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Photodynamic inactivation of *Lasiodiplodia theobromae*: lighting the way towards an environmentally friendly phytosanitary treatment

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The fungus *Lasiodiplodia theobromae* is one of the main causal agents of trunk canker and dieback of grapevine. The objective of this work was to evaluate the efficiency of photodynamic inactivation (PDI) of *L. theobromae* with synthetic and natural photosensitizers and irradiation with either sunlight or artificial photosynthetically active radiation. Although the growth of the mycelium could not be completely prevented with natural sunlight irradiation, phenothiazine dyes (methylene blue, MB; toluidine blue O, TBO), riboflavin and a cationic porphyrin (Tetra-Py$^+$-Me) caused complete inhibition under continuous irradiation with artificial light. Free radicals were the main cytotoxic agents in the PDI with MB, indicating the predominance of the type I mechanism. PDI with MB or Tetra-Py$^+$-Me may represent a promising approach for the sanitation of vine material in greenhouse nurseries, in order to reduce the risk of infection upon grafting.

1. Introduction

Lasiodiplodia theobromae (Pat.) Griff. & Maubl. [1] is the causative agent of trunk canker in vine, being currently considered as a tropical to sub-tropical vine pathogen of global incidence [2]. Infection occurs mainly through wounds produced during pruning [3]. The symptoms include cankers, wood necrosis, spur dieback, retarded growth, foliar chlorosis and necrosis, and fruit rot [4].

Current strategies for the control of *L. theobromae* are mainly preventive. In vineyards, the chemical control of *L. theobromae* is the prevailing approach. However, most, if not all, of the efficient chemical fungicides have been progressively banned. Sodium arsenite, formerly used to control grapevine trunk diseases, is now prohibited [5,6] and DuPont Escudo®, a commercial formulation of carbendazime and flusilazol has also been withdrawn from the market [7]. Preventive treatment with azoles and synthetic resins are, for the moment, the only option [7,8] with the major drawback of the selection and dissemination of azole-resistant plant pathogens [9].

Photodynamic inactivation (PDI) of microorganisms relies on the interaction between light, molecular oxygen and a light-sensitive molecule (photosensitizer, PS) that leads to the production of highly cytotoxic radicals (type I) or singlet oxygen (type II) which cause lethal damage to the target cells [10]. Although initially developed for clinical applications, PDI has rapidly expanded beyond the medical scope, and phytosanitary applications represent an emerging field [11–14].

The objective of this work was to provide an alternative to the chemical prophylaxis of grapevine trunk disease caused by *L. theobromae*, using non-toxic photoantimicrobials.

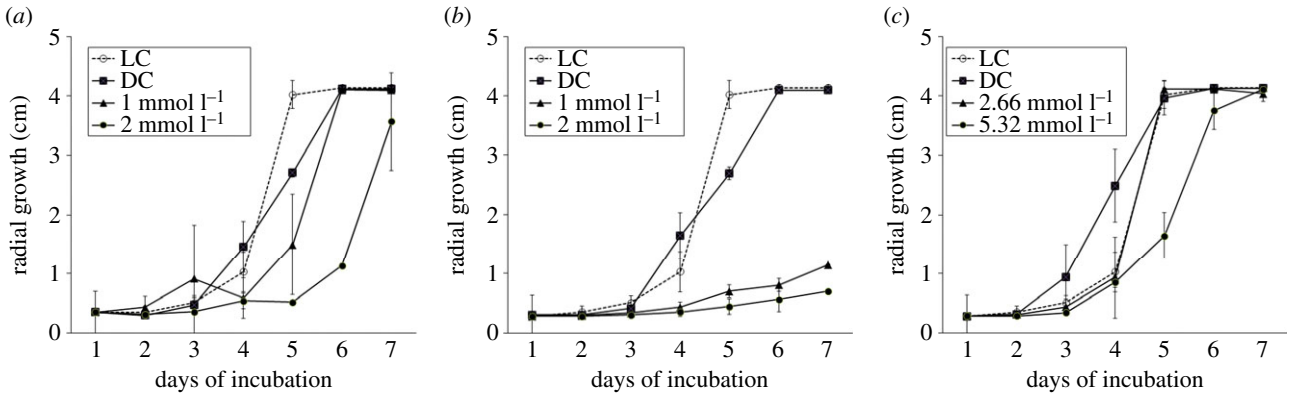

Figure 1. Radial mycelium growth of *Lasiodiplodia theobromae* cultivated in the presence of (*a*) TBO, (*b*) MB or (*c*) riboflavin, under natural sunlight irradiation. LC, light control; DC, dark control. Values represent the mean of three independent assays with five replicates, and error bars represent the standard deviation.

