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1 **ABSTRACT**

2 Wheat crops are constantly challenged by the pathogen *Zymoseptoria tritici*
3 responsible for Septoria tritici Blotch (STB) disease. The present study reports the
4 evaluation of five elicitor compounds (λ -carrageenan, CpG-ODN, glycine betaine,
5 *Spirulina platensis* and ergosterol) for the protection of wheat against STB in order to
6 offer new alternative tools to farmers for sustainable crop protection. Screening of
7 elicitors of wheat defenses was carried out through a succession of experiments:
8 biocidal *in vitro* tests enabled to check for any fungicidal activities; glasshouse
9 experiments allowed to determine the efficacy of a given compound in protecting
10 wheat against STB; qRT-PCR biomolecular tests investigated the relative expression
11 of 23 defense genes in treated *versus* untreated plants. We therefore demonstrated
12 that λ -carrageenan, CpG-ODN, glycine betaine, *Spirulina platensis* and ergosterol
13 are potential elicitors of wheat defenses. Foliar treatment with these compounds
14 conferred protection of wheat by up to approximately 70 % against *Z. tritici* under
15 semi-controlled conditions and induced both SA- and/or JA-dependent signaling
16 pathways in the plant. These findings contribute to extend the narrow list of potential
17 elicitors of wheat defenses against *Z. tritici*.

18

19 **KEYWORDS**

20 *Triticum aestivum*; elicitor; *Zymoseptoria tritici*; Integrated pest management;
21 alternative plant protection tools

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23

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26 Wheat is the most cultivated crop in Europe and in the world, with up to 734 million
27 tons produced in 2015-2016 (Fones and Gurr 2015; Satger 2016). This
28 monocotyledonous plant must however face a threatening disease in the field:
29 Septoria tritici Blotch (STB), caused by the fungal pathogen *Zymoseptoria tritici*
30 (teleomorph: *Mycosphaerella graminicola*) (Torriani et al. 2015). STB is considered
31 as the main disease affecting wheat crops in the European Union (EU) by inflicting
32 up to 40 % of yield losses each year (Eyal 1999; Rudd 2015). So far, fungicide use is
33 the most effective control method as no wheat cultivar is currently totally resistant to
34 *Z. tritici* (Fraaije et al. 2005; Palmer and Skinner 2002). About 70% of EU fungicides
35 are used to that end (Fones and Gurr 2015). However, reducing pesticide use in
36 agricultural practices has become a key priority for multiple countries (including the
37 EU) in the prospect of environmental sustainability and health protection (Dayan et
38 al. 2009; European Commission 2012). In this respect, elicitors are promising and
39 alternative plant protection tools which have a prominent place in current research
40 (Lyon et al. 2014). Contrary to fungicides, elicitors are natural immune-stimulating
41 compounds which indirectly target a broad spectrum of pathogens by enhancing the
42 defensive state of the plant (Thakur and Sohal 2013). As such, elicitors are intended
43 as preventive treatments complementary to the use of reduced fungicide rates
44 (Walters et al. 2013; Walters et al. 2014). For now, few elicitor products are
45 registered on the EU market for the sustainable management of wheat diseases,
46 aside from Vacciplant® (laminarin-based product; Goëmar, France) and
47 BION®50WG (synthetic Acibenzolar-S-methyl elicitor; Syngenta, Switzerland) (Le
48 Mire et al. 2016). The present study thus focuses on the screening of elicitors of
49 wheat defenses against *Z. tritici*, as a response to the urgent need for a larger panel
50 of alternative tools for sustainable wheat protection (Walters et al. 2013). Five

51 different compounds were selected for this work: λ -carrageenan, cytosine-phosphate-
52 guanine oligodesoxynucleotide motifs (CpG ODN), *Spirulina (Arthrospira) platensis*,
53 glycine betaine and ergosterol. As it appears, none of these compounds have been
54 tested before as elicitors on the pathosystem wheat-*Z. tritici*, although previous
55 research has indeed demonstrated their elicitor properties on other plant species
56 and/or animals. λ -carrageenan stimulates the resistance of tobacco, tomato, and
57 thale-cress plants against pathogens such as *Botrytis cinerea* and Tobacco mosaic
58 virus (TMV) (Mercier et al. 2001; Sangha et al. 2010, 2015; Vera et al. 2011). A
59 recent study reported that bacterial CpG-ODN induced defense responses in thale-
60 cress (Yakushiji et al. 2009), while unmethylated CpG-ODN was also proven to
61 induce animal innate immunity against pathogenic bacteria, viruses and protozoans
62 (Carrington and Secombes 2006; Covello et al. 2012). The cyanobacterium *S.*
63 *platensis* (*Spirulina*) showed beneficial health effects for humans and animals
64 (poultry, mammals, and fish) thanks to its immune-stimulating properties, notably *via*
65 the production of antibodies and cytokines (Frag et al. 2016; Wan et al. 2016). The
66 recent work of Věchet and Šerá (2015) revealed that glycine betaine (GB) stimulates
67 wheat defenses against powdery mildew (Věchet and Šerá 2015). Lastly, ergosterol
68 is a well-known elicitor of dicotyledonous plants (*e.g.* tomato, tobacco and sugar
69 beet) (Amborabé et al. 2003; Lochman and Mikes 2006; Rossard et al. 2010). This
70 study was conducted in two steps: (i) we first investigated the efficacy of the five
71 compounds in protecting wheat against STB under glasshouse conditions. Each
72 compound was tested at three different concentrations in order to assess any dose-
73 dependent effects; (ii) in order to confirm their elicitor potential, we carried out an
74 independent experiment to explore the defense signaling pathways triggered in
75 treated *versus* untreated plants. To that end, an innovative biomolecular tool

76 developed by INRA (France) was used to study the expression of 23 wheat defense
77 genes (Brisset and Duge De Bernonville 2011; Dugé de Bernonville et al. 2014). The
78 recognition of an elicitor by the plant indeed triggers a characteristic cascade of
79 defense signals resulting in plant induced resistance (Muthamilarasan et al. 2013).
80 Conducting such biomolecular test for screening purposes provides further evidence
81 as to the elicitor properties of the tested compounds. Moreover, we examined the
82 correlation between the effectiveness of each compound to protect wheat and the
83 expression of defense genes in the treated plants.

84

85 MATERIALS AND METHODS

86 Plant and fungal materials

87 The experiments were conducted on wheat (*Triticum aestivum* L.) plants of the
88 susceptible cv. Avatar. Plants were grown in the glasshouse under semi-controlled
89 conditions (natural photoperiod supplemented with artificial light if needed, with 20 °C
90 ± 5 according to the sunlight). Screening experiments and plant defense induction
91 experiments were undertaken independently. For the screening of elicitors for their
92 protective efficacy, seeds were sown in 25 x 15 cm plastic pots filled with loam (10
93 plants per pot). The study of induced plant defenses was carried out at another
94 location (INRA facilities in Angers) where seeds were sown in 30 x 20 cm plastic
95 boxes filled with loam (40 plants per box).

96 The *Z. tritici* strain T01187 (isolated in 2009 from Northern France) was used for
97 plant inoculation. Fungal culture was performed on potato dextrose agar medium for
98 eight days at 18 °C with a 12 h : 12 h day : night cycle. Inocula were prepared by
99 washing the cultures with 10 ml sterile distilled water and the resulting spore
100 suspension was adjusted to desired concentrations using Malassez cell.

101 Elicitor preparation

102 The sources and characteristics of the five compounds examined in this study are
 103 provided in Table 1. To be noted that the compound CpG-ODN consists of CpG-28
 104 (sequence 5'-TAAACGTTATAACGTTATGACGTCAT- 3') synthesized with a wholly
 105 phosphorothioate backbone (Carpentier et al. 2003). For each compound, the tested
 106 concentrations were not the same as we selected the average effective doses which
 107 were generally used in previous studies to demonstrate their respective elicitor
 108 potential. All treatment solutions were freshly prepared before use in distilled water
 109 supplemented with 0.1 % (v : v) of spreading agent Break-Thru®S240 (polyether
 110 trisiloxane, Evonik Industries), and 0.05 % (v : v) of solubilizing agent Tween 20
 111 (polyoxyethylene-sorbitan monolaurate, Sigma Aldrich). Control plants were treated
 112 with distilled water alone. In addition, other control plants were treated respectively
 113 with 0.75 % (v : v) of the epoxiconazole-based fungicide Opus® (BASF Agro, France)
 114 or with the synthetic elicitor Bion®50WG (abbreviated to Bion) at 0.6 mg ml⁻¹
 115 (recommended doses according to <https://ephy.anses.fr>). It should be noted that
 116 preliminary *in vitro* experiments according to Siah et al (2010) have been conducted
 117 to rule out any compound showing a direct biocidal activity towards the fungal
 118 pathogen *Z. tritici* (see supplementary Fig. S1).

119

120 Glasshouse elicitor screening: plant treatment and inoculation

121 At the three-four leaf stage (third leaf fully expanded), the plants of each pot were
 122 sprayed to run-off with 30 ml of the treatment solutions using a hand sprayer. Each
 123 elicitor candidate was tested at three different concentrations in order to assess any
 124 dose-dependent protection effects. Plant inoculation was performed five days after
 125 treatment, by spraying the plants of each pot to run-off with 30 ml of a spore

126 suspension (10^6 spores ml^{-1} in distilled water) amended with 0.05 % (v : v) Tween 20.
127 Immediately after inoculation, each pot was covered with a transparent polyethylene
128 bag for three days in order to ensure water-saturated conditions compatible with
129 spore germination. A visual estimation of the disease severity was carried out at 28
130 days post-inoculation according to Siah et al. (2010). The disease severity was
131 measured as the percentage of the third leaf area covered with symptomatic lesions
132 (necrosis and chlorosis) bearing pycnidia. For each treatment, at least two
133 independent biological experiments were performed in the glasshouse, with 40
134 technical repetitions (plants). An incomplete block design was carried out due to the
135 large number of compounds to be tested (Lawal 2014). Results of combined
136 experiments were analyzed with linear mixed effect models (Galecki and
137 Burzykowski 2013), and ANOVA and the Tukey multiple comparison procedure at P
138 = 0.05 were used to compare the mean disease severity of the plants treated with the
139 different products. Reported values correspond to the average infection levels of the
140 treated plants.

