bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 **Article title:**

2 # *Botrytis cinerea* identifies host plants via the recognition of antifungal capsidiol to
3 induce expression of a specific detoxification gene

4

5 Teruhiko Kuroyanagi[1], Abriel Bulasag[1,2], Keita Fukushima[1], Takamasa Suzuki[3], Aiko Tanaka[1],
6 Maurizio Camagna[1], Ikuo Sato[1], Sotaro Chiba[1], Makoto Ojika[1] and Daigo Takemoto[1*]

7 [1] Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, 464-8601,
8 Japan

9 [2] College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna,
10 Philippines 4031

11 [3] College of Bioscience and Biotechnology, Chubu University, Kasugai, Aichi 478-8501, Japan

12

13 [*]Corresponding author: Daigo Takemoto

14 Email: dtakemo@agr.nagoya-u.ac.jp

15

16 Author Contributions: T.K., A.B., K.F., I.S, S.C., M.O., and D.T., designed research; T.K., A.B.,
17 K.F., T.S., M.O., and D.T. performed research; T.K., K.F., T.S, A.T, M.C., and D.T. analyzed data;
18 T.S., M.C., M.O., and D.T. contributed new reagents/analytic tools; M.C. and D.T. wrote the
19 paper.

20 Competing Interest Statement: The authors declare no competing interest.

21

22 Keywords: capsidiol, detoxification, phytoalexin, polyxenous pathogen, Solanaceae plants.

23

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

24 **Abstract**

25 The gray mold pathogen *Botrytis cinerea* has a broad host range, causing disease
26 in over 400 plant species, but it is not known how this pathogen evolved this
27 polyxenous nature. *B. cinerea* can metabolize a wide range of phytoalexins,
28 including the stilbenoid, resveratrol, and the sesquiterpenoids capsidiol in tobacco,
29 and rishitin in potato and tomato. In this study, we analyzed the metabolism of
30 sesquiterpenoid phytoalexins by *B. cinerea*. Capsidiol was dehydrogenated to
31 capsenone which was then further oxidized, while rishitin was directly oxidized to
32 epoxy- or hydroxy-rishitins indicating that *B. cinerea* has separate mechanisms to
33 detoxify structurally similar sesquiterpenoid phytoalexins. RNAseq analysis
34 revealed that a distinct set of genes were induced in *B. cinerea* when treated with
35 capsidiol or rishitin, suggesting that *B. cinerea* can distinguish structurally similar
36 phytoalexins to activate appropriate detoxification mechanisms. The gene most
37 highly upregulated by capsidiol treatment encoded a dehydrogenase, designated
38 *Bccpdh*. Heterologous expression of *Bccpdh* in a capsidiol-sensitive plant
39 symbiotic fungus, *Epichloë festucae*, resulted in an acquired tolerance of capsidiol
40 and the ability to metabolize capsidiol to capsenone, while *B. cinerea Δbccpdh*
41 mutants became relatively sensitive to capsidiol. The *Δbccpdh* mutant showed
42 reduced virulence on the capsidiol producing *Nicotiana* and *Capsicum* species but
43 remained fully pathogenic on potato and tomato. Homologs of *Bccpdh* are not
44 found in taxonomically distant Ascomycota fungi but not in related Leotiomycete
45 species, suggesting that *B. cinerea* acquired the ancestral *Bccpdh* by horizontal
46 gene transfer, thereby extending the pathogenic host range of this polyxenous
47 pathogen to capsidiol-producing plant species.

48

49 **Significance Statement**

50 *B. cinerea* can metabolize a wide range of phytoalexins, however, the extent to
51 which phytoalexin detoxification contributes to pathogenicity is largely unknown. In
52 this study, we have shown that *B. cinerea* recognizes structurally resembling
53 sesquiterpenoid phytoalexins, rishitin and capsidiol, to activate appropriate
54 detoxification mechanisms. We identify *Bccpdh*, encoding a dehydrogenase for
55 capsidiol detoxification, which is upregulated in *B. cinerea* exclusively during the
56 infection of capsidiol producing plant species, and is required to exert full virulence.
57 Analysis of the *Bccpdh* locus implicates that the gene was acquired via horizontal
58 gene transfer. This work highlights that the polyxenous plant pathogen *B. cinerea*
59 can distinguish its host plants by its anti-microbial compounds, to activate
60 appropriate mechanisms for enhanced virulence.

