). Recent studies report that fagopyrin extracted from buckwheat (*Fagopyrum esculentum* Moench) and hypericin extracted from Saint John's wort (*Hypericum perforatum* Linnaeus) have photosensitising properties that may affect the proliferation of microbial cells (Simonetti et al. 2016; Süntar et al. 2016; Sytar et al. 2016). The hypericin uptake by a microorganism can cause a variety of photodynamic actions when it is photo-activated with spectrum-specific light exposure (Nakajima & Kawashima 2012).

Biologically active extracts of higher plants were reported to exhibit prominent antifungal properties and they might be considered alternative solutions to synthetic fungicides in crop protection management (Yanar et al. 2011; Díaz et al. 2018). These compounds can retard the reproduction and growth of plant pathogenic fungi including oomycetes of the genus *Phytophthora* (Stephan & Koch 2002; Krebs et al. 2006; Yanar et al. 2011). Specifically, caripyrin, which is a pyridyloxirane, being isolated from *Gymnopus montagnei* (Berk.) Redhead (2014) was found to inhibit both the conidial germination and appressorium formation in *P. oryzae* (Rieger et al. 2010). Besides, the adaptation of pathogens against stressful conditions involves the formation of multicellular communities known as biofilms (Huang et al. 2018). *Botrytis cinerea* Persoon 1794 during the infection of tomato stems created intensively layered hyphal networks, which were embedded in an exopolymeric matrix (Harding et al. 2010). The existence of similar heterogeneous hyphae and extracellular polysaccharide architectures were revealed during the *Fusarium oxysporum* Schlechtendal f.sp. *cucumerinum* interaction with the plant hosts (Peiqian et al. 2014).

Herein, we report the biological effects of fagopyrin and hypericin on the growth and morphology of *P. citrophthora* in solid and liquid cultures. Furthermore, in order to assess the pathogenic potential of *P. citrophthora* zoospores treated with these photosensitisers, we performed leaf bioassays on pear seedlings coupled with epifluorescence microscopy.

MATERIAL AND METHODS

Isolate and culture conditions. *P. citrophthora* (strain No. 1915), which was previously isolated from *Pyrus communis* bark, was kindly provided by the *Phytophthora* collection of the Benaki Phytopathological Institute. The isolate was cultured on Corn Meal Agar (CMA) plates at 24 °C and maintained by transferring mycelial plugs from the fully-grown culture to fresh plates every 2 weeks.

Isolation of the photosensitisers. Fagopyrin and hypericin were purified with silica and Sephadex LH-20 column chromatography according to Agostinis et al. (1996) and both phytochemicals were characterised employing HPLC (high-performance liquid chomatography) analysis and UV/VIS (ultraviolet-visible) spectrometry (Sytar et al. 2016). The stock solutions of hypericin and fagopyrin (3 mg/mL) were prepared in dimethyl sulfoxide (DMSO) and stored at −20 °C under dark conditions.

Effects of the photosensitisers on the mycelial colony growth. The inhibitory effects of fagopyrin and hypericin in the radial mycelial growth of *P. citrophthora* were assigned *in vitro* by using three different concentrations (0.5, 1 and 2% v/v) of each photosensitiser on CMA plates. Thus, the appropriate serial dilutions of the stock concentrations of each photosensitiser were prepared accordingly. The pathogen was inoculated by transferring 2 mm diameter plugs from the growing margin of the 10-day-old colonies and placing them in the centre of the 6-cm-diameter CMA plates containing either (*i*) the DMSO solvent-only (control treatment, C), (*ii*) the fagopyrin (F) or (*iii*) the hypericin (H); each photosensitiser was supplemented at the three above concentrations. All the subcultures were incubated at 24 °C under light conditions for up to14 days. Each treatment was examined in eight replicates to ensure the application consistency of the two photosensitisers. At the end of the above growth period, the diameter of each colony was recorded and converted to radial growth.

Effect of the photosensitisers on the mycelial dry weight. Liquid culture experiments were conducted to assess the hyphal morphologies in the presence of the photosensitisers. The photosensitizers were add-

ed from the stock solutions in V8 medium (50 mL) to give 2% concentration and *P. citrophthora* was inoculated by transferring one plug of mycelium (5 mm diameter) from the margins of a colony. The hyphal morphologies after stationary incubation in the dark for 7 days at 24 °C were microscopically examined and then the cultures were minced and 1 mL of these minced mycelia were added to 25 mL of the medium in conical flasks of 100-mL in volume. The cultures were harvested after 10 days, filtered through Whatman filter paper and the mycelial mats were thoroughly rinsed with ddH$_2$O (double-distilled water) to remove any salts associated with the mycelia. Their dry weights were recorded after oven drying the filter papers along with the mycelial mats. All the experiments were performed in eight replicates.