2. Material and methods

(a) Biological material and chemical reagents

The *L. theobromae* strain LA-SV1 used in this study was isolated from a grapevine in Peru [15]. Starter cultures in oatmeal agar (OA) were incubated at 28°C in the dark for 5 days.

All reagents were purchased from Merck, except when otherwise indicated. The cationic porphyrin 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin tetra-iodide (Tetra-Py$^+$-Me) was synthesized and purified according to the literature [16]. Stock solutions of toluidine blue O (TBO), methylene blue (MB, AppliChem) and Tetra-Py$^+$-Me were prepared in dimethylsulfoxide (DMSO). Riboflavin was dissolved in water, and so were D-mannitol and sodium azide, used as a free radical scavenger and $^1O_2$ quencher, respectively.

(b) Photodynamic inactivation under natural (discontinuous) or artificial (continuous) light

The PDI of *L. theobromae* was assessed as the inhibition of mycelium radial growth in a double-layered solid medium, during 7 days of incubation. The PS was incorporated in soft OA (0.5% agar) overlays. Plates (9 cm diameter) were inoculated with 6 mm mycelium plugs from starter cultures, and the assays were conducted at room temperature (approx. 25°C) for 7 days.

For the assays with solar light (natural daylight), the PSs TBO (1.0 and 2.0 mmol l^{-1}), MB (1.0 and 2.0 mmol l^{-1}) and riboflavin (5.3 mmol l^{-1}) were tested. Cultures were exposed to indirect sunlight through window glass, corresponding to a photosynthetically active radiation (PAR) irradiance of 600 W m^{-2} [17]. Experiments with continuous artificial light tested the PSs TBO (1.0 mmol l^{-1}), MB (1.0 mmol l^{-1}), riboflavin (2.66 mmol l^{-1}) and the cationic porphyrin Tetra-Py$^+$-Me (50 µmol l^{-1}), as a reference PS. The cultures were incubated under an array of 13 fluorescent lamps (OSRAM 21 18 W) continuously delivering PAR (380–700 nm, 25 W m^{-2}) [17].

Light controls (LC), exposing the fungus to the same light conditions as the test but without PS, dark controls (DC) exposing the fungus to each PS in the dark, and a control (+), in which the fungus was cultivated in the dark, without any PS, were included. Three independent assays, each including five replicates for each experimental condition, were conducted.

(c) Effect of photodynamic inactivation on biomass production and mechanism of photosensitization

Liquid cultures (50 ml) were incubated at room temperature for 7 days, under continuous irradiation with PAR (380–700 nm, 25 W m^{-2}). Oatmeal broth was amended with MB (50 µmol l^{-1}) or Tetra-Py$^+$-Me (5.0 µmol l^{-1}). Sets of five replicates for each experimental condition were inoculated with plugs of starter cultures. LC, DC and (+) controls were included. In order to determine the type of photosensitization mechanism (type I or type II), parallel sets of test cultures containing each PS were amended with either 100 mmol l^{-1} D-mannitol or 100 mmol l^{-1} of sodium azide. For the determination of biomass dry weight, fungal material was collected on pre-weighed gauze and dried at 50°C until a constant weight was reached (approx. 3–4 days). Five replicates for each experimental condition were conducted.

(d) Statistical analysis

Significant differences in radial growth and biomass production between different experimental conditions were assessed by univariate ANOVA with the IBM SPSS Statistics 25 package with a 5% significance threshold. Normality and homogeneity of variances were checked by the Kolmogorov–Smirnov and the Levene tests, respectively.