61
62
63

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

64 **Introduction**

65

66 The antimicrobial secondary metabolites produced in plants during the induction
67 of disease resistance are collectively termed phytoalexins (1, 2). Several hundred
68 phytoalexins of diverse structures have been identified from a wide range of plant
69 species, which include terpenoids, flavonoids and indoles (3, 4). Many of these
70 phytoalexins are considered to exhibit their antimicrobial activity by targeting the
71 cell wall or cell membrane of pathogens (5, 6), but their mechanisms of action
72 remain largely unknown.
73 In plants belonging to the Solanaceae family, the major phytoalexins are
74 sesquiterpenoids, such as capsidiol in *Nicotiana* and *Capsicum* species and rishitin
75 in *Solanum* species (2, 7, 8). In *Nicotiana* sp., production of capsidiol is strictly
76 controlled by regulating the gene expression for capsidiol biosynthesis, such as
77 *EAS* (5-*epi*-aristolochene synthase) and *EAH* (5-*epi*-aristolochene dihydroxylase),
78 encoding the enzymes dedicated to the production of capsidiol (9, 10). In *N.*
79 *benthamiana*, expression of *EAS* and *EAH* genes is hardly detected in healthy
80 tissues, but their expression is rapidly induced during infection by pathogens or the
81 treatment with elicitor protein (11, 12). Produced phytoalexins are secreted locally
82 via ABC (ATP-binding cassette) transporters at the site of pathogen attack (13,
83 14). For plants, such temporal and spatial control of phytoalexins is probably
84 critical as the toxicity of phytoalexins is often not specific to microorganisms but is
85 also harmful to plant cells (13, 15, 16). For example, rishitin affects the permeability
86 of plant liposomal membranes and disrupts chloroplasts (17). Therefore, timely
87 production and efficient transport of phytoalexins to the site of pathogen attack are
88 important for plants to apply these double-edged weapons effectively.
89 It has been reported that various phytopathogenic fungi can metabolize and
90 detoxify phytoalexins (18). Although many studies have shown an approximate
91 correlation between the ability of strains to detoxify phytoalexins and their virulence
92 (19–21), the importance of phytoalexin detoxification for pathogen virulence is
93 largely unproven for most plant-pathogen interactions. The best-studied
94 phytoalexin metabolizing enzyme is pisatin demethylase (PDA) of *Nectria*
95 *haematococca*. Deletion of the *PDA* gene resulted in reduced virulence of *N.*
96 *haematococca* on pea, directly proving the importance of PDA for the virulence of
97 this pathogen (22). It has also been reported that sesquiterpenoid phytoalexins are
98 metabolized by pathogenic fungi. Capsidiol is metabolized to less toxic capsenone
99 via dehydration by pathogens such as the gray mold pathogen *Botrytis cinerea*
100 and *Fusarium oxysporum* (23). *Gibberella pulicaris*, the dry rot pathogen of potato
101 tubers, oxidizes rishitin to 13-hydroxy rishitin and 11,12-epoxyrishitin (24).
102 However, the enzymes involved in the detoxification of sesquiterpenoid
103 phytoalexins have not been isolated to date, and their importance for pathogenicity
104 has not been demonstrated.
105 In this study, we first investigated some pathogens isolated from Solanaceae and
106 non-Solanaceae plants on their tolerance of capsidiol and rishitin, as well as their
107 ability to detoxify/metabolize these phytoalexins. Among the pathogens that can
108 metabolize both capsidiol and rishitin, we chose *Botrytis cinerea* for further

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

109 analysis to investigate the importance of its ability to detoxify capsidiol for the
110 pathogenicity on plant species that produce capsidiol.
111
112

113 **Results**

114

115 **Metabolization of sesquiterpenoid phytoalexins capsidiol and rishitin by**
116 **fungal plant pathogens.**