Statistical analysis. A one-way ANOVA was conducted for assessing the effects of the photosensitisers on the hyphal growth at the three tested concentrations, as well as in the experiments for the determination of the mycelial dry weights. The statistical differences among each treatment were revealed by comparing the mean values of the radial growth and of the mycelial dry weight with a significance threshold setting at $P < 0.05$. Tukey's post-hoc analysis followed to determine which means differed significantly. The statistical analyses were performed with the GraphPad Prism software (version 5).

Morphological characteristics of *P. citrophthora* after exposure to the photosensitisers. The hyphal colonies were aseptically analysed by bright field (BF) and epifluorescence microscopy for the estimation of the potential morphological alterations upon the photosensitiser exposure at concentrations of 2% v/v. The microscopic observations images were captured using a Zeiss Axioscope 40 epifluorescence microscope (Zeiss, Germany) coupled with a ProgRes CF cool camera (Jenoptik, Germany), and ProgRes CapturePro software (version 2.0). The BF images were obtained for each treatment to facilitate the morphology identification. Epifluorescence microscopy was employed to evaluate the cellular uptake and subcellular distribution of the photosensitisers in the *P. citrophthora* cells using an excitation wavelength of 405 nm with an emission wavelength of 455 nm employing a specific blue (DAPI) filter channel.

Plant material, zoospore production and leaf bioassays. Ten-week-old *P. communis* seedlings (pear rootstock OHF-333) grown in pots in a greenhouse under controlled conditions (24 °C with a 16 h photoperiod at 75% rel. humidity. Prior to the leaf inoculation with the *P. citrophthora* zoospore suspensions, the leaves were washed three times with ddH$_2$O. Two independent bioassays were performed with the intact leaf samples and the experiment was repeated three times.

The zoospore production was performed according to Larousse et al. (2014) with minor modifications. Briefly, the mycelium of *P. citrophthora* was cultured for 10 days in a V8 liquid medium at 24 °C under continuous light and then it was macerated and incubated on 2% water agar plates, which were previously supplemented with either fagopyrin (2%) or hypericin (2%). Similar subcultures were obtained by only incubating with *P. citrophthora* (control treatments). All the subcultures were incubated for a further four days under dark or light conditions. Then, a heat shock treatment was performed in order to induce the release of the zoospores, which involved incubation for 1.5 h at 4 °C followed by incubation for 30 min at 37 °C with iced water. The zoospore suspensions of *P. citrophthora* (10^6 cells/mL) of all the subcultures were used for the *in planta* infection bioassays by inoculating the upper surfaces of the young intact leaves with a 10 μL suspension of zoospores in a Tween 20 (0.01%) solution. Microscopic observations of the leaf disks in the infection spots were performed after 10 days.

RESULTS

Effect of the photosensitisers on the colony growth. Assessment of the *in vitro* effects of the photosensitiser uptake by *P. citrophthora* was determined by measuring the mean radial growth of the mycelial colonies on the CMA plates under the three tested concentrations of fagopyrin or hypericin (Figure 1A). Overall, the presence of the photosensitisers in the CMA medium caused a significant ($P < 0.001$) decrease in the colony radial growth. This response was highly dependent on the concentrations of the photosensitisers with the highest inhibition of radial growth occurring in the concentrations of 2% v/v for both the photosensitisers (Figure 1A). The representative CMA plates with the expansion of the colonies are shown in Figure 1B. It was evident that fagopyrin at the above concentration was more efficient than hypericin to inhibit the colony growth of *P. citrophthora*.