141

142 **Induction of defense gene expression: plant treatment and leaf sampling**

143 The investigation of the plant signaling pathways triggered by the various elicitor
144 treatments was performed in the glasshouse located in INRA facilities. The elicitor
145 compounds were prepared and applied in the same way as in screening tests, but
146 were tested only at their medium concentration C2 (Table 1) due to space limitations.
147 Control plants were treated with distilled water alone. According to the INRA
148 experimental design devoted to the use of the qPFD tool, each treatment was applied
149 on one box of 40 plants (40 repetitions), and two independent biological experiments
150 were performed. In order to test for priming activities, the plants on the half of each

151 box were sprayed one day later with a solution containing 40 nm of hydrogen
152 peroxide (H₂O₂) which mimics a pathogenic attack, as described by Dugé de
153 Bernonville et al. (2014). Elicitor priming is characterized by the non-triggering of
154 plant defense mechanisms directly after elicitor recognition. Instead, strong and rapid
155 host defense reactions are activated only upon a subsequent challenge (Van Loon et
156 al. 2006). Such phenomenon prevents fitness costs to the plant in the absence of an
157 infection. In the present case, if a tested compound exerts a priming activity, the
158 application of H₂O₂ shortly after would strongly induce the expression of defense
159 genes. As a reactive oxygen species (ROS), H₂O₂ is indeed involved in plant
160 oxidative stress and acts as a key mediator in defense gene activation (Halliwell
161 2006). Besides, its use to mimic an STB infection is justified by the work of Shetty et
162 al. (2007) who found that wheat infected by *Z. tritici* exhibited an important and early
163 accumulation of H₂O₂ in incompatible interactions (Shetty et al. 2007). We sampled
164 the third leaf of five distinct seedlings at day 1 after treatment (*i.e.*, 24 hours after
165 treatment), right before H₂O₂ application on the half of each box. Similarly, the third
166 leaf of five distinct seedlings was sampled at day 2 and day 3 after treatment on the
167 whole boxes, for plants treated with H₂O₂ or not. All samples were immediately
168 pooled, frozen and stored at - 80 °C until use. To be noted that supplementary
169 experiments were carried out and confirmed that the application of water
170 supplemented with the adjuvants (0.1 % Break-Thru S240 and 0.05 % Tween 20) on
171 wheat plants did not induce the expression of defense genes (data not shown).

172

173 **RNA extraction and quantification of gene expression by real-time RT-PCR**

174 Total RNA was extracted from around 100 mg of plant tissue using the
175 Nucleospin®RNA Plant Kit (Macherey-Nagel). Reverse-transcription of total RNA

176 was carried out using the M-MLV Reverse Transcriptase (ref M1701, Promega,
177 Madison USA), according to the manufacturer protocol. Real-time qPCR was
178 performed with MESA BLUE qPCR MasterMix (ref RT-SY2X-03+WOUFLB,
179 Eurogentec, Liège, Belgique) according to manufacturer instructions and using the
180 INRA biomolecular tool developed by Brisset and Duge De Bernonville (2011) on a
181 Biorad MyiC detection system. Cycling conditions consisted of a first step of DNA
182 polymerase activation (95 °C for 5 min), followed by 35 repeated cycles of
183 denaturing, annealing and extending (95 °C for 15 min, 60 °C for 1min). The
184 biomolecular study focused on twenty three different genes involved in several plant
185 defense mechanisms, including pathogenesis-related (PR) proteins, secondary
186 metabolism, oxidative stress, and defense signaling pathways (Van Loon and Van
187 Strien 1999; Vogt 2009; Weber 2002). Relative gene expression was obtained using
188 the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008). Values correspond to the average
189 difference of expression of a given gene between treated and water control plants at
190 each time point (data obtained from the combination of two independent
191 experiments). Three internal reference genes were used for normalization (e.g.,
192 TubA, GAPDH, and Actin). The effect of plant treatment on the wheat defense
193 responses was evaluated by Multivariate ANOVA. In order to visualize and analyze
194 relative gene expression, a heatmap representation was performed using
195 dissimilarity distance (1-corr(X, Y)). Moreover, the identification of sets of genes that
196 may be similarly expressed across all conditions within the dataset was realized by
197 clustering gene expression. Finally, clustering results were confirmed by Principal
198 Component Analysis (PCA) of the gene expression data. PCA is a multivariate
199 statistical technique for simplifying complex data sets in which observations are
200 described by several dependent variables (in this study, the twenty three defense-

201 related genes) (Abdi and Williams 2010). The dimensionality of the data is reduced
202 by identifying new variables, also called directions or principal components (PCs),
203 which are uncorrelated and ordered. The first few PCs explain most of the data
204 variation and are expected to show the cluster structure of the original data set. The
205 first principal components are the directions along which samples show the greatest
206 variation. Consequently, plotting samples along the first two PCs makes it possible to
207 visually assess similarities and differences between samples. The statistical
208 programming environment R was used to analyze the data for all experiments. The
209 lme4 and multcomp packages were used to analyze greenhouse screening results
210 and the FactoMineR package was used for the PCA.

211

212 **Correlation between glasshouse and biomolecular results**

213 Correlation analysis is a method of statistical evaluation used to study the strength of
214 a relationship between at least two variables. The reliability of the elicitor screening
215 experiments carried out in this study can be strengthened by checking that the
216 protective effectiveness of a given compound is correlated to the induction of defense
217 responses in the treated wheat plant. Such correlation increases the chances of a
218 compound of being short-listed for further experiments under practical conditions. We
219 therefore examined for each compound to what extent its protective efficacy was
220 correlated with defense induction levels in treated wheat plants. The correlation was
221 assessed at each qRT-PCR analysis date. The level of gene expression was
222 estimated with the help of the PCA dimension which described the largest variability.
223 The average level of gene expression used for the correlation test thus consisted of
224 values of projected individuals (treatments) on the corresponding dimension. The
225 protection efficacy values corresponded only to compounds tested at their medium

226 concentration C2 (Table 1) since they were only tested at this concentration during
227 biomolecular experiments.

228

229 RESULTS

230 Protection efficacy against *Zymoseptoria tritici* under glasshouse conditions

231 The mean disease severity assessed during these screening experiments
232 corresponds to the percentage of symptomatic lesions scored on the third leaf
233 surface of wheat plants. As a result, a mean disease severity of 23 % was scored on
234 the water control (Fig. 1). Plants treated with the fungicide epoxiconazole or the
235 elicitor reference Bion showed an average disease severity of 0.1 % and 6 %,
236 respectively. The mean disease severity of wheat plants treated with the five elicitor
237 candidates ranged from 5 % to 15 %. Overall, plants sprayed with the various
238 treatments showed significantly less disease symptoms of *Z. tritici* compared to the
239 control (Tukey test, $P = 0.05$). The protection efficacy of a treatment corresponds to
240 the difference of mean disease severity between the control and the treated plants.
241 Consequently, the fungicide treatment showed the greatest protection efficacy (99.6
242 %), followed by Bion (74%). All five elicitor treatments were as efficient as the
243 commercial elicitor Bion in protecting wheat against STB: their protective
244 effectiveness ranged between 69 % and 72 %. However, the disease severity scored
245 on plants treated with CpG-ODN (B) and glycine betaine (D) was not statistically
246 different to that of control plants at the following concentrations: medium
247 concentration C2 for CpG-ODN ($9.5 \times 10^{-4} \text{ g l}^{-1}$) and highest concentration C3 for
248 CpG-ODN and glycine betaine (0.0095 g l^{-1} and 12 g l^{-1} respectively).

249

250

251 Induction of plant immune responses

252 Independently from screening trials, we monitored the expression level of 23
 253 defense-related genes of wheat at 1, 2 and 3 days after treatment with the five
 254 compounds (labelled A to E; Table 1). MANOVA tests showed that plant treatment
 255 had a significant effect on the expression of defense genes (Hotelling-Lawley test, *p*-
 256 *value* < 10⁻³). We then measured the difference of average expression level of each
 257 gene between treated plants and the water control, and represented it on a heatmap
 258 profile (Fig. 2). Overall, elicitor treatments generally triggered plant defense
 259 mechanisms at day 2 after treatment. Besides, no priming activities were observed
 260 as the application of H₂O₂ had no supplementary effect to the application of one of
 261 the elicitor treatments on the expression of wheat defense genes. Three major
 262 patterns were highlighted by the hierarchical clustering of genes according to their
 263 expression levels. The first pattern (1) includes genes involved in cell wall
 264 reinforcement, in the mevalonate pathway for the biosynthesis of isoprenoids and in
 265 the phenylpropanoid pathway for the biosynthesis of antimicrobial compounds. In
 266 particular, we observed that plants treated with the elicitor reference Bion showed a
 267 3-fold upregulation of HMGR gene expression (hydroxymethyl glutarate-CoA
 268 reductase) across all experimental conditions and a 2-fold downregulation of CHS
 269 gene expression (chalcone synthase) at day 2 and 3 after treatment, with or without
 270 subsequent application of H₂O₂. HMGR and CHS are key regulators of isoprenoid
 271 and flavonoid biosynthesis respectively (Antolín-Llovera et al. 2011; Dao et al. 2011).
 272 Conversely, plants treated with λ-carrageenan (A) showed a downregulation of these
 273 two genes, at day 1 and 2 after treatment, with or without H₂O₂.
 274 The second pattern (2) includes genes coding for PR proteins. A strong upregulation
 275 of PR4 and PR5 gene expression across all experimental conditions was observed

276 for plants treated with λ -carrageenan (A), CpG-ODN (B), glycine betaine (D) and
277 ergosterol (E). These genes code respectively for the synthesis of hevein-like and
278 thaumatin-like proteins displaying antimicrobial activities. PR1 and PR8 gene
279 expression were also significantly upregulated by λ -carrageenan and ergosterol with
280 6-fold and 4-fold increase, respectively. PR1 is a well-known marker of salicylic acid-
281 dependent defense responses, while PR8 consists of class III chitinases (Van Loon
282 and Van Strien 1999). Conversely, spirulina (C) induced a 6-fold downregulation of
283 PR1 gene expression at day 2 after treatment, with or without H₂O₂ applied
284 afterwards. Interestingly, plants treated with Bion and water-treated plants
285 subsequently sprayed with H₂O₂ showed no difference in PR gene expression
286 compared to the control.