117 Four oomycetes and 12 fungal species were evaluated for their ability to
118 detoxify/metabolize sesquiterpenoid phytoalexins capsidiol and rishitin, produced
119 by Solanaceae plant species. Four *Phytophthora* species isolated from
120 Solanaceae host plants, including potato late blight pathogen *P. infestans*, *P.*
121 *nicotiana* isolated from tobacco, *P. capsici* isolated from green pepper and *P.*
122 *cryptogea* isolated from nipplefruit (*Solanum mammosum*), are all sensitive to
123 capsidiol and rishitin. The amount of capsidiol and rishitin after the incubation with
124 these oomycete pathogens did not decrease, indicating that they are neither
125 capable of metabolizing nor tolerant to capsidiol and rishitin (Fig. 1 and
126 Supplemental Fig. S1). Among 12 fungal plant pathogens (eight isolated from
127 Solanaceae plants), seven and eight fungal strains can metabolize capsidiol and
128 rishitin, respectively, and showed increased resistance to phytoalexins. In some
129 cases, pathogens can metabolize phytoalexins that are not produced by their host,
130 indicating that the ability to detoxify phytoalexins does not always correlate with
131 their host range (Supplemental Figs. S1-3 and Supplementary Notes 1 and 2).
132 *Botrytis cinerea* and *Sclerotinia sclerotiorum* are well-known as pathogens with a
133 wide host range and are capable of metabolizing both capsidiol and rishitin. In this
134 study, *B. cinerea* was selected as the pathogen to further investigate the role of
135 detoxification of phytoalexins on the pathogenicity of this polyxenous pathogen.
136

137 **Metabolization of capsidiol and rishitin by *B. cinerea.***

138 Metabolization of capsidiol and rishitin by *B. cinerea* were evaluated by LC/MS.
139 Under the experimental conditions of this study, capsidiol was metabolized to
140 capsenone by dehydrogenation within 12 h, and oxidized capsenone was detected
141 at 24 h, whereas oxidized capsidiol was not detected (Fig. 1C and Supplemental
142 Figs. S4). In contrast, rishitin was directly oxidized within 6 h and at least four
143 different forms of oxidized rishitin were detected within 24 h (Fig. 1C and
144 Supplemental Figs. S5), indicating that despite the structural similarity of these
145 phytoalexins, *B. cinerea* detoxifies/metabolizes capsidiol and rishitin by different
146 mechanisms (Fig. 1C).
147

148 **Unique sets of genes are upregulated in *B. cinerea* treated with capsidiol,**
149 **rishitin or resveratrol.**

150 To identify *B. cinerea* genes upregulated during the detoxification of
151 sesquiterpenoid phytoalexins, RNAseq analysis was performed for mycelia of *B.*
152 *cinerea* cultured in minimal media supplemented with capsidiol or rishitin. A
153 stilbenoid phytoalexin, resveratrol, produced in grape was also used for

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

154 comparison. Mycelia of *B. cinerea* were grown in liquid CM media supplemented
155 with either 500 μM rishitin, 500 μM resveratrol, or 100 μM capsidiol, as 500 μM
156 capsidiol caused significant growth defects in *B. cinerea* (Fig. 1A, Supplemental
157 Fig. S6). The mycelial tissue was then used to perform an RNAseq analysis.
158 Among 11,707 predicted *B. cinerea* genes (25), 25, 27 or 23 genes were
159 significantly upregulated (Log2 fold change >2, p <0.05) by the treatment with
160 capsidiol, rishitin or resveratrol, respectively. Unexpectedly, distinctive sets of
161 genes were upregulated even between *B. cinerea* treated with capsidiol and rishitin
162 which resemble each other structurally (Fig. 2A, Supplemental Table S1-3),
163 indicating that *B. cinerea* can either distinguish the structural difference of capsidiol
164 and rishitin or the damage caused by these sesquiterpenoid phytoalexins. For
165 instance, two genes Bcin08g00930 and Bcin12g01750 encoding hypothetical
166 proteins containing a motif for dehydrogenases were specifically induced by
167 capsidiol, while the treatment of rishitin significantly induced Bcin07g05430,
168 encoding a cytochrome P450 gene (Fig. 2B). Expression of *BcatrB* encoding an
169 ABC transporter involved in the tolerance of *B. cinerea* against structurally
170 unrelated phytoalexins resveratrol, camalexin and fungicides fenpiclonil and
171 fludioxonil (26, 27), is upregulated by treatment with rishitin and resveratrol, but
172 not by capsidiol. In contrast, Bcin15g00040, encoding a major facilitator
173 superfamily (MFS)-type transporter was upregulated specifically by capsidiol (Fig.
174 2B). Interestingly, treatment of *B. cinerea* with phytoalexins also activated genes
175 predicted to be involved in pathogenicity to plants. For example, capsidiol
176 treatment induced expression of a hydrophobin gene Bcin06g00510.1 (Bhp3),
177 while rishitin treatment activated the expression of Bcin01g00080.1 (Bcboa8),
178 encoding an enzyme for biosynthesis of a phytotoxin, botcinic acid (Supplemental
179 Tables S1 and S2). Given that capsidiol is metabolized to capsenone by a
180 dehydrogenation reaction in *B. cinerea* (Fig. 1C, Supplemental Fig. S4), the
181 function of Bcin08g00930 and Bcin12g01750, both encoding a predicted short-
182 chain dehydrogenase/reductase (SDR) were further analyzed in this study.
183