Specifically, the control cultures of *P. citrophthora* showed an even distribution of the hyphal biomass within the colony. Contrary, the fagopyrin treatment (2% v/v) caused a significant inhibition
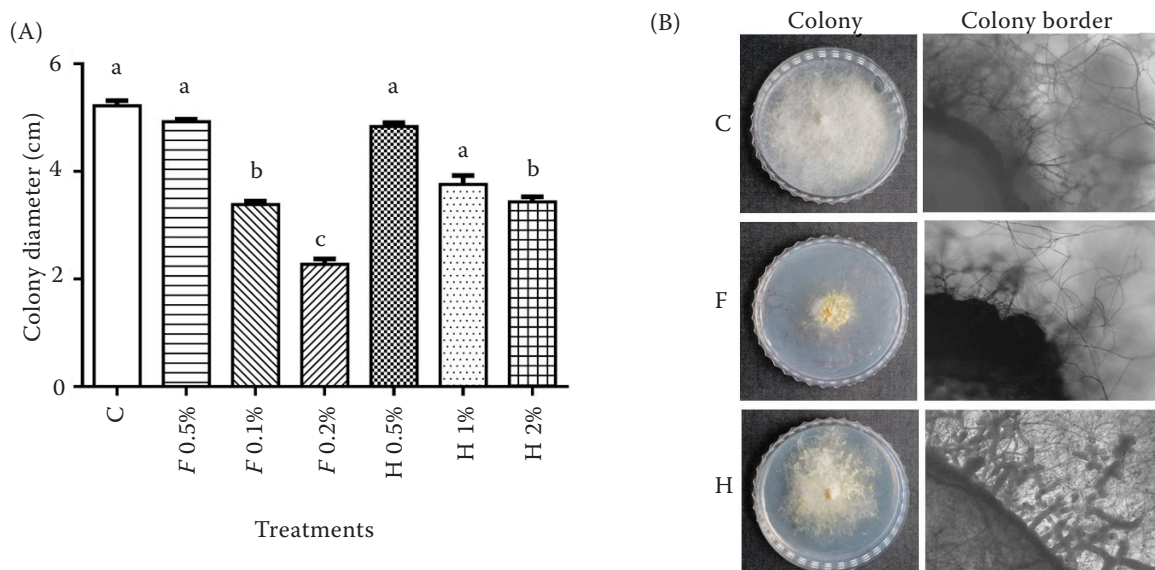
Figure 1. The effect of the photosensitisers on the hyphal growth and peripheral morphology of *P. citrophthora* colonies under light conditions

(A) The colony radial growth on the CMA plates after exposure to variable concentrations of fagopyrin (F) and hypericin (H) ranging from 0.5% to 2% and incubation at 24 °C for 14 days; (B) The representative solid CMA substrate supplemented with either 2% fagopyrin (F) or 2% hypericin (H). The 14-day-old colonies of the CMA cultures mock-inoculated with *P. citrophthora* served as the control (C); the peripheral morphologies of these colonies are shown in the right panel

($P < 0.001$) of the radial growth by almost 2.5-fold, while the colonies appeared compact with a reduced density at their periphery, and the biomass was allocated at the interior of the colony (Figure 1B, right panel). A significant ($P < 0.001$), but less pronounced, inhibition in the radial growth was evident in the hypericin-treated cultures (Figure 1B). Notably, these colonies displayed an irregular biomass distribution at their periphery and a reduced mycelium density along with enlarged cell formations (Figure 1B, right panel).

Alterations in the hyphal density, organisation and fluorescence signal. Based on the above results, a thorough microscopic investigation was conducted upon the biological effects of the photosensitisers at the 2% v/v concentrations, which appeared to be the most potent for both of them. The BF microscopic observations showed a rather dense mycelium in the control cultures with the hyphal networks forming lattices for their extension. The hyphal density was reduced in the photosensitiser-treated cultures with fewer and smaller networks compared to the control cultures (Figure 2).
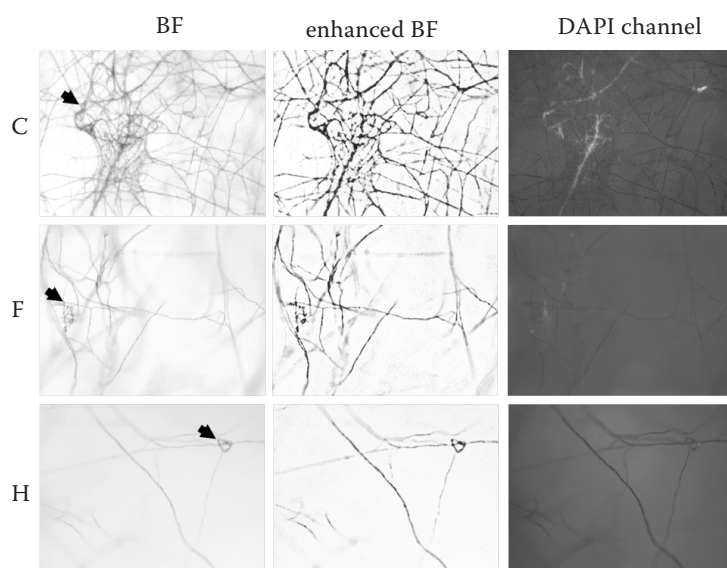

Figure 2. The effect of (F) fagopyrin and (H) hypericin on the *P. citrophthora* mycelium organisation