287 A last pattern (3) includes genes involved in anti-oxidative processes and plant
288 defense-signaling. The expression of LOX2 (13-lipoxygenase 2) was strongly
289 induced by Bion (10-fold upregulation) and by CpG-ODN, spirulina, GB and
290 ergosterol (between 7 and 9-fold upregulation). Similarly, a 6-fold upregulation of
291 LOX2 expression was induced by λ -carrageenan but only at day 1 after treatment. It
292 is noteworthy that the lipoxygenase enzyme coded by the LOX2 gene is involved in
293 jasmonic acid-dependent defense signaling (Wasternack and Hause 2013). On the
294 other hand, expression of genes PAL (phenylalanine ammonia-lyase), POX
295 (peroxidase) and PR15 (oxalate oxidase) were upregulated by Bion and by the other
296 tested compounds, with the exception of CpG-ODN. Such upregulation occurred
297 generally at day 1 and/or day 2 after treatment, with or without H₂O₂ application. The
298 enzyme PAL is involved in the biosynthesis of phenolic compounds, including
299 salicylic acid, whereas peroxidases and oxalate oxidases are antioxidant enzymes

300 (Halliwell 2006; La Camera et al. 2004). The remaining genes were found to be very
301 weakly influenced by the treatments.

302 Confirmation of clustering results was provided by PCA (Fig. 3). Genes were used as
303 variables and treatments as individuals. The first two principal components described
304 the largest variability (26.8 % and 17.2 % of initial variation, respectively). They
305 enabled to separate the treatments and cluster the genes into three similar groups
306 with strong correlation coefficients ($|r| > 0.5$, p -value $< 10^{-3}$). Genes coding for PR
307 proteins were once again clearly separated into a distinct group. Similarly, genes
308 involved in anti-oxidative processes or in plant defense-signaling were gathered
309 close to one another. Variables with significant correlation coefficients (p -value $< 10^{-3}$)
310 were those which showed strong gene up- and/or down-regulation in the heatmap
311 profile.

312

313 **Correlation between protection efficacy and gene induction**

314 The PCA first component score described the largest variability (26.8%) and was
315 thus used for the correlation test (Fig. 4). A correlation found between two variables
316 is characterized by the fact that a change in one is accompanied by a change in
317 another. As it appears, the higher the coordinates were on dimension 1, the higher
318 the protective efficacy. Overall, protection efficacy and defense induction were
319 positively correlated at each qRT-PCR sampling date for all compounds. The
320 strongest and most significant correlation was obtained for day 1 (linear regression
321 analysis, $r^2 = 0.73$, p -value = 0.03). Treatments with Bion and spirulina were the most
322 effective to protect wheat against STB while at the same time inducing significant
323 defense responses in the plant. For the two other sampling dates, the correlations
324 were positive but less significant (p -value > 0.05).

325 **DISCUSSION**326 **Efficacious protection of wheat against STB under glasshouse conditions**

327 Each of the five elicitor treatments containing respectively λ -carrageenan, CpG-ODN,
328 spirulina, glycine betaine and ergosterol, significantly protected wheat against *Z. tritici*
329 under semi-controlled conditions. The STB disease severity was broadly reduced by
330 up to 70%, thus providing a consistent protection of the plant. Besides, these elicitor
331 treatments were as efficient as the commercialized elicitor product Bion. We
332 highlighted that high concentrations of applied CpG-ODN and GB did not effectively
333 protect wheat against the pathogen. Such finding could be related to the fact that
334 elicitor compounds are generally effective at low concentrations and within a given
335 window (Trotel-Aziz et al. 2006; Thakur and Sohal 2013). Moreover, we
336 demonstrated in preliminary assays that none of the compounds behaved as
337 biofungicides at the concentrations used for glasshouse screening. It is therefore
338 likely that all five tested compounds indeed acted as elicitors of wheat defenses.
339 Such result is in line with previous researches which underlined their elicitor potential
340 on other pathosystems.

341 For instance, among the three main types of marine carrageenans extracted from red
342 algae, λ -carrageenan was proven to be the most efficient in stimulating plant defense
343 responses due to its high degree of sulfation (41 % of total weight) (Shukla et al.
344 2016; Vera et al. 2011). We could assume that its efficacy to protect wheat against *Z.*
345 *tritici* could be linked to such high sulfate content, and further studies would allow a
346 better understanding of its mode of action. Concerning CpG-ODN, no research has
347 yet been reported for its ability to protect a crop plant against a fungal pathogen.
348 However, previous studies have demonstrated that CpG-ODN acts as a pathogen-
349 associated molecular pattern (PAMP) in mammalian cells and is recognized by

350 pattern recognition receptor (PRR) Toll-Like Receptor 9 (TLR9) (Carrington and
 351 Secombes 2006). A similar recognition process of CpG-ODN by specific wheat
 352 receptors can be assumed. On the other hand, the elicitor properties of spirulina are
 353 likely due its high content in secondary metabolites such as carotenoids, superoxide
 354 dismutase, glycolipids and sulfolipids, as suggested in other studies (Priyadarshani
 355 and Rath 2012). Most interestingly, and to our knowledge, the transkingdom potential
 356 of spirulina in inducing the defense mechanisms of both animals and plants had
 357 never been established. In 1995, Kulik already assumed that cyanobacteria could
 358 represent valuable biocontrol agents in agriculture, but since then no studies had yet
 359 demonstrated the potential of spirulina as a plant resistance inducer (Kulik 1995).
 360 Concerning GB, most studies carried out up to now have reported its potential to
 361 induce plant resistance to abiotic stresses. The fact that this plant osmolyte may also
 362 play a role in enhancing plant defenses against diseases opens up a whole new
 363 range of possibilities in IPM strategies. Conversely, ergosterol is a well-known elicitor
 364 and our results confirm that this compound presents a clear benefit for sustainable
 365 plant protection extended to crop plants.

366

367 **SA and/or JA defense signaling pathways are triggered in wheat**

368 Biomolecular tests on wheat defense responses confirm that each elicitor treatment
 369 was indeed perceived by the plant: the expression of genes coding for antimicrobial
 370 compounds was upregulated; the expression of genes involved in the synthesis of
 371 signal hormones such as salicylic acid (SA) and jasmonic acid (JA) were induced
 372 concomitantly and/or at different time scales in treated wheat plants. Signaling
 373 pathways mediated by the hormones SA, JA and ethylene (ET) play indeed a major
 374 role in plant defense responses (Muthamilarasan et al. 2013).

375 In dicotyledonous plants, SA-dependent defense signaling occurs upon infection by
376 biotrophic or hemibiotrophic pathogens (Glazebrook 2005), whereas defense
377 responses mediated by JA and ET are triggered upon infection by necrotrophic
378 pathogens and phloem-feeding insects (Adie et al. 2007; Van der Ent et al. 2009).
379 Defense genes can be differentially expressed through these defense signaling
380 pathways. PR1 and PR5 are systemic acquired resistance (SAR) marker genes
381 which are characteristically induced during SA-dependent defense responses,
382 whereas LOX2 and PR4 induction is generally linked to JA-dependent signaling
383 (Glazebrook 2005; Van Loon and Van Strien 1999). Most studies have reported the
384 existence of a mutual antagonism between SA and JA signaling pathways, although
385 synergistic interactions have also been described in thale-cress (Niu et al. 2011;
386 Schenk et al. 2000; Van Wees et al. 2000). However, most investigations have been
387 carried out on dicotyledonous plants and less is known about SA/JA crosstalk in
388 monocotyledonous plants. Central elements of induced resistance pathways are
389 conserved between dicotyledonous and monocotyledonous plants but their regulation
390 and interaction with other plant pathways seem to have undergone particular
391 evolutionary adaptations (Balmer et al. 2013; Kogel and Langen 2005). Knowledge
392 on chemical and molecular mechanisms of induced resistance in monocotyledonous
393 plants is still missing. For instance, the role of SA during SAR has yet to be
394 elucidated, and the PR1 family has undergone important diversifications (Balmer et
395 al. 2013). Recently, Ding et al (2016) showed that wheat was able to finely tune its
396 defense responses depending on its pathogenic invader (Ding et al. 2016). They
397 reported that SA and JA were able to act synergistically or antagonistically in order to
398 influence the expression of wheat defense genes.