184 **Bcin08g00930 encodes a short-chain dehydrogenase for the detoxification**
185 **of capsidiol.**

186 To investigate the function of SDR genes induced by capsidiol treatment, an
187 endophytic fungus *Epichloë festucae* was employed for the heterologous
188 expression of these genes. Bcin08g00930 and Bcin12g01750 were expressed in
189 *E. festucae* under the control of the constitutive *TEF* promoter (28). The growth of
190 wild type and control *E. festucae* expressing DsRed was severely inhibited in the
191 100 μM capsidiol (Fig. 3A and B). *E. festucae* transformants expressing
192 Bcin12g01750 also hardly grew in 100 μM capsidiol, however, *E. festucae* became
193 tolerant to capsidiol by the expression of Bcin08g00930 (Fig. 3A and B). The
194 amount of capsidiol was not altered by wild type, DsRed or Bcin12g01750
195 expressing *E. festucae*, while capsidiol was metabolized to capsenone by the *E.*
196 *festucae* transformant expressing Bcin08g00930 (Fig. 3C). These results indicated
197 that Bcin08g00930 encodes a dehydrogenase that can detoxify capsidiol, thus the

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

198 SDR encoded by Bcin08g00930 was designated as BcCPDH standing for *B.*
199 *cinerea* capsidiol dehydrogenase.
200

201 **Expression of *Bccpdh* Is specifically induced by capsidiol.**

202 To further investigate the expression pattern of *Bccpdh*, *B. cinerea* was
203 transformed with a reporter construct for GFP expression under the control of the
204 1 kb proximal *Bccpdh* promoter (P_*Bccpdh:GFP*). P_*Bccpdh:GFP* transformants
205 showed no obvious expression of GFP in water, but a significant increase of GFP
206 fluorescence was detected in 500 μM capsidiol (Fig. 4A). The P_*Bccpdh:GFP*
207 transformant was incubated with other anti-microbial terpenoids produced by
208 Solanaceae species, including rishitin, debneyol (29), sclareol (30) and capsidiol
209 3-acetate (31). Among the terpenoids tested, treatment of capsidiol and capsidiol
210 3-acetate induced expression of GFP in the P_*Bccpdh:GFP* transformant. This
211 result further indicated that *B. cinerea* specifically reacts to capsidiol and its
212 derivative capsidiol 3-acetate. As the *Bccpdh* promoter was activated by capsidiol
213 3-acetate, we investigated whether BcCPDH could metabolize capsidiol 3-acetate
214 to capsenone 3-acetate. However, capsidiol 3-acetate was not metabolized by *E.*
215 *festucae* expressing *Bccpdh* (Bcin08g00930) (Supplemental Fig. S7A).
216 Consistently, capsidiol 3-acetate was directly oxidized by *B. cinerea* and at least
217 two different forms of oxidized capsidiol 3-acetate, but not capsenone 3-acetate,
218 were detected (Supplemental Fig. S7B), indicating that capsidiol and capsidiol 3-
219 acetate were metabolized in *B. cinerea* by distinctive pathways.