3. Results

(a) Photodynamic inactivation efficiency

Exposure to MB (1.0 or 2.0 mmol l^{-1}) induced a reduction of 72–83% (ANOVA, $p > 0.05$) in growth, in relation to the control (figure 1). TBO (2.0 mmol l^{-1}) caused an initial delay in growth, in relation to the controls (87% reduction at day 5; ANOVA, $p < 0.05$), but growth recovered during the subsequent incubation. Riboflavin was the least efficient PS. Delay in mycelium growth, in relation to the controls, was observed only at the highest concentration, and the difference between tests and controls was only significant at day 5 (40% growth of LC; ANOVA, $p < 0.05$).

All tested PSs caused complete inhibition of mycelium growth (figure 2). There was also a slight inhibition in the LC and DC, in relation to the control (+). Mycelium growth in the LC was delayed by 2–3 days, when compared with the control (+), with a growth reduction of 26%. In the DC, the inhibition was maximal after 2–3 days of incubation, with an average mycelium radius corresponding to 15–31% of the corresponding value in control (+). However, at the end of the experiment, only the DC of Tetra-Py$^+$-Me remained significantly different from control (+) (ANOVA, $p < 0.05$).

(b) Biomass production and photodynamic inactivation mechanism

In liquid cultures, biomass production in 7 days (table 1) was significantly reduced in the presence of either MB (48.5 mg),