399 In the present study, JA-dependent signaling was induced in plants treated with λ -
 400 carrageenan during the first 24 hours before giving way to SA-dependent defense
 401 responses. Previous studies have demonstrated that λ -carrageenan could protect
 402 tomato plants and thale-cress by triggering the expression of JA-related genes
 403 (Sangha et al. 2010, 2015). In addition, Vera et al (2011) showed that carrageenans
 404 were able to suppress a disease at a systemic scale by triggering SA-related defense
 405 responses such as an increased PAL enzymatic activity and the accumulation of
 406 phenolic compounds (Vera et al. 2011). Besides, Ray et al (2003) reported that
 407 infection of susceptible and resistant wheat cultivars by *Z. tritici* induced a strong
 408 upregulation of the LOX gene expression up to 3 hours after plant infection before
 409 quickly decreasing (Ray et al. 2003). On the other hand, SA and JA defense
 410 signaling pathways were induced simultaneously in wheat plants treated with GB,
 411 CpG-ODN or ergosterol. Our results therefore confirm the existence of intricate
 412 hormone crosstalk in plant innate immunity (Balmer et al. 2013; Ding et al. 2016;
 413 Lochman and Mikes 2006; Thaler et al. 2012). On the other hand, only JA-dependent
 414 signaling seems to have been strongly induced in plants treated with spirulina and
 415 Bion. The effect of spirulina on plant induced resistance had never been investigated
 416 previously. However, numerous studies have already been dedicated to the elicitor
 417 potential of Bion and our results are surprisingly in contradiction with these previous
 418 findings. As a chemical elicitor consisting of Acibenzolar-S-methyl, Bion shows
 419 functional analogy to the plant hormone SA and was shown to induce a long lasting
 420 SAR with a characteristic accumulation of PR1 and PR5 proteins and an increase in
 421 PAL and CHS activity (Görlach et al. 1996; Hofgaard et al. 2005). The fact that our
 422 results rather suggest the involvement of JA mediated signaling in wheat treated with

423 Bion could be linked to the genotype of the cultivar used in this study (Ors et al.
424 2017) and/or to the fine tuning of signal hormones in the plant (Ding et al. 2016).
425 Further investigations, including biochemical experiments, would probably help to
426 better understand how these five elicitor treatments and Bion contributed to induce
427 such defense responses in this major European crop. Research on induced
428 resistance of monocots is slowly emerging (Balmer et al. 2013). Yet, the primary
429 objective of the present study was mainly to rapidly identify interesting elicitor
430 compounds which are effective to protect the wheat plant against STB. The qPFD
431 represented an interesting biomolecular tool as a useful complement to glasshouse
432 trials for thorough elicitor screening. The efficacy of the five elicitor treatments to
433 protect wheat under semi-controlled conditions and their ability to trigger SA and/or
434 JA signaling pathways in the plant make them attractive candidates for the
435 development of additional elicitor tools against STB. Our results are even more
436 interesting as these compounds are already available on the market. Their common
437 use has already required various toxicological tests (Bode et al. 2011; EFSA
438 FEEDAP Panel 2013; Marles et al. 2011; Weiner 2014). For instance, λ -carrageenan
439 is widely used in the food industry as an additive due to its jellifying and emulsifying
440 properties (Weiner 2014), whereas spirulina is used as a concentrated and nutritious
441 food supplement (Small 2011). CpG-ODN is generally used at low doses for medical
442 purposes as an adjuvant for vaccines targeting infectious diseases and cancer (
443 (Bode et al. 2011; Carpentier et al. 2003; Maubant et al. 2011). As an additional
444 product of the sugar beet processing industry, GB is a commercially important
445 compound with multiple applications in agriculture, medicine and animal husbandry
446 (Eklund et al. 2005; Mäkelä 2004). Ergosterol is generally extracted from yeast and is
447 of industrial and commercial importance as a precursor of therapeutically useful

448 substances such as vitamin D₂ (Ethiraj 2013). On a pragmatic basis, the main
 449 interest of implementing elicitors as alternative plant protection tools in agriculture is
 450 to reduce the use of agrochemicals in the field and promote environment-friendly IPM
 451 strategies. Consequently, field trials would represent the next logical step to this
 452 study in order to check the potential of these compounds under real conditions.
 453 Numerous environmental parameters such as plant developmental stage, plant
 454 genotype and disease pressure are indeed known to influence the protection efficacy
 455 of elicitors in the field (Walters et al. 2005).

456

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460

461 **LITERATURE CITED**

- 462 Abdi, H., and Williams, L. J. 2010. Principal component analysis. Wiley Interdiscip.
 463 Rev. Comput. Stat. 2:433–459. doi: 10.1002/wics.101
- 464 Adie, B., Chico, J., Rubio-Somoza, I., and Solano, R. 2007. Modulation of plant
 465 defenses by ethylene. J. Plant Growth Regul. 26:160–177. doi: 10.1007/s00344-007-
 466 0012-6
- 467 Amborabé, B.-E., Rossard, S., Pérault, J.-M., and Roblin, G. 2003. Specific
 468 perception of ergosterol by plant cells. C. R. Biol. 326:363–370.
- 469 Antolín-Llovera, M., Leivar, P., Arró, M., Ferrer, A., Boronat, A., and Campos, N.
 470 2011. Modulation of plant HMG-CoA reductase by protein phosphatase 2A. Plant
 471 Signal. Behav. 6:1127–1131. doi: 10.4161/psb.6.8.16363

- 472 Balmer, D., Planchamp, C., and Mauch-Mani, B. 2013. On the move: Induced
473 resistance in monocots. *J. Exp. Bot.* 64:1249–1261. doi: 10.1093/jxb/ers248
- 474 Bode, C., Zhao, G., Steinhagen, F., Kinjo, T., and Klinman, D. M. 2011. CpG DNA as
475 a vaccine adjuvant. *Expert Rev. Vaccines.* 10:499–511. doi: 10.1586/erv.10.174
- 476 Carpentier, A. F., Auf, G., and Delattre, J.-Y. 2003. CpG-oligonucleotides for cancer
477 immunotherapy: Review of the literature and potential applications in malignant
478 glioma. *Front. Biosci.* 8:e116–e128.
- 479 Carrington, A. C., and Secombes, C. J. 2006. A review of CpGs and their relevance
480 to aquaculture. *Vet. Immunol. Immunopathol.* 112:87–101. doi:
481 10.1016/j.vetimm.2006.03.015
- 482 Covello, J. M., Friend, S. E., Purcell, S. L., Burka, J. F., Markham, R. J. F., Donkin, A.
483 W., Groman, D. B., and Fast, M. D. 2012. Effects of orally administered
484 immunostimulants on inflammatory gene expression and sea lice (*Lepeophtheirus*
485 *salmonis*) burdens on Atlantic salmon (*Salmo salar*). *Aquaculture.* 366:9–16. doi:
486 10.1016/j.aquaculture.2012.08.051
- 487 Dao, T., Linthorst, H., and Verpoorte, R. 2011. Chalcone synthase and its functions
488 in plant resistance. *Phytochem. Rev.* 10:397–412. doi: 10.1007/s11101-011-9211-7
- 489 Dayan, F. E., Cantrell, C. L., and Duke, S. O. 2009. Natural products in crop
490 protection. *Bioorg. Med. Chem.* 17:4022–4034. doi: 10.1016/j.bmc.2009.01.046
- 491 Ding, L.-N., Yang, G.-X., Yang, R.-Y., Cao, J., and Zhou, Y. 2016. Investigating
492 interactions of salicylic acid and jasmonic acid signaling pathways in monocots
493 wheat. *Physiol. Mol. Plant Pathol.* 93:67–74. doi: 10.1016/j.pmpp.2016.01.002
- 494 Dugé de Bernonville, T., Marolleau, B., Staub, J., Gaucher, M., Brisset, M.-N. 2014.
495 Using molecular tools to decipher the complex world of plant resistance inducers: an
496 apple case study. *J. Agric. Food Chem.* 62:11403–11411.

- 497 EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used
 498 in Animal Feed). 2013. Scientific Opinion on the safety and efficacy of betaine
 499 (betaine anhydrous and betaine hydrochloride) as a feed additive for all animal
 500 species based on a dossier submitted by VITAC EEIG. EFSA J. 11:3210. doi:
 501 10.2903/j.efsa.2013.3210
- 502 Eklund, M., Bauer, E., Wamatu, J., and Mosenthin, R. 2005. Potential nutritional and
 503 physiological functions of betaine in livestock. Nutr. Res. Rev. 18:31–48. doi:
 504 10.1079/NRR200493
- 505 Ethiraj, S. 2013. Fermentative production of ergosterol using *Saccharomyces*
 506 *cerevisiae*. J. Appl. Sci. Res. 9:1214–1221.
- 507 Eyal, Z. 1999. The *Septoria tritici* and *Stagonospora nodorum* Blotch diseases of
 508 wheat. Eur. J. Plant Pathol. 105:629–641. doi: 10.1023/A:1008716812259
- 509 Farag, M. R., Alagawany, M., El-Hack, M. E. A., and Dhama, K. 2016. Nutritional and
 510 healthical aspects of *Spirulina (Arthrospira)* for poultry, animals and human. Int. J.
 511 Pharmacol. 12:36–51. doi: 10.3923/ijp.2016.36.51
- 512 Fones, H., and Gurr, S. 2015. The impact of *Septoria tritici* Blotch disease on wheat:
 513 An EU perspective. Fungal Genet. Biol. 79:3–7. doi: 10.1016/j.fgb.2015.04.004
- 514 Fraaije, B. A., Cools, H. J., Fountaine, J., Lovell, D. J., Motteram, J., West, J. S., and
 515 Lucas, J. A. 2005. Role of ascospores in further spread of Qol-resistant cytochrome
 516 b alleles (G143A) in field populations of *Mycosphaerella graminicola*.
 517 Phytopathology. 95:933–941. doi: 10.1094/PHYTO-95-0933
- 518 Gałecki, A., and Burzykowski, T. 2013. Linear mixed-effects model. In Linear Mixed-
 519 Effects Models Using R, Springer Texts in Statistics, Springer New York, p. 245–273.
 520 doi: 10.1007/978-1-4614-3900-4_13