220 Analysis of different lengths of *Bccpdh* promoters indicated that 250 bp of
221 promoter sequence are sufficient for specific activation of the promoter by capsidiol
222 treatment, and the cis-element required for capsidiol-specific expression was
223 shown to be within -250 and -200 bp upstream of the start codon (Supplemental
224 Figs. S8 and 9). By further analysis using *B. cinerea* transformant P_*Bccpdh:Luc*
225 using luciferase as a quantitative marker, it was shown that *Bccpdh* promoter was
226 activated within 2 h of capsidiol treatment, and the expression activity decreased
227 as capsidiol was metabolized (Supplemental Figs. S10, Supplementary Notes 3).
228

229 **Expression of *Bccpdh* is specifically induced during the infection of**
230 **capsidiol producing plant species.**

231 Leaves of *Nicotiana benthamiana*, which produce capsidiol as the major
232 phytoalexin (13), were inoculated with conidia of the *B. cinerea* transformant
233 P_*Bccpdh:GFP*. No GFP signal was detected in germinating *B. cinerea* conidia
234 grown on the surface of *N. benthamiana* leaves 2 h after the inoculation, but
235 obvious GFP expression was detected in the appressoria at 8 h after the
236 inoculation (Fig. 4B). Growing hyphae in the leaf tissue of *N. benthamiana* showed
237 GFP fluorescence, and the intensity of GFP fluorescence in hyphae was stronger
238 near the edge of the lesion compared with that of hyphae growing in the areas of
239 dead tissue within the lesions (Fig. 4B), probably because capsidiol was eventually
240 detoxified in these areas, which are heavily infected with *B. cinerea*.

241 To examine the possibility whether polyxenous *B. cinerea* activates *Bccpdh* for
242 detoxification of other phytoalexin or anti-microbial compounds produced in other

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

243 plant species, the *B. cinerea* P_*Bccpdh:GFP* transformant was inoculated on a
244 wide variety of plants. Over 50 plant species were used for the inoculation test
245 including 9 Solanaceae, 6 Brassicaceae, 6 Rosaceae, 5 Fabaceae and 6
246 Asteraceae plants, and development of disease symptoms was observed in all
247 tested plant species. Among the tested plants, expression of GFP under the control
248 of *Bccpdh* promoter was only detected during the infection in three *Nicotiana* and
249 two *Capsicum* species (Fig. 4C, Supplemental Fig. S11 and Table S4), all of which
250 are reported to produce capsidiol (8, 32–34). These results further indicated that
251 *B. cinerea* specifically recognizes capsidiol for the induction of *Bccpdh*.
252
253 **BcCPDH metabolizes and detoxifies capsidiol to capsenone in *B. cinerea*.**
254 *B. cinerea Bccpdh* KO mutant (*Δbccpdh*) was produced to investigate the function
255 of BcCPDH (Supplemental Fig. S12). Mycelia of *B. cinerea* wild type and *Δbccpdh*
256 were incubated with capsidiol and the metabolites were detected by LC/MS. While
257 capsidiol was metabolized to capsenone in the wild type strain, most of the
258 capsidiol remained unmetabolized two days after incubation with the *Δbccpdh*
259 strain (Fig. 5A). Instead, oxidized capsidiol, which was not detectable in the wild
260 type strain, was detected in *Δbccpdh* incubations (Supplemental Fig. S13 and
261 Supplementary Notes 4). While the growth of *B. cinerea* hyphae was not affected
262 by the disruption of the *Bccpdh* gene in 100 μM capsidiol, growth of *Δbccpdh* was
263 diminished compared with that of wild type *B. cinerea* at higher concentrations of
264 capsidiol (Fig. 5B). These results confirmed that BcCPDH is the enzyme
265 responsible for the detoxification of capsidiol in *B. cinerea*.
266
267 **Epoxidation of capsidiol and capsenone is mediated via Bcin16g01490.**
268 The incubation of the *Δbccpdh* knockout strain in capsidiol revealed the presence
269 of a potential secondary capsidiol degradation pathway, which resulted in the
270 accumulation of oxidized capsidiol. Since we were unable to find any indication for
271 such a pathway in our RNAseq results for 100 μM capsidiol treated incubations,
272 we extended our search to a preliminary RNAseq analysis which used 500 μM
273 capsidiol treatments. We identified the gene Bcin16g01490 encoding a
274 cytochrome P450, which was significantly upregulated under these elevated
275 capsidiol concentrations (Supplemental Table S1 and Fig. S14A). Heterologous
276 expression of Bcin16g01490 in *E. festucae*, resulted in the conversion of capsidiol
277 to oxidized capsidiol as detected during *Δbccpdh* strain incubations (Supplemental
278 Figs. S13 and 14, Supplementary Notes 4). Moreover, sequential incubation of
279 capsidiol with the *Bccpdh* expressing *E. festucae* transformant followed by a
280 Bcin16g01490 expressing transformant reproduced