Each photosensitiser was supplemented at a 2% concentration on the CMA plates while (C) the untreated cultures served as the control; the arrowheads denote the presence of an intricate hyphal network in the mycelium and blunt ends in the hyphae; the enhanced BF images are also shown, while the separate fluorescent images were recovered after irradiation by the DAPI channel
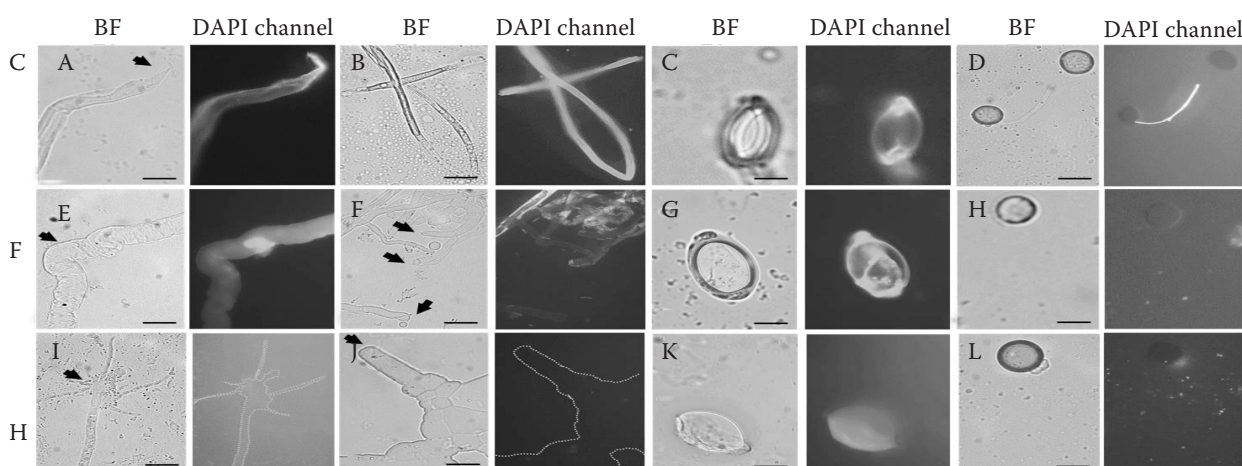
Figure 3. The micrographs of the *P. citrophthora* cultures showing the morphologies of the asexual structures with alterations in the hyphal shapes and fluorescence intensities after exposure to the photosensitisers

(C) The control cultures A–D; (F) the fagopyrin-treated cultures E–H; (H) the hypericin-treated cultures I–L; the scale bars = 10 μm; the separate fluorescent images show the emission intensities recovered by the DAPI channel

Remarkably, the control mycelium exhibited strong fluorescence in the lattices, whereas the photosensitiser-treated cultures showed a decreased fluorescence signal (Figure 2). These results, underscore the biological effects of both photosensitisers on the hyphal organisation within the mycelium.

Phenotypic effects of the photosensitisers on the asexual structures and mycelial dry weights. The control cultures typically displayed invasive hyphae with pointed apices (Figure 3A) and strong fluorescence localised on the sides of the polarised hyphal structures (Figure 3B, right panel) and germinating chlamydospores (Figure 3D, right panel). In contrast, the fagopyrin-treated cultures exhibited clubbed hyphae (Figure 3E), which appeared dilated with a diffused fluorescence pattern (Figure 3E, right panel) compared to the control. In all the treatments, fluorescence was observed at the papillated sporangia (Figure 3C, G, K), but not at the chlamydospores (Figure 3D, H, L). Notably, the hypericin-treated cultures displayed defects in the hyphal morphology. Firstly, they produced an increased number of secondary polarising tubes in the subapical regions (Figure 3I), whereas the control cultures exhibited a dominant polarity axis of growth. Secondly, a large fraction of the cells switched to putative BLS (biofilm-like structures) collective forms, characterised by irregular shapes and an abnormally enlarged size (Figure 3J). Experiments in the liquid cultures showed similar results regarding the effect of the photosensitisers on *P. citrophthora* in terms of the hyphal morpholo-

gies. Remarkably, the fagopyrin-treated hyphae exhibited a diffused fluorescence pattern, whereas the hypericin-treated ones were characterised by irregular shapes and an abnormally enlarged size (Figure 4). The photosensitisers also had a pronounced inhibitory effect on the mycelial dry weights in the liquid cultures (Table 1). Specifically, in the liquid V8 cultures, fagopyrin caused a 49.63% inhibition of the mycelial dry weight, while hypericin caused a 17.11% inhibition of the mycelial dry weight.