- 521 Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and
 522 necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43:205–227. doi:
 523 10.1146/annurev.phyto.43.040204.135923
- 524 Görlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K. H.,
 525 Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996.
 526 Benzothiadiazole, a novel class of inducers of systemic acquired resistance,
 527 activates gene expression and disease resistance in wheat. *Plant Cell.* 8:629–643.
 528 doi: 10.1105/tpc.8.4.629
- 529 Halliwell, B. 2006. Reactive species and antioxidants. Redox biology is a
 530 fundamental theme of aerobic life. *Plant Physiol.* 141:312–322. doi:
 531 10.1104/pp.106.077073
- 532 Hofgaard, I. S., Ergon, Å., Wanner, L. A., and Tronsmo, A. M. 2005. The effect of
 533 chitosan and Bion on resistance to pink snow mould in perennial ryegrass and winter
 534 wheat. *J. Phytopathol.* 153:108–119. doi: 10.1111/j.1439-0434.2005.00937.x
- 535 Kogel, K-H., Langen, G. 2005. Induced disease resistance and gene expression in
 536 cereals. *Cell Microbio.* 7:1555–1564. doi:10.1111/j.1462-5822.2005.00592.x
- 537 Kulik, M. M. 1995. The potential for using cyanobacteria (blue-green algae) and
 538 algae in the biological control of plant pathogenic bacteria and fungi. *Eur. J. Plant*
 539 *Pathol.* 101:585–599. doi: 10.1007/BF01874863
- 540 La Camera, S., Gouzerh, G., Dhondt, S., Hoffmann, L., Fritig, B., Legrand, M., et al.
 541 2004. Metabolic reprogramming in plant innate immunity: the contributions of
 542 phenylpropanoid and oxylipin pathways. *Immunol. Rev.* 198:267–284. doi:
 543 10.1111/j.0105-2896.2004.0129.x

- 544 Lawal, B. 2014. Incomplete block design. In *Applied Statistical Methods in*
 545 *Agriculture, Health and Life Sciences*, Springer, Cham, p. 639–659. doi:
 546 10.1007/978-3-319-05555-8_16
- 547 Le Mire, G., Nguyen, M., Fassotte, B., Du Jardin, P., Verheggen, F., Delaplace, P.,
 548 and Jijakli, M. H. 2016. Implementing plant biostimulants and biocontrol strategies in
 549 the agroecological management of cultivated ecosystems. A review. *Biotechnol.*
 550 *Agron. Société Environ.* 20(S1):299–313.
- 551 Lochman, J., and Mikes, V. 2006. Ergosterol treatment leads to the expression of a
 552 specific set of defence-related genes in tobacco. *Plant Mol. Biol.* 62:43–51. doi:
 553 10.1007/s11103-006-9002-5
- 554 Lyon, G. D., Newton, A. C., and Walters, D. R. 2014. Induced resistance in crop
 555 protection: The future, drivers and barriers. In *Induced Resistance for Plant Defense*,
 556 eds. Dale R. Walters, Adrian C. Newton, and Gary D. Lyon. John Wiley & Sons, Ltd,
 557 p. 316–325. doi: 10.1002/9781118371848.ch14
- 558 Mäkelä, P. 2004. Agro-industrial uses of glycine betaine. *Sugar Tech.* 6:207–212.
 559 doi: 10.1007/BF02942500
- 560 Marles, R. J., Barrett, M. L., Barnes, J., Chavez, M. L., Gardiner, P., Ko, R., Mahady,
 561 G. B., Low Dog, T., Sarma, N. D., Giancaspro, G. I., Sharaf, M., and Griffiths, J.
 562 2011. United States pharmacopeia safety evaluation of *Spirulina*. *Crit. Rev. Food Sci.*
 563 *Nutr.* 51:593–604. doi: 10.1080/10408391003721719
- 564 Maubant, S., Banissi, C., Beck, S., Chauvat, A., and Carpentier, A. F. 2011. Adjuvant
 565 properties of cytosine-phosphate-guanosine oligodeoxynucleotide in combination
 566 with various polycations in an ovalbumin-vaccine model. *Nucleic Acid Ther.* 21:231–
 567 240. doi: 10.1089/nat.2011.0291

- 568 Mercier, L., Lafitte, C., Borderies, G., Briand, X., Esquerré-Tugayé, M. T., and
 569 Fournier, J. 2001. The algal polysaccharide carrageenans can act as an elicitor of
 570 plant defence. *New Phytol.* 149:43–51. doi: 10.1046/j.1469-8137.2001.00011.x
- 571 Muthamilarasan, M., and Prasad, M. 2013. Plant innate immunity: An updated insight
 572 into defense mechanism. *J. Biosci.* 38:433–449.
- 573 Niu, D. D., Liu, H. X., Jiang, C. H., Wang, Y. P., Wang, Q. Y., Jin, H. L., and Guo, J.
 574 H. 2011. The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces
 575 systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate-
 576 and jasmonate/ethylene-dependent signaling pathways. *Mol. Plant. Microbe Interact.*
 577 24:533–542. doi: 10.1094/MPMI-09-10-0213
- 578 Ors, M. E., Randoux, B., Selim, S., Couleaud, G., Maumené, C., and Reignault, P.
 579 2017. Cultivar-dependent partial resistance and associated defence mechanisms in
 580 wheat against *Zymoseptoria tritici*. *Plant Pathol.* doi: 10.1111/ppa.12760
- 581 Palmer, C. L., and Skinner, W. 2002. *Mycosphaerella graminicola*: Latent infection,
 582 crop devastation and genomics. *Mol. Plant Pathol.* 3:63–70. doi: 10.1046/j.1464-
 583 6722.2002.00100.x
- 584 Priyadarshani, I., and Rath, B. 2012. Bioactive compounds from microalgae and
 585 cyanobacteria: Utility and applications. *Int. J. Pharm. Sci. Res.* 3:4123-4130. doi:
 586 10.13040/IJPSR.0975-8232.3(11).4123-30
- 587 Ray, S., Anderson, J., Urmeev, F., and Goodwin, S. 2003. Rapid induction of a
 588 protein disulfide isomerase and defense-related genes in wheat in response to the
 589 hemibiotrophic fungal pathogen *Mycosphaerella graminicola*. *Plant Mol. Biol.* 53:741–
 590 754. doi: 10.1023/B:PLAN.0000019120.74610.52

- 591 Rossard, S., Roblin, G., and Atanassova, R. 2010. Ergosterol triggers characteristic
 592 elicitation steps in *Beta vulgaris* leaf tissues. J. Exp. Bot. 61:1807–1816. doi:
 593 10.1093/jxb/erq047
- 594 Rudd, J. J. 2015. Previous bottlenecks and future solutions to dissecting the
 595 *Zymoseptoria tritici*–wheat host-pathogen interaction. Fungal Genet. Biol. 79:24–28.
 596 doi: 10.1016/j.fgb.2015.04.005
- 597 Sangha, J. S., Ravichandran, S., Prithiviraj, K., Critchley, A. T., and Prithiviraj, B.
 598 2010. Sulfated macroalgal polysaccharides λ -carrageenan and ι -carrageenan
 599 differentially alter *Arabidopsis thaliana* resistance to *Sclerotinia sclerotiorum*. Physiol.
 600 Mol. Plant Pathol. 75:38–45. doi: 10.1016/j.pmpp.2010.08.003
- 601 Sangha, J. S., Kandasamy, S., Khan, W., Bahia, N. S., Singh, R. P., Critchley, A. T.,
 602 et al. 2015. λ -Carrageenan suppresses tomato chlorotic dwarf viroid (TCDVd)
 603 replication and symptom expression in tomatoes. Mar. Drugs. 13:2875–2889. doi:
 604 10.3390/md13052875
- 605 Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S.
 606 C., and Manners, J. M. 2000. Coordinated plant defense responses in *Arabidopsis*
 607 revealed by microarray analysis. Proc. Natl. Acad. Sci. 97:11655–11660. doi:
 608 10.1073/pnas.97.21.11655
- 609 Schmittgen, T., and Livak, K. 2008. Analyzing real-time PCR data by the comparative
 610 CT method. Nat. Protoc. 3:1101–8. doi: 10.1038/nprot.2008.73
- 611 Shetty, N. P., Mehrabi, R., Lütken, H., Haldrup, A., Kema, G. H. J., Collinge, D. B.,
 612 and Jørgensen H. J. 2007. Role of hydrogen peroxide during the interaction between
 613 the hemibiotrophic fungal pathogen *Septoria tritici* and wheat. New Phytol. 174:637–
 614 647. doi: 10.1111/j.1469-8137.2007.02026.x