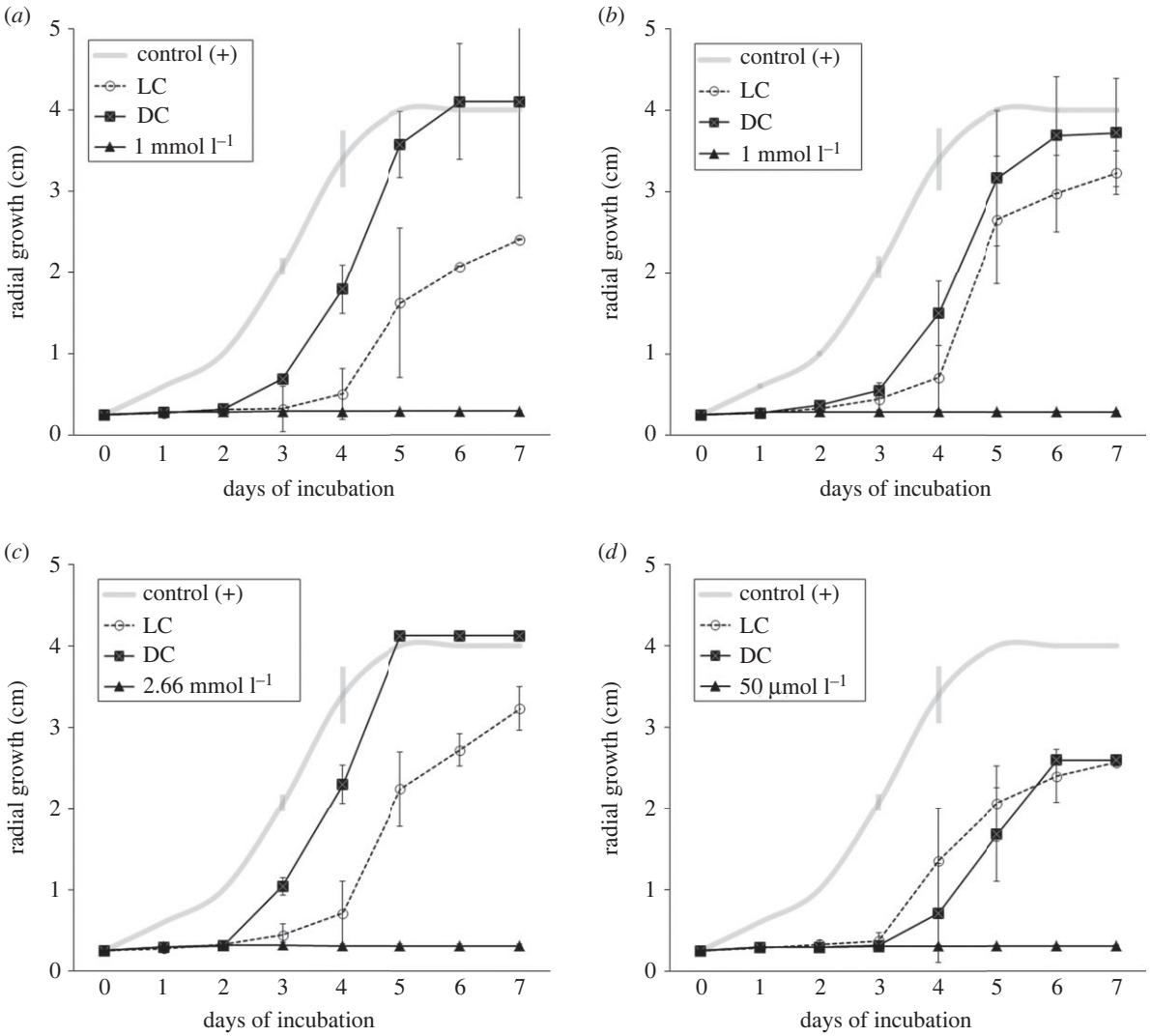

Figure 2. Radial mycelium growth of *Lasiodiplodia theobromae* cultivated in the presence of (*a*) TBO, (*b*) MB, (*c*) riboflavin or (*d*) cationic porphyrin Tetra-Py^{+}-Me, under continuous irradiation with artificial PAR (380–700 nm, 25 W m^{-2}). Control (+) values are repeated in all the graphs for comparison. Values represent the mean of three independent assays, and error bars represent the standard deviation.

or Tetra-Py^{+}-Me (50.6 mg), corresponding to 36 and 38% of the biomass in the control (+). Light alone (LC) or exposure to the PS in the dark (DC) did not significantly affect biomass production.

The addition of sodium azide did not significantly affect photosensitization with MB. However, in the presence of D-mannitol (+M condition), the biomass production (176.3 mg) was significantly different from that of the MB test (table 2). Neither D-mannitol nor sodium azide attenuated the photosensitization with Tetra-Py^{+}-Me. With the porphyrin, biomass production was not significantly different between the three tested PDI conditions.

4. Discussion

PDI of *L. theobromae* was assessed in view of the emergence of tolerance to common antifungals among phytopathogenic fungi and the need to reduce the use of chemical biocides. The PSs were incorporated in a soft OA overlay to facilitate the contact between the PS and the penetrative zone of the mycelium responsible for nutrient uptake [18].

Assays with natural sunlight showed no significant inactivation with either TBO or riboflavin, and mycelium development was only slightly delayed (approx. 1 day). Riboflavin was tested because, as an authorized food additive (E101), it is an interesting PS for food-related applications. However, it is quickly degraded by light [19], and it lacks the positive charge that would increase affinity to the cell wall of fungi [20]. TBO efficiency varies among fungal species [21], and the effects may differ from those caused by MB [22,23]. The differences observed may have had species- and/or medium-related causes. MB caused significant attenuation of mycelium growth under natural light in a concentration-dependent manner, but not a complete fungicidal effect, although the MB concentrations used were much higher than normally used for fungal PDI [22,24]. At very high concentrations, the target sites of the PS become saturated, leaving a substantial amount of unbound PS that will competitively absorb light [25]. Additionally, at high concentrations, MB has intrinsic antibacterial and antifungal activity [26,27]. Therefore, MB concentrations for PDI are limited by the self-shading and dark toxicity of this dye.

Considering that incomplete inhibition under natural sunlight may have been due to recovery of photodynamic damage during the night period, experiments with continuous artificial light (PAR, 380–700 nm) were conducted. Mycelium growth was completely inhibited with all tested PSs under continuous artificial light. Noticeably, considering

Table 1. Biomass of *Lasiodiplodia theobromae* produced in 50 ml liquid cultures (oatmeal broth) after 7 days of continuous irradiation with PAR (380–700 nm, 25 W m^{-2}), expressed as absolute value (mg dry weight) or in relation to the biomass produced in the control culture (% control (+)). Test, PDI conditions (PS + light); LC, light controls; DC, dark controls; control (+), dark conditions without PS. Values represent the mean ± standard deviation of five replicates. * indicates a significant difference (ANOVA, $p < 0.05$) in relation to control (+).

photosensitizer	biomass	LC	DC	test	control (+)
MB	mg dry weight	147.1 ± 10.6	115.0 ± 4.1	48.5 ± 26.9*	133.9 ± 4.5 mg
50 µmol l^{-1}	% control (+)	110	86	36	
Tetra-Py^{+}-Me	mg dry weight	135.6 ± 14.3	148.2 ± 34.7	50.6 ± 18.5*	
5 µmol l^{-1}	% control (+)	101	111	38	