Leaf bioassays. The leaf bioassays revealed a differential host response which was dependent on whether light or dark conditions were applied during the zoospore production. Specifically, under dark conditions, the photosensitiser-pretreated zoospores induced the mycelial growth with a high fluorescence signal when examined microscopically under UV exposure. This mycelial growth formed layered hyphal networks consisting mostly of short hyphae. Notably, there were no visible lesions in the leaves, even 10 days after the zoospore inoculation, implying the induction of a non-invasive accumulation of dense hyphae. In the case of the

Table 1. The effect of the photosensitisers on the mycelial dry weight of *Phytophthora citrophthora*

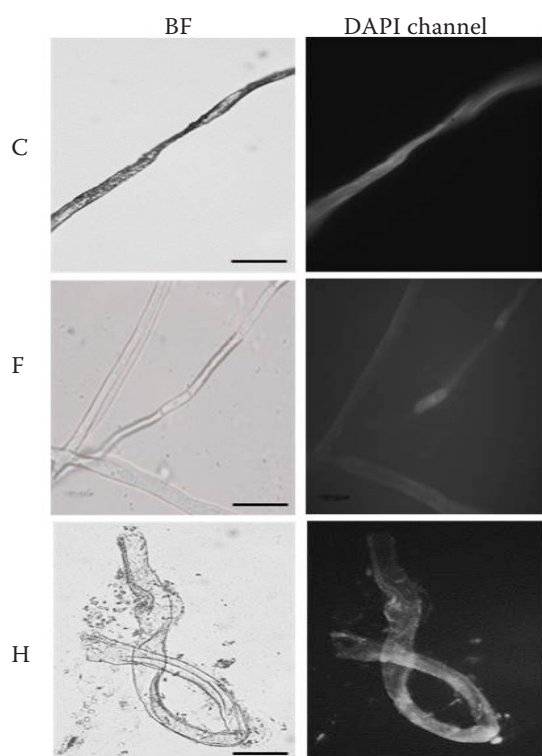| Treatments	Dry weight (mg/25 mL)	Inhibition (%)
Control	151.3 ± 1.58[a]	–
Fagopyrin 2%	76.2 ± 1.46[b]	49.63
Hypericin 2%	125.4 ± 1.21[c]	17.11

Figure 4. The effect of the photosensitisers on the *P. citrophthora* hyphal morphology in the 7-day-old liquid cultures on the V8 medium at 24 °C under light conditions

(C) the control culture; (F) the fagopyrin-treated culture; (H) the hypericin-treated culture; the scale bars = 10 μm; the separate fluorescent images show the emission intensities recovered by the DAPI channel; each photosensitiser was supplemented at a 2% concentration

zoospores produced under light conditions, no signs of the zoospore penetration and hyphal formation in the leaves were observed. Finally, in the control bioassays, typical necrotic lesions were observed resembling a hypersensitive response of an incompatible *P. citrophthora*-host interaction (Figure 5).

DISCUSSION

In this study, we investigated the biological effects of two structurally related photosensitisers against *P. citrophthora.* Particularly, we investigated their impact on the colony growth, mycelial dry weight, morphology of the asexual structures and the pathogenic potential of the *P. citrophthora* zoospores in the leaf bioassays. Nowadays, there is ongoing research on phytochemical compounds with promising antifungal properties (Yanar et al. 2011; Borges et al. 2016; Díaz et al. 2018). Naphthodianthrones, such as fagopyrin and hypericin, are photodynamic substrates acting as photosensitisers inside the cells (Sytar et al. 2016). Besides their photodynamic properties, they also have some biological activity in specific cellular compartments without irradiation (Jendželovská et al. 2016). Our results showed that the photosensitisers had a significant inhibitory effect, especially at their higher tested concentrations (2%) on the colony growth and mycelial dry weight in the solid and liquid cultures. These data provide the first evidence of the fagopyrin and hypericin inhibitory effects on the hyphal growth of *P. citrophthora,* whereas
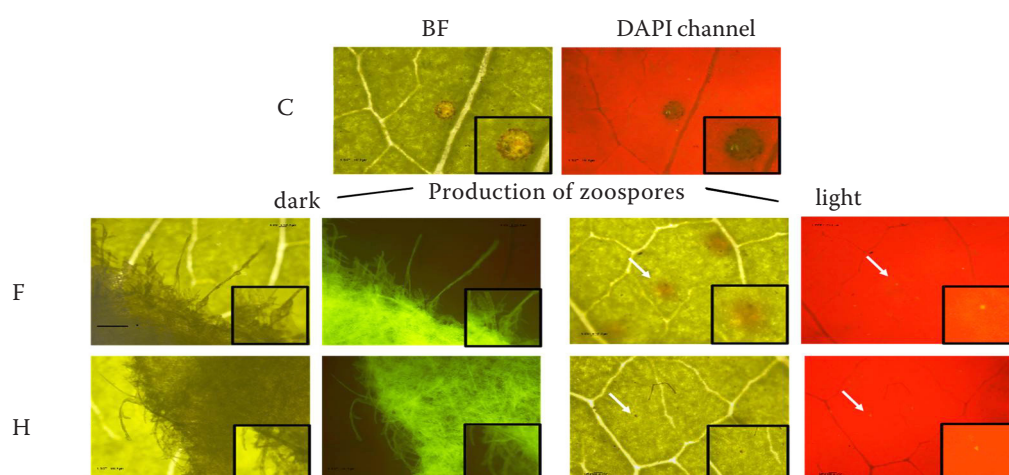