- 615 Shukla, P. S., Borza, T., Critchley, A. T., and Prithiviraj, B. 2016. Carrageenans from
 616 red seaweeds as promoters of growth and elicitors of defense response in plants.
 617 *Front. Mar. Sci.* 3:81. doi: 10.3389/fmars.2016.00081
- 618 Siah, A., Deweer, C., Morand, E., Reignault, P., and Halama, P. 2010. Azoxystrobin
 619 resistance of French *Mycosphaerella graminicola* strains assessed by four *in vitro*
 620 bioassays and by screening of G143A substitution. *Crop Prot.* 29:737–743. doi:
 621 10.1016/j.cropro.2010.02.012
- 622 Siah, A., Deweer, C., Duyme, F., Sanssené, J., Durant, R., Halama, P., and
 623 Reignault, P. 2010. Correlation of in planta endo-beta-1,4-xylanase activity with the
 624 necrotrophic phase of the hemibiotrophic fungus *Mycosphaerella graminicola*. *Plant*
 625 *Pathol.* 59:661-670.
- 626 Small, E. 2011. 37. Spirulina – food for the universe. *Biodiversity.* 12:255–265. doi:
 627 10.1080/14888386.2011.642735
- 628 Thakur, M., and Sohal, B. S. 2013. Role of elicitors in inducing resistance in plants
 629 against pathogen infection: A review. *ISRN Biochem.* 2013:762412. doi:
 630 10.1155/2013/762412
- 631 Thaler, J. S., Humphrey, P. T., and Whiteman, N. K. 2012. Evolution of jasmonate
 632 and salicylate signal crosstalk. *Trends Plant Sci.* 17:260–270. doi:
 633 10.1016/j.tplants.2012.02.010
- 634 Torriani, S. F. F., Melichar, J. P. E., Mills, C., Pain, N., Sierotzki, H., and Courbot, M.
 635 2015. *Zymoseptoria tritici*: A major threat to wheat production, integrated approaches
 636 to control. *Fungal Genet. Biol.* 79:8–12. doi: 10.1016/j.fgb.2015.04.010
- 637 Trotel-Aziz, P., Couderchet, M., Vernet, G., and Aziz, A. 2006. Chitosan stimulates
 638 defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*.
 639 *Eur. J. Plant Pathol.* 114:405–413. doi: 10.1007/s10658-006-0005-5

- 640 Van der Ent, S., Van Wees, S. C. M., and Pieterse, C. M. J. 2009. Jasmonate
 641 signaling in plant interactions with resistance-inducing beneficial microbes.
 642 *Phytochemistry*. 70:1581–1588. doi: 10.1016/j.phytochem.2009.06.009
- 643 Van Loon, L. C., and Van Strien, E. A. 1999. The families of pathogenesis-related
 644 proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol.*
 645 *Mol. Plant Pathol.* 55:85–97. doi: 10.1006/pmpp.1999.0213
- 646 Van Loon, L. C., Rep, M., and Pieterse, C. M. J. 2006. Significance of inducible
 647 defense-related proteins in infected plants. 44:135–162. doi:
 648 10.1146/annurev.phyto.44.070505.143425
- 649 Van Wees, S. C. M., De Swart, E. A. M., Van Pelt, J., Van Loon, L. C., and Pieterse,
 650 C. M. J. 2000. Enhancement of induced disease resistance by simultaneous
 651 activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis*
 652 *thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 97:8711–8716. doi: 10.1073/pnas.130425197
- 653 Věchet, L., and Šerá, B. 2015. Effectiveness of both synthetic compounds and
 654 biological extracts against powdery mildew (*Blumeria graminis* f. sp. *tritici*) on winter
 655 wheat. *Agrociencia*. 49:77–85.
- 656 Vera, J., Castro, J., Gonzalez, A., and Moenne, A. 2011. Seaweed polysaccharides
 657 and derived oligosaccharides stimulate defense responses and protection against
 658 pathogens in plants. *Mar. Drugs*. 9:2514–2525. doi: 10.3390/md9122514
- 659 Vogt, T. 2009. Phenylpropanoid biosynthesis. *Mol. Plant*. 3:2–20. doi:
 660 10.1093/mp/ssp106
- 661 Walters, D., Walsh, D., Newton, A., and Lyon, G. 2005. Induced resistance for plant
 662 disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology*.
 663 95:1368–1373. doi: 10.1094/PHYTO-95-1368

- 664 Walters, D. R., Ratsep, J., and Havis, N. D. 2013. Controlling crop diseases using
 665 induced resistance: challenges for the future. *J. Exp. Bot.* 64:1263–1280. doi:
 666 10.1093/jxb/ert026
- 667 Walters, D. R., Havis, N. D., Paterson, L., Taylor, J., Walsh, D. J., and Sablou, C.
 668 2014. Control of foliar pathogens of spring barley using a combination of resistance
 669 elicitors. *Front. Plant Sci.* 5:241. doi: 10.3389/fpls.2014.00241
- 670 Wan, D., Wu, Q., and Kuča, K. 2016. Chapter 42 - Spirulina. In *Nutraceuticals*, ed.
 671 Ramesh C. Gupta. Boston: Academic Press, p. 569–583.
- 672 Wasternack, C., and Hause, B. 2013. Jasmonates: biosynthesis, perception, signal
 673 transduction and action in plant stress response, growth and development. An
 674 update to the 2007 review in *Annals of Botany*. *Ann. Bot.* 111:1021–1058. doi:
 675 10.1093/aob/mct067
- 676 Weber, H. 2002. Fatty acid-derived signals in plants. *Trends Plant Sci.* 7:217–224.
- 677 Weiner, M. L. 2014. Food additive carrageenan: Part II: A critical review of
 678 carrageenan *in vivo* safety studies. *Crit. Rev. Toxicol.* 44:244–269. doi:
 679 10.3109/10408444.2013.861798
- 680 Yakushiji, S., Ishiga, Y., Inagaki, Y., Toyoda, K., Shiraishi, T., and Ichinose, Y. 2009.
 681 Bacterial DNA activates immunity in *Arabidopsis thaliana*. *J. Gen. Plant Pathol.*
 682 75:227–234. doi: 10.1007/s10327-009-0162-4
- 683
- 684 **Author-Recommended Internet Resources**
- 685 Brisset, M. N., and Duge De Bernonville, T. 2011. Patent WO/2011/161388. Device
 686 for determining or studying the state of stimulation of the natural defences of plants
 687 or portions of plants.
 688 <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2011161388&recNum=21>

689 0&docAn=FR2011051470&queryString=(CL/antibod*)%2520OR%2520(DE/antibod*)
 690 %2520OR%2520(ET/antibod*)%2520&maxRec=145490.

691 European Commission. 2012. Sustainable agriculture for the future we want. In Rio:
 692 European Commission. [http://ec.europa.eu/agriculture/events/2012/rio-side-](http://ec.europa.eu/agriculture/events/2012/rio-side-event/brochure_en.pdf)
 693 [event/brochure_en.pdf](http://ec.europa.eu/agriculture/events/2012/rio-side-event/brochure_en.pdf)

694 Satger, O. 2016. L'offre mondiale excédentaire en 2015/2016 et la perspective de
 695 bonnes récoltes en 2016 continuent de faire pression sur les prix des grains.

696 Agreste. 287:6. <http://agreste.agriculture.gouv.fr/IMG/pdf/conjsynt287201605cult.pdf>

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715 **Table 1.** Characteristics of the compounds used in the study

Active ingredient	Supplier	Characteristics	General use	Concentration tested (g l ⁻¹)		
				C1	C2	C3
λ -carrageenan	SIGMA	Linear polysaccharide extracted from red algae	Gelling and emulsifying agent in the food industry	0.1	1	5
CpG-ODN	Pr. A. Carpentier, Paris, France	Short single-stranded synthetic DNA molecules (CpG-28, sequence 5'-TAAACGTTATAAC GTTATGACGTCAT-3')	Human vaccine adjuvant	9.5 x 10 ⁻⁵	9.5 x 10 ⁻⁴	9.5 x 10 ⁻⁴
<i>Spirulina platensis</i>	Djrbalgue, Tunisia	Dried cyanobacterium	Human dietary supplement	0.3	3	30
Glycine betaine	lthec	Organic osmolyte extracted from beetroot	Protectant of plants against abiotic stress	0.12	1.2	12
Ergosterol	SIGMA	Fungal sterol	Therapeutic vitamin D precursor	0.002	0.08	0.03
BION®50WG (Acibenzolar-S-methyl; 50 % w : w)	Syngenta, Europe	Synthetic elicitor	Plant resistance inducer		0.6	
Fungicide Opus® (Epoiconazole)	BASF Agro, France	Triazole fungicide	Broad-spectrum systemic fungicide		0.75 (v :v)	

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721 **Fig. 1.** Efficacy of five candidate elicitors (λ -carrageenan, CpG-ODN, Spirulina,
 722 Glycine betaine, Ergosterol) tested at three different concentrations to protect wheat
 723 leaves against the disease *Septoria tritici* Blotch (STB) under glasshouse conditions.
 724 Preventive treatment of wheat with various compounds significantly reduced *Z. tritici*
 725 disease severity compared to the control. Compounds CpG-ODN and Glycine
 726 betaine showed no difference with the control at high concentrations. Data are
 727 percentages of the third leaf surface of plants exhibiting symptomatic STB lesions
 728 (necrosis and/or chlorosis). Medians are represented by black horizontal lines in
 729 each box, and means are represented by “+” symbol ($n \geq 80$, e.g., incomplete block
 730 design with five pots of eight plants and two independent experiments at least per
 731 treatment). Boxes tagged with the same letters correspond to means that are not
 732 significantly different using the Tukey test at $P = 0.05$.

733
 734 **Fig. 2.** Heatmap profiling of the average expression level of 23 defense-related
 735 genes of wheat across all experimental conditions (product, \pm H₂O₂, day post-
 736 treatment). Data obtained by the combination of two independent experiments ($n =$
 737 32). Hierarchical clustering of gene expression highlighted three main patterns. The
 738 Log₂ expression levels above or under 0 represent respectively an induction (in
 739 green) or a repression (in red) of gene expression in treated plants compared to the
 740 water control. Hydrogen peroxide (H₂O₂) was applied (+) or not (-) on plants at 1 day
 741 after treatment to mimic a pathogen attack. The list of genes examined in this study
 742 is the following: Apox, ascorbate peroxidase; CalS, callose synthase; CHS, chalcone
 743 synthase; CAD, cinnamyl-alcohol dehydrogenase; CSL, cysteine sulfoxide; EIN3,
 744 EIN3-binding F box protein; EDS1, enhanced disease susceptibility 1; Far, (E,E)- α -
 745 farnesene synthase; FPPS, farnesyl pyrophosphate synthase; GST, glutathione S-

746 transferase; HMGR, hydroxymethyl glutarate-CoA reductase; JAR, jasmonate
 747 resistant 1; Lox2, 13-lipoxygenase 2; PAL, phenylalanine ammonia-lyase; PR,
 748 pathogenesis-related protein; PPO, polyphenol oxidase; POX, peroxidase; WRKY,
 749 WRKY transcription factor 30.