Table 2. Fungal biomass (dry weight) produced in 50 ml liquid cultures (oatmeal broth) after 7 days of continuous irradiation with PAR (380–700 nm, 25 W m^{-2}), with or without the free radical scavenger D-mannitol (M) or the $^1O_2$ quencher sodium azide (SA). Test, PDI conditions (PS + light) without capture molecules; control (+), dark conditions without PS; values were repeated from table 1, for comparison. Values represent the mean ± standard deviation of five replicates. * indicates a significant difference (ANOVA, $p < 0.05$) in relation to the corresponding test.

photosensitizer	biomass	test	+SA	+M	control (+)
MB	mg dry weight	36.0 ± 22.7	20.9 ± 1.6	176.3 ± 30.4*	133.9 ± 4.5 mg
50 µmol l^{-1}	% control (+)	27	16	132	
Tetra-Py^{+}-Me	mg dry weight	26.3 ± 2.9	21.7 ± 2.0*	30.8 ± 5.6	
5 µmol l^{-1}	% control (+)	20	16	23	

average light irradiance estimates for similar experimental conditions [17], the total energy dose applied during continuous irradiation with artificial light (approx. 2400 J cm^{-2}) was approximately 5% of that applied in the experiments with natural sunlight. The results agree with other reports of significant or complete inhibition of fungi by MB and artificial light [28–31]. However, light, *per se*, had a negative effect on the growth of *L. theobromae* and all tested PSs showed some degree of dark toxicity. Light regulates important physiological processes in fungi, and it has been shown that biomass production is higher in the dark than under white light [32,33]. MB, TBO, riboflavin and porphyrins are considered to have low dark toxicity against fungi but most literature results refer to yeasts or suspensions of conidia [34], less susceptible to antimicrobial compounds than active vegetative hyphae [35].

PDI experiments in liquid cultures were conducted to discriminate the mechanism of mycelium photosensitization, using the reduction of biomass as a proxy for inactivation. Only the most effective PSs (MB and Tetra-Py^{+}-Me) were tested because the concentrations had to be lowered to 1/100 of those used in solid medium. Preliminary tests showed that high PS concentrations produced an intense coloration of the medium, which attenuated light penetration. Additionally, the PSs visibly precipitated on the surface of the hyphae, making it impossible to obtain an accurate determination of the mycelium weight (data not shown).

MB (50 µmol l^{-1}) and Tetra-Py^{+}-Me (5.0 µmol l^{-1}) induced a significant reduction in biomass. Dark toxicity (DC) and the direct inhibition by light (LC) were attenuated in relation to the assays in solid medium. The lower concentrations of PS, the complete immersion of the mycelium in the slightly turbid liquid medium and the glass walls of the flask may have granted some protection from direct light.

PDI of fungi may involve type I (free radicals) or type II ($^1O_2$) reactions, depending on the PS and the reaction medium, and often both processes occur concomitantly [34].

The addition of D-mannitol had a significant protective effect against the photosensitization with MB, but sodium azide, a $^1O_2$ quencher, had no effect, indicating that the PDI with MB occurred almost exclusively by a type I mechanism. MB is known to act via type I and type II mechanisms. However, considering the high concentration of MB, and the duration of the experiment (7 days), some aggregation of the PS may have occurred [36]. The formation of dimers limits $^1O_2$ generation, and as such, the type II mechanism may have been diverted to type I [36–38]. PDI with the porphyrin Tetra-Py^{+}-Me was not attenuated by either trapping molecule, although porphyrins are in general considered to act by type II mechanism [39]. The results may indicate that both type I and type II mechanisms contributed to the photosensitization effect of the porphyrin, but this hypothesis needs further testing. Oatmeal broth is rich in phenolic compounds that act as $_1O^2$ quenchers and free radical scavengers [40], which may have provided an anti-oxidative background that complicates the discrimination of the effect of the trapping molecules.