Figure 5. The *in planta* leaf bioassays of the pear seedlings with the *P. citrophthora* zoospore suspensions produced in the absence of the photosensitisers, (C) the control treatments, and in cultures supplemented with either 2% (F) fagopyrin or (H) hypericin. The inset pictures at the right bottom show the magnified symptoms

The fluorescent microscopy images captured by the bright field or by the DAPI channel. The scale bar = 400 μm

the antimicrobial properties of these two compounds have been recently evaluated on other microbes (Simonetti et al. 2016; Süntar et al. 2016; Sytar et al. 2016; Huang et al. 2018).

The photosensitiser treatments reduced the density of the mycelia, the number of hyphal lattices and their fluorescence intensity. Previously, the role of the hypericin-mediated photodynamic antimicrobial effect was previously documented in some strains of spoilage yeasts (Yow et al. 2012). In our study, the photosensitiser uptake did not appear to be cytotoxic to the *P. citrophthora* cells. Although the inhibitory effect of hypericin is in agreement with a previous study (Bilia et al. 2001), we additionally showed the formation of enlarged cells with irregular shapes growing collectively as a BLS. These cells colonised and settled on the solid substrate, but lacked invasive polarised structures. Remarkably, the hypericin treatment impaired the formation of a single axis of hyphal polarity, resulting in a loss of apical dominance and a reduced lateral branching when compared with the control cultures, which extended radially and formed a dense and evenly expanded mycelium. The mechanism by which the sub-apical hyphal sites are selected for branching is unclear, but there is evidence that the localised accumulation of calcium or ROS (Reactive Oxygen Species) might promote the formation of new tips at the sub-apical sites (Semighini & Harris 2008). This is in agreement with the known function of the photosensitisers to generate ROS (Bilkis et al. 2018).

Fagopyrin had a more pronounced inhibitory effect on the radial colony growth and the mycelial dry weight of *P. citrophthora*. The hyphal dilation, clubbed appearance and round apices were continuously observed in the fagopyrin-treated cultures along with alterations in the localisation of the fluorescence, indicating changes in the hyphal metabolic activity. Similar results regarding the hyphal morphologies and fluorescence densities were also observed in the liquid cultures containing 2% concentrations of the photosensitisers.

Therefore, our results strongly suggest that these two photosensitisers, which are both lipid-soluble, accumulated in the cell membranes and appeared quite stable when briefly irradiated. A parallel investigation in other *Phytophthora* strains (*Phytophthora nicotianae* Breda de Haan 1896 and *Phytophthora palmivora* E.J. Butler 1919), sharing a broad host range, revealed similar results in terms of the hyphal morphology and fluorescence intensities in solid cultures that were amended with these two photosensitisers. This evidence further supports the conservation of the inhibitory effect of the photosensitisers across the genus *Phytophthora*. Specifically, the control cultures displayed hyphae with pointed apices and strong fluorescence localised on the sides of the polarised hyphal structures. In contrast, diffused fluorescence patterns and defects in the hyphal morphology in terms of irregular shapes were observed in the fagopyrin and hypericin-treated cultures (Figure 6).

Leaf bioassays were performed in order to elucidate the effects of fagopyrin and hypericin during the *P. citrophthora* zoospores infection on pear seedlings and to decipher whether these
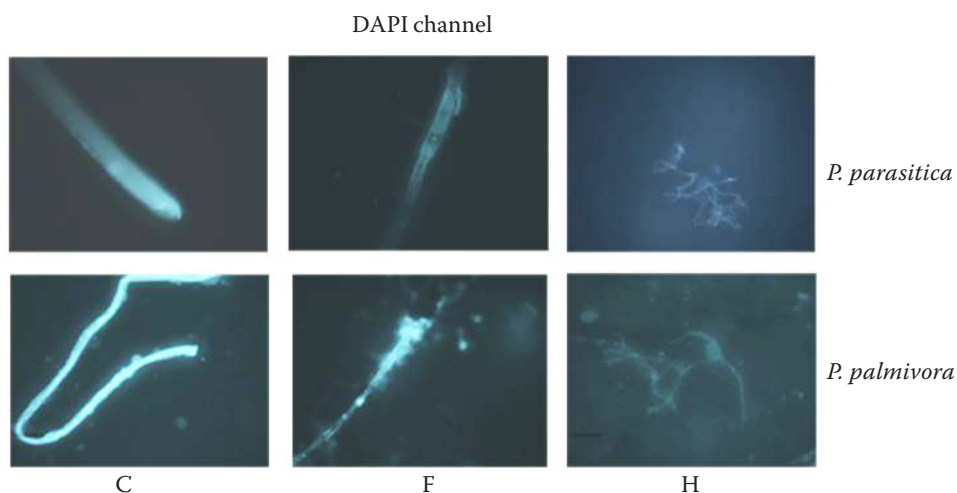
DAPI channel