750

751 **Fig. 3.** Principal component analysis (PCA) of gene expression across all
 752 experimental conditions compared to water control (product, \pm H₂O₂, day post-
 753 treatment). Three major groups are highlighted in different colors (red, blue and
 754 black). The variables correspond to the 23 defense-related genes of wheat examined
 755 by heatmap profiling (n = 32) and hierarchical clustering. Variables are projected on
 756 the first and second principal component scores (Dim1 and Dim2) and were
 757 significantly correlated to one of the dimensions.

758

759 **Fig. 4.** Relationship between the expression of wheat defense genes and the efficacy
 760 of five compounds in protecting wheat against *Zymoseptoria tritici*: λ -carrageenan
 761 (A); CpG-ODN (B); Spirulina (C); Glycine betaine (D); Ergosterol (E). Positive
 762 correlations are highlighted and are significant at day 1 after plant treatment. Gene
 763 expression was obtained by qRT-PCR while protection efficacy data was a result of
 764 glasshouse elicitor screening. Hydrogen peroxide (H₂O₂) applications were realized
 765 to test for priming activity: grey, without; black, with. Coordinates on PCA first
 766 component score are averaged across biological replicates and were used as a
 767 measure of induced gene expression. Correlation coefficients and p-values of linear
 768 models are indicated in each plot (n \geq 80 for screening tests and n = 32 for gene
 769 expression studies).

770

771 **Fig. S1.** *In vitro* assessment of the biocidal effect of λ -carrageenan, CpG-ODN,
772 *Spirulina platensis*, glycine betaine and ergosterol towards the pathogen
773 *Zymoseptoria tritici*. Values correspond, respectively, to the average percentage of
774 germinated spores and average fungal diameter (cm) of the pathogen scored on
775 potato dextrose agar (PDA) culture medium amended with the five different
776 compounds. For each compound, five decreasing concentrations were tested, with C
777 being the highest concentration: λ -carrageenan, 5 g l⁻¹; CpG-ODN, 0.0095 g l⁻¹;
778 *Spirulina platensis*, 30 g l⁻¹; glycine betaine, 12 g l⁻¹; ergosterol, 0.03 g l⁻¹. The
779 control corresponds to PDA medium without tested compounds (0 g l⁻¹). Means
780 tagged with the same letters are not significantly different using the Tukey test at $P =$
781 0.05.

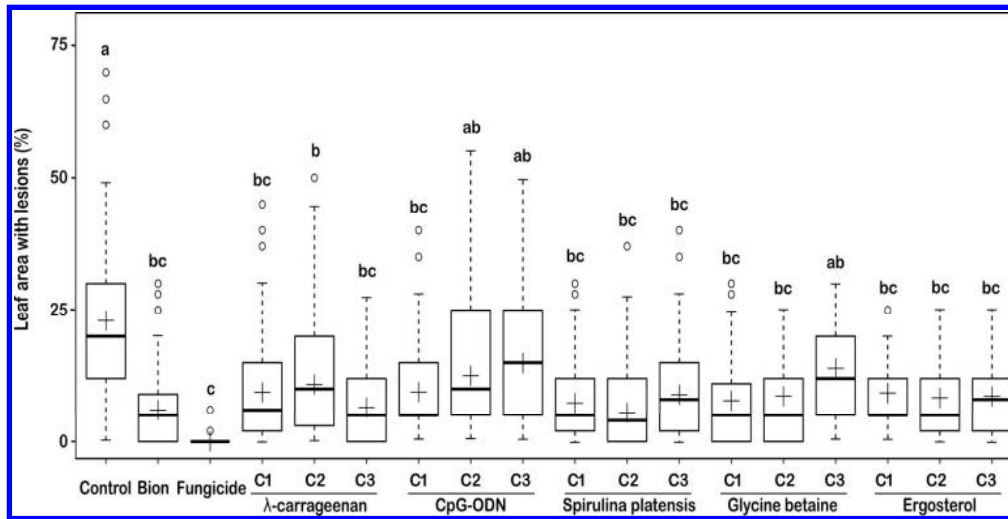


Fig. 1. Efficacy of five candidate elicitors (λ -carrageenan, CpG-ODN, Spirulina, Glycine betaine, Ergosterol) tested at three different concentrations to protect wheat leaves against the disease *Septoria tritici* Blotch (STB) under glasshouse conditions. Preventive treatment of wheat with various compounds significantly reduced *Z. tritici* disease severity compared to the control. Compounds CpG-ODN and Glycine betaine showed no difference with the control at high concentrations. Data are percentages of the third leaf surface of plants exhibiting symptomatic STB lesions (necrosis and/or chlorosis). Medians are represented by black horizontal lines in each box, and means are represented by "+" symbol ($n \geq 80$, e.g., incomplete block design with five pots of eight plants and two independent experiments at least per treatment). Boxes tagged with the same letters correspond to means that are not significantly different using the Tukey test at $P = 0.05$.

279x140mm (191 x 191 DPI)

Comment citer ce document :

Le Mire, G., Siah, A., Marolleau, B., Gaucher, M., Maumené, C., Brostaux, Massart, Brisset, M.-N., Jijakli (2019). Evaluation of -carrageenan, CpG-ODN, glycine betaine, Spirulina platensis and ergosterol as elicitors for the control of Zymoseptoria tritici in wheat. Phytopathology, 109 (3), 409-417. . DOI : 10.1094/PHYTO-11-17-0367-R

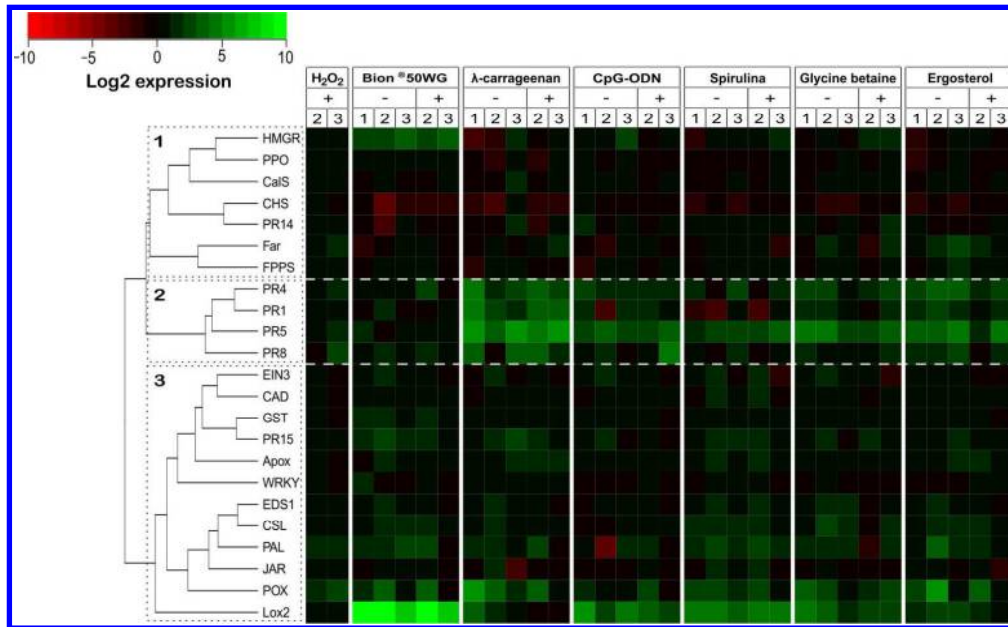


Fig. 2. Heatmap profiling of the average expression level of 23 defense-related genes of wheat across all experimental conditions (product, \pm H₂O₂, day post-treatment). Data obtained by the combination of two independent experiments (n = 32). Hierarchical clustering of gene expression highlighted three main patterns. The Log₂ expression levels above or under 0 represent respectively an induction (in green) or a repression (in red) of gene expression in treated plants compared to the water control. Hydrogen peroxide (H₂O₂) was applied (+) or not (-) on plants at 1 day after treatment to mimic a pathogen attack. The list of genes examined in this study is the following: Apox, ascorbate peroxidase; CalS, callose synthase; CHS, chalcone synthase; CAD, cinnamyl-alcohol dehydrogenase; CSL, cysteine sulfoxide; EIN3, EIN3-binding F box protein; EDS1, enhanced disease susceptibility 1; Far, (E,E)- α -farnesene synthase; FPPS, farnesyl pyrophosphate synthase; GST, glutathione S-transferase; HMGR, hydroxymethyl glutarate-CoA reductase; JAR, jasmonate resistant 1; Lox2, 13-lipoxygenase 2; PAL, phenylalanine ammonia-lyase; PR, pathogenesis-related protein; PPO, polyphenol oxidase; POX, peroxidase; WRKY, WRKY transcription factor 30.