This study demonstrates that *L. theobromae* is susceptible to PDI with MB, TBO, riboflavin and the cationic porphyrin Tetra-Py^{+}-Me, and that under continuous irradiation with artificial light, growth was completely suppressed. Therefore, although in field conditions PDI may still not provide the same degree of protection as chemical biocides, it represents a promising, eco-friendly and cost-effective alternative for the sanitation of vine material in greenhouse nurseries, where plants are particularly susceptible to agrochemicals. Considering the advantage of the minimal environmental impacts of PDI, further exploration of PDI against *L. theobromae* should be conducted. Incorporating PSs in films or resin coatings to be applied on pruning or

grafting wounds could delay the early phases of hyphal growth, allowing plant defences to be activated, and thus reducing the risk of trunk infections.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.905qfttjk [41].

Authors' contributions. A.C., A.C.E. and M.G. conceived the work and designed the experiments. M.G. and B.D. conducted the experiments. M.G., I.N.S.-G. and A.C. analysed data and interpreted the results. A.C. and M.A.F.F. wrote the manuscript. M.A.F.F. and A.A. led the projects that provided material support to the work. All authors reviewed and approved the final manuscript, agreeing to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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References

1. Alves A, Crous PW, Correia A, Phillips A. 2008 Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Divers.* **28**, 1–13.
2. Mehl J, Wingfield M, Roux J, Slippers B. 2017 Invasive everywhere? Phylogeographic analysis of the globally distributed tree pathogen *Lasiodiplodia theobromae*. *Forests* **8**, 145. (doi:10.3390/f8050145)
3. Serra S, Mannoni MA, Ligios V. 2008 Studies on the susceptibility of pruning wounds to infection by fungi involved in grapevine wood diseases in Italy. *Phytopathol. Mediterranea* **47**, 234–246.
4. Burruano S, Mondello V, Conigliaro G, Alfonzo A, Spagnolo A, Mugnai L. 2008 Grapevine decline in Italy caused by *Lasiodiplodia theobromae*. *Phytopathol. Mediterranea* **47**, 132–136. (doi:10.14601/Phytopathol_Mediterr-2616)
5. Bencko V, Slámová A. 2007 Best practices for promoting farmers' health: the case of arsenic history. *J. Public Health* **15**, 279–288. (doi:10.1007/s10389-007-0123-3)
6. Mondello V, Songy A, Battiston E, Pinto C, Coppin C, Trotel-Aziz P, Clément C, Mugnai L, Fontaine F. 2018 Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Dis.* **102**, 1189–1217. (doi:10.1094/PDIS-08-17-1181-FE)
7. Fontaine F, Gramaje D, Armengol J, Smart R, Nagy ZA, Borgo M, Rego C, Corio-Costet MF. 2016 *Grapevine trunk diseases. A review*. Paris, France: OIV publications.
8. Amponsah NT, Jones E, Ridgway HJ, Jaspers MV. 2012 Evaluation of fungicides for the management of *Botryosphaeria* dieback diseases of grapevines. *Pest Manage. Sci.* **68**, 676–683. (doi:10.1002/ps.2309)
9. Mohd-Assaad N, McDonald BA, Croll D. 2016 Multilocus resistance evolution to azole fungicides in fungal plant pathogen populations. *Mol. Ecol.* **25**, 6124–6142. (doi:10.1111/mec.13916)
10. Hamblin MR. 2016 Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes. *Curr. Opin. Microbiol.* **33**, 67–73. (doi:10.1016/j.mib.2016.06.008)
11. Martins D, Mesquita MQ, Neves MG, Faustino MA, Reis L, Figueira E, Almeida A. 2018 Photoinactivation of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit plants by cationic porphyrins. *Planta* **248**, 409–421. (doi:10.1007/s00425-018-2913-y)
12. Gomes MC *et al.* 2011 Photodynamic inactivation of *Penicillium chrysogenum* conidia by cationic porphyrins. *Photochem. Photobiol. Sci.* **10**, 1735–1743. (doi:10.1039/c1pp05174a)
13. Vandresen CC, Gonçalves AG, Ducatti DRB, Murakami FS, Noseda MD, Duarte MER, Barreira SMW. 2016 *In vitro* photodynamic inactivation of conidia of the phytopathogenic fungus *Colletotrichum graminicola* with cationic porphyrins. *Photochem. Photobiol. Sci.* **15**, 673–681. (doi:10.1039/C5PP00372E)