Figure 6. The hyphal morphologies and fluorescence intensities of *P. parasitica* and *P. palmivora* after exposure to the photosensitisers (C) Control; (F) Fagopyrin-treated (2%) and (H) Hypericin-treated (2%) cultures in CMA plates incubated at 24 °C for 14 days. Emission intensities recovered at DAPI channel

phytochemical influences affect the pathogenicity in some way. Necrotic leaf lesions that closely resembled a hypersensitive response were observed in the control treatments and were indicative of the establishment of an incompatible interaction at the point of the zoospore penetration. When the zoospores were produced under dark conditions, the photosensitisers favoured a non-invasive accumulation of dense hyphae. Presumably, under these conditions, the effects of the photosensitisers were diminished at their tested concentrations. However, under light conditions, a few lesions were observed, albeit not necrotic, implying that the virulence of the zoospores was inactivated in the presence of photosensitisers which effectively prevented any hyphal formation. The present results suggest that both photosensitisers interfere biologically during the *P. citrophthora* zoospore production. A previous study in *P. capsici* documented that zoospores are highly induced on pepper roots in hydroponic culture under dark when compared to light cycle conditions (Nielsen et al. 2006). Our observations are consistent with the light-dependent photodynamic and antimicrobial effects of the photosensitisers (Simonetti et al. 2016; Süntar et al. 2016; Sytar et al. 2016). Therefore, the leaf bioassays with the *P. citrophthora* zoospores revealed a differential host response which was dependent on whether light or dark conditions were applied during the zoospore production.

Overall, and despite that fagopyrin and hypericin are structurally related, our results revealed that their effects are not identical on the growth, morphology and pathogenicity of *P. citrophthora*. Although the molecular basis of the mechanism that regulates this biological interference remains to be elucidated in the future, the present study highlights the differential and inhibitory effects of these photosensitisers on *P. citrophthora*.

REFERENCES

Agostinis P., Donella-Deana A., Cuveele J., Vandenbogaerde A., Sarno S., Merlevede W., Witte P. (1996): A Comparative analysis of the photosensitized inhibition of growth-factor regulated protein kinases by hypericin-derivatives. Biochemical and Biophysical Research Communications, 220: 613–617.

Bilia R., Bergonzi C., Morgenni F., Mazzi G.,Vincieri F. (2001): Evaluation of chemical stability of St. John's wort commercial extract and some preparations. International Journal of Pharmaceutics, 213: 199–208.

Bilkis I., Silman I., Weiner L. (2018): Generation of reactive oxygen species by photosensitizers and their modes of action on proteins. Current Medicinal Chemistry, 25: 5528–5539.

Blair E., Coffey D., Park Y., Geiser M., Kang S. (2008): A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genetics and Biology, 45: 266–277.

Borges A., Abreu C., Dias C., Saavedra J., Borges F., Simões M. (2016): New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms. Molecules, 21: 877–918.

Bush A., Stromberg L., Hong C., Richardson A., Kong P. (2006): Illustration of key morphological characteristics of *Phytophthora* species identified in Virginia nursery irrigation water. Plant Health Progress, 7: 1–12.

Cacciola O., Lio M. di S. (2008): Management of Citrus diseases caused by *Phytophthora* spp. In: Ciancio A., Mukerji K.G. (eds): Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria. Dordrecht, Springer: 61–84.

Díaz K., Espinoza J., Espinoza L., González C., Madrid A. (2018): Labdanes: antifungal compounds isolates from the resinous exudate of *Madia sativa* against *Phytophthora cinnamomi* Rands. Natural Product Research, 25: 1–5.

Elena K., Paplomatas E. (1999): Collar rot caused by *Phytophthora citrophthora* on pear trees in Greece. Phytoparasitica, 27: 291–298.

Harding W., Marques R., Howard J., Olson E. (2010): Biofilm morphologies of plant pathogenic fungi. The Americas Journal of Plant Science and Biotechnology, 4: 43–47.