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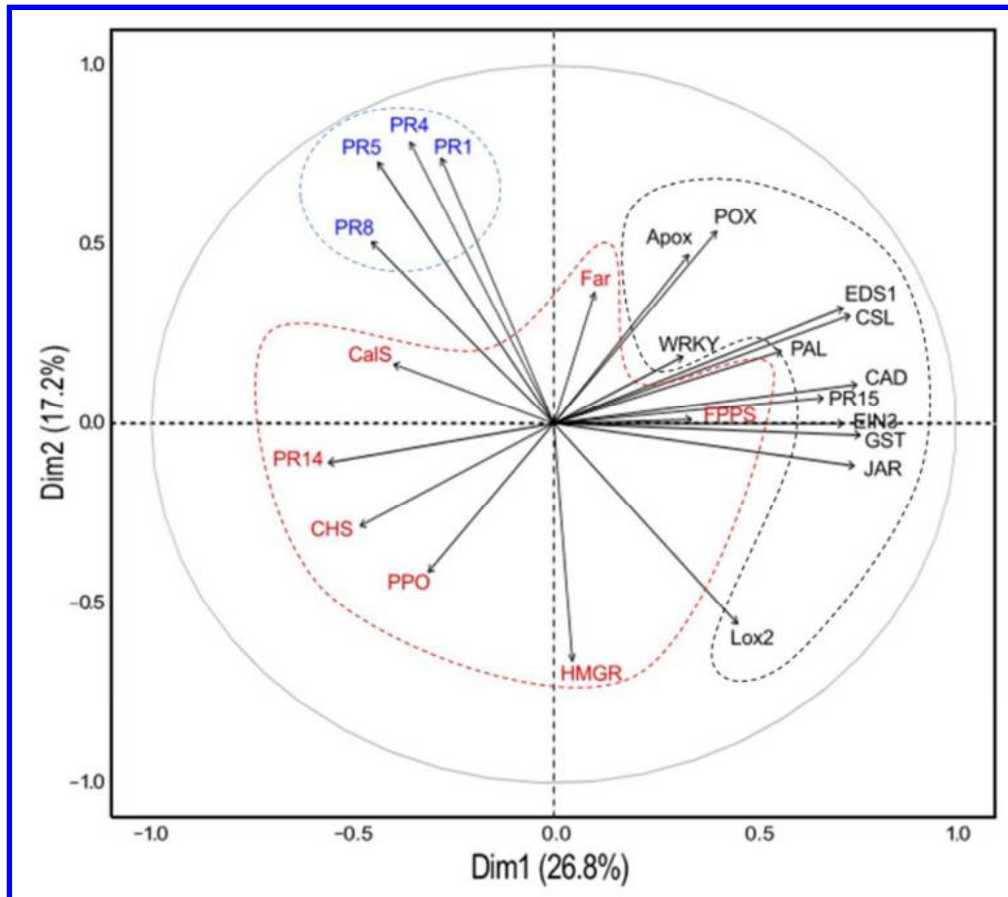


Fig. 3. Principal component analysis (PCA) of gene expression across all experimental conditions compared to water control (product, \pm H₂O₂, day post-treatment). Three major groups are highlighted in different colors (red, blue and black). The variables correspond to the 23 defense-related genes of wheat examined by heatmap profiling ($n = 32$) and hierarchical clustering. Variables are projected on the first and second principal component scores (Dim1 and Dim2) and were significantly correlated to one of the dimensions.

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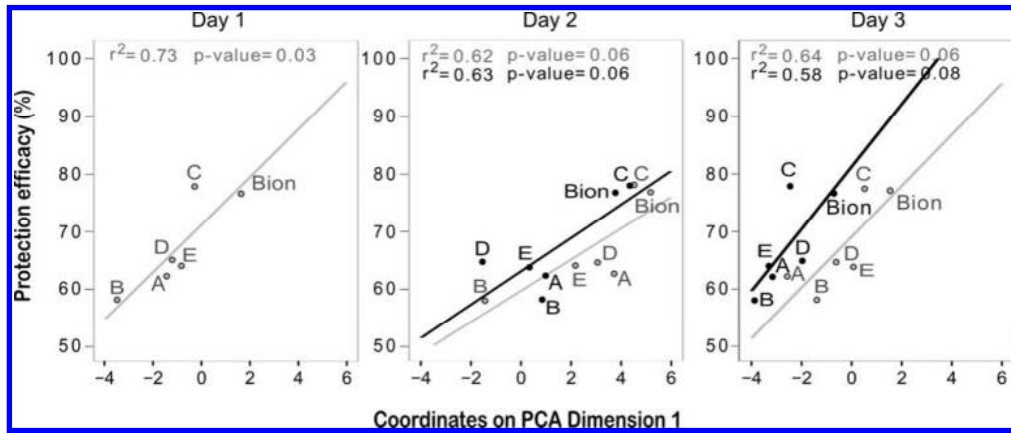


Fig. 4. Relationship between the expression of wheat defense genes and the efficacy of five compounds in protecting wheat against *Zymoseptoria tritici*: λ -carrageenan (A); CpG-ODN (B); Spirulina (C); Glycine betaine (D); Ergosterol (E). Positive correlations are highlighted and are significant at day 1 after plant treatment. Gene expression was obtained by qRT-PCR while protection efficacy data was a result of glasshouse elicitor screening. Hydrogen peroxide (H_2O_2) applications were realized to test for priming activity: grey, without; black, with. Coordinates on PCA first component score are averaged across biological replicates and were used as a measure of induced gene expression. Correlation coefficients and *p-values* of linear models are indicated in each plot ($n \geq 80$ for screening tests and $n = 32$ for gene expression studies).

236x98mm (113 x 113 DPI)

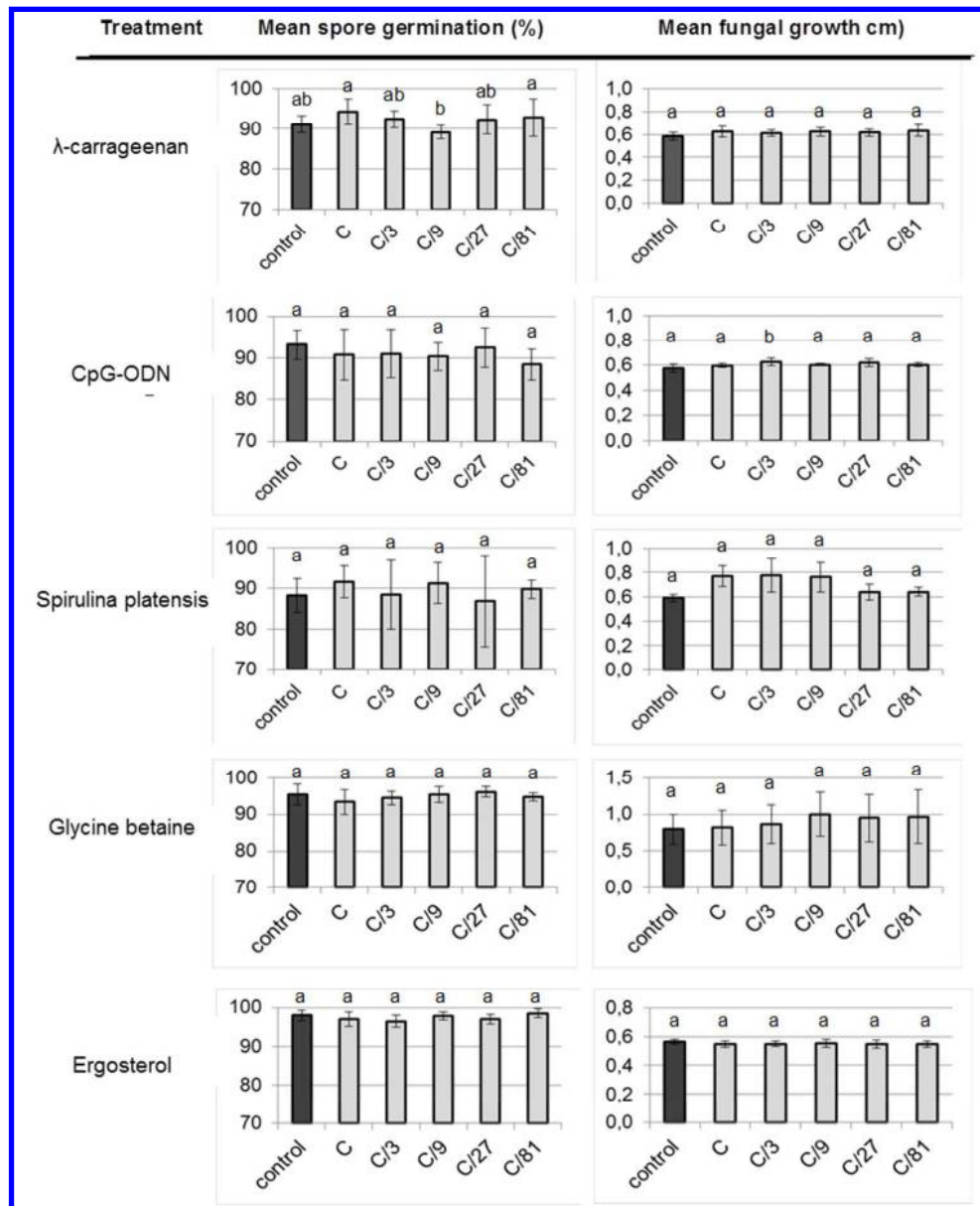


Fig. S1. *In vitro* assessment of the biocidal effect of λ -carrageenan, CpG-ODN, *Spirulina platensis*, glycine betaine and ergosterol towards the pathogen *Zymoseptoria tritici*. Values correspond, respectively, to the average percentage of germinated spores and average fungal diameter (cm) of the pathogen scored on potato dextrose agar (PDA) culture medium amended with the five different compounds. For each compound, five decreasing concentrations were tested, with C being the highest concentration: λ -carrageenan, 5 g l⁻¹; CpG-ODN, 0.0095 g l⁻¹; *Spirulina platensis*, 30 g l⁻¹; glycine betaine, 12 g l⁻¹; ergosterol, 0.03 g l⁻¹. The control corresponds to PDA medium without tested compounds (0 g l⁻¹). Means tagged with the same letters are not significantly different using the Tukey test at $P = 0.05$.

355x434mm (150 x 150 DPI)

Comment citer ce document :

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