14. de Menezes HD, Tonani L, Bachmann L, Wainwright M, Braga GÚL, von Zeska Kress MR. 2016 Photodynamic treatment with phenothiazinium photosensitizers kills both ungerminated and germinated microconidia of the pathogenic fungi *Fusarium oxysporum*, *Fusarium moniliforme* and *Fusarium solani*. *J. Photochem. Photobiol. B Biol.* **164**, 1–12. (doi:10.1016/j.jphotobiol.2016.09.008)
15. Rodríguez-Gálvez E, Maldonado E, Alves A. 2015 Identification and pathogenicity of *Lasiodiplodia theobromae* causing dieback of table grapes in Peru. *Eur. J. Plant Pathol.* **141**, 477–489. (doi:10.1007/s10658-014-0557-8)
16. Alves E *et al.* 2008 Photodynamic inactivation of recombinant bioluminescent *Escherichia coli* by cationic porphyrins under artificial and solar irradiation. *J. Ind. Microbiol. Biotechnol.* **35**, 1447–1454. (doi:10.1007/s10295-008-0446-2)
17. Costa L, Carvalho CMB, Faustino MAF, Neves MG, Tomé JPC, Tomé AC, Cavaleiro JA, Cunha Â, Almeida A. 2010 Sewage bacteriophage inactivation by cationic porphyrins: influence of light parameters. *Photochem. Photobiol. Sci.* **9**, 1126–1133. (doi:10.1039/c0pp00051e)
18. Balmant W, Sugai-Guérios MH, Coradin JH, Krieger N, Junior AF, Mitchell DA. 2015 A model for growth of a single fungal hypha based on well-mixed tanks in series: simulation of nutrient and vesicle transport in aerial reproductive hyphae. *PLoS ONE* **10**, e0120307. (doi:10.1371/journal.pone.0120307)
19. Sheraz MA, Kazi SH, Ahmed S, Anwar Z, Ahmad I. 2014 Photo, thermal and chemical degradation of riboflavin. *Beilstein J. Org. Chem.* **10**, 1999–2012. (doi:10.3762/bjoc.10.208)
20. Li X-S, Guo J, Zhuang J-J, Zheng B-Y, Ke M-R, Huang J-D. 2015 Highly positive-charged zinc(II) phthalocyanine as non-aggregated and efficient antifungal photosensitizer. *Bioorg. Med. Chem. Lett.* **25**, 2386–2389. (doi:10.1016/j.bmcl.2015.04.004)
21. Alananbeh KM, BouQellah NA, Al Harbi MR, Ouf SA. 2018 The efficacy of photosensitizers on mycelium growth, mycotoxin and enzyme activity of *Alternaria* spp. *Jordan J. Biol. Sci.* **11**, 499–510.
22. Gonzales FP, da Silva SH, Roberts DW, Braga GU. 2010 Photodynamic inactivation of conidia of the fungi *Metarhizium anisopliae* and *Aspergillus nidulans* with methylene blue and toluidine blue. *Photochem. Photobiol.* **86,** 653–661. (doi:10.1111/j.1751-1097.2009.00689.x)
23. Carvalho GG, Felipe MP, Costa MS. 2009 The photodynamic effect of methylene blue and toluidine blue on *Candida albicans* is dependent on medium conditions. *J. Microbiol.* **47**, 619. (doi:10.1007/s12275-009-0059-0)
24. Teichert MC, Jones JW, Usacheva MN, Biel MA. 2002 Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **93**, 155–160. (doi:10.1067/moe.2002.120051)
25. Jackson Z, Meghji S, MacRobert A, Henderson B, Wilson M. 1999 Killing of the yeast and hyphal forms of *Candida albicans* using a light-activated antimicrobial agent. *Laser Med. Sci.* **14**, 150–157. (doi:10.1007/s101030050037)
26. Calzavara-Pinton P, Venturini M, Sala R. 2005 A comprehensive overview of photodynamic therapy in the treatment of superficial fungal infections of the skin. *J. Photochem. Photobiol. B Biol.* **78**, 1–6. (doi:10.1016/j.jphotobiol.2004.06.006)
27. Wainwright M, Crossley K. 2002 Methylene blue - a therapeutic dye for all seasons? *J. Chemother.* **14**, 431–443. (doi:10.1179/joc.2002.14.5.431)
28. de Souza SC, Junqueira JC, Balducci I, Koga-Ito CY, Munin E, Jorge AOC. 2006 Photosensitization of different *Candida* species by low power laser light. *J. Photochem. Photobiol. B Biol.* **83**, 34–38. (doi:10.1016/j.jphotobiol.2005.12.002)
29. Peloi LS, Soares RR, Biondo CE, Souza VR, Hioka N, Kimura E. 2008 Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue. *J. Biosci. (Bangalore)* **33**, 231–237. (doi:10.1007/s12038-008-0040-9)
30. Pupo YM, Gomes GM, Santos EB, Chaves L, Michel MD, Kozlowski VA, Gomes OM, Gomes JC. 2011

Susceptibility of *Candida albicans* to photodynamic therapy using methylene blue and toluidine blue as photosensitizing dyes. *Acta Odontol. Latinoam.* **24**, 188–192.

31. Ouf SA, Abdel-Kader MH, Shokeir HA, El-Adly AA. 2003 Study of solar photosensitization processes on dermatophytic fungi. *Acta Microbiol. Pol.* **52**, 65–80.
32. Velmurugan P, Lee YH, Venil CK, Lakshmanaperumalsamy P, Chae J-C, Oh B-T. 2010 Effect of light on growth, intracellular and extracellular pigment production by five pigment-producing filamentous fungi in synthetic medium. *J. Biosci. Bioeng.* **109**, 346–350. (doi:10.1016/j.jbiosc.2009.10.003)
33. Rodriguez-Romero J, Hedtke M, Kastner C, Müller S, Fischer R. 2010 Fungi, hidden in soil or up in the air: light makes a difference. *Annu. Rev. Microbiol.* **64**, 585–610. (doi:10.1146/annurev.micro.112408.134000)
34. Calzavara-Pinton P, Rossi MT, Sala R, Venturini M. 2012 Photodynamic antifungal chemotherapy. *Photochem. Photobiol.* **88**, 512–522. (doi:10.1111/j.1751-1097.2012.01107.x)
35. Martinez-Rossi NM, Peres NTA, Rossi A. 2008 Antifungal resistance mechanisms in dermatophytes. *Mycopathologia* **166**, 369. (doi:10.1007/s11046-008-9110-7)
36. da Collina GA, Freire F, da Costa Santos TP, Sobrinho NG, Aquino S, Prates RA, Da Silva DFT, Tempestini Horliana ACR, Pavani C. 2018 Controlling methylene blue aggregation: a more efficient alternative to treat *Candida albicans* infections using photodynamic therapy. *Photochem. Photobiol. Sci.* **17**, 1355–1364. (doi:10.1039/C8PP00238J)
37. Tardivo JP, Del Giglio A, De Oliveira CS, Gabrielli DS, Junqueira HC, Tada DB, Severino D, de Fátima Turchiello R, Baptista MS. 2005 Methylene blue in photodynamic therapy: from basic mechanisms to clinical applications. *Photodiagn. Photodyn. Ther.* **2**, 175–191. (doi:10.1016/S1572-1000(05)00097-9)
38. Sabbahi S, Alouini Z, Jemli M, Boudabbous A. 2008 The role of reactive oxygen species in *Staphylococcus aureus* photoinactivation by methylene blue. *Water Sci. Technol.* **58**, 1047–1054. (doi:10.2166/wst.2008.471)
39. Almeida A, Cunha A, Faustino MAF, Tomé AC, Neves MGPMS. 2011 Porphyrins as antimicrobial photosensitizing agents. In *Photodynamic inactivation of microbial pathogens* (eds MR Hamblin, G Jori), pp. 83–160. Cambridge, UK: RCS Publisher.
40. Razzaghi-Asl N, Garrido J, Khazraei H, Borges F, Firuzi O. 2013 Antioxidant properties of hydroxycinnamic acids: a review of structure-activity relationships. *Curr. Med. Chem.* **20**, 4436–4450. (doi:10.2174/09298673113209990141)
41. Cunha A, Garcia M, David B, Sierra-Garcia IN, Alves A, Esteves AC. 2021 Data from: Photodynamic inactivation of *Lasiodiplodia theobromae*: lighting the way towards an environmentally friendly phytosanitary treatment. Dryad Digital Repository. (doi:10.5061/dryad.905qfttjk)