Huang C., Shen M., Huang Y.J., Lin H.C., Chen C.T. (2018): Photodynamic inactivation potentiates the susceptibility of antifungal agents against the planktonic and biofilm cells of *Candida albicans*. International Journal of Molecular Sciences, 19: 434–446.

Jendželovská Z., Jendželovský R., Kuchárová B., Fedoročko P. (2016): Hypericin in the light and in the dark: two sides of the same coin. Frontiers in Plant Science, 7: 1–20.

Krebs H., Dorn B., Forrer H.R. (2006): Control of late blight of potato with medicinal plant suspensions. Agrarforschung, 13: 16–21.

Larousse M., Govetto B., Séassau A., Etienne C., Industri B., Theodorakopoulos N., Deleury E., Ponchet M., Panabières F., Galiana E. (2014): Characterization of PPMUCL1/2/3, Three members of a new oomycete-specific mucin-like protein family residing in *Phytophthora parasitica* biofilm. Protist, 165: 275–292.

Martin F.N., Blair J.E., Coffey M.D. (2014): A combined mitochondrial and nuclear multilocus phylogeny of the genus *Phytophthora*. Fungal Genetics and Biology, 66: 19–32.

Nakajima N., Kawashima N. (2012): A basic study on hypericin-PDT *in vitro*. Photodiagnosis and Photodynamic Therapy, 9: 196–203.

Nielsen C.J., Ferrin D.M., Stanghellini M.E. (2006): Cyclic production of sporangia and zoospores by *Phytophthora capsici* on pepper roots in hydroponic culture. Canadian Journal of Plant Pathology, 28: 461–466.

Pane A., Cacciola S., Scibetta S., Bentivenga G., Magnano di San Lio G. (2009): Four *Phytophthora* species causing foot and root rot of apricot in Italy. Plant Disease, 93: 844–845.

Peiqian L., Xiaoming P., Huifang S., Jingxin Z., Ning H., Birun L. (2014): Biofilm formation by *Fusarium oxysporum* f. sp. *cucumerinum* and susceptibility to environmental stress. FEMS Microbiology Letters, 350: 138–145.

Rieger P.H., Liermann J.C., Opatz T., Anke H., Thines E. (2010): Caripyrin, a new inhibitor of infection-related morphogenesis in the rice blast fungus *Magnaporthe oryzae*. Journal of Antibiotics, 63: 285–289.

Semighini P., Harris D. (2008): Regulation of apical dominance in *Aspergillus nidulans* hyphae by reactive oxygen species. Genetics, 179: 1919–1932.

Simonetti G., Tocci N., Valletta A., Brasili E., D'Auria F. D., Idoux A., Pasqua G. (2016): *In vitro* antifungal activity of extracts obtained from *Hypericum perforatum* adventitious roots cultured in a mist bioreactor against planktonic cells and biofilm of *Malassezia furfur*. Natural Product Research, 30: 544–550.

Stephan D., Koch E. (2002): Screening of plant extracts, microrganisms and commercial prepartions for biocontrol of *Phytophthora infestans* on detached potato leaves. Bulletin OILB/SROP, 25: 341–394.

Süntar I., Oyardi O., Akkol E.K., Ozçelik B. (2016): Antimicrobial effect of the extracts from *Hypericum perforatum* against oral bacteria and biofilm formation. Pharmaceutical Biology, 54: 1065–1070.

Sytar O., Švedienė J., Ložienė K., Paškevičius A., Kosyan A., Taran N. (2016): Antifungal properties of hypericin, hypericin tetrasulphonic acid and fagopyrin on pathogenic fungi and spoilage yeasts. Pharmaceutical Biology, 54: 3121–3125.

Villacorta R.B., Roque K.F.J., Tapang G.A., Jacinto S.D. (2017): Plant extracts as natural photosensitizers in photodynamic therapy: in vitro activity against human mammary adenocarcinoma MCF-7 cells. Asian Pacific Journal of Tropical Biomedicine, 7: 358–366.

Yanar Y., Kadioğlu I., Gökçe A., Demirtaş B., Gören N., Cam H., Whalon M. (2011): *In vitro* antifungal activities of 26 plant extracts on mycelial growth of *Phytophthora infestans* (Mont.) de Bary. African Journal of Biotechnology, 10: 2625–2629.

Yang X., Tyler B.M., Hong C. (2017): An expanded phylogeny for the genus *Phytophthora*. IMA Fungus, 8: 355–384.

Yow C.M.N., Tang H.M., Chu E.S.M., Huang, Z. (2012): Hypericin-mediated photodynamic antimicrobial effect on clinically isolated pathogens. Photochemistry and Photobiology, 88: 626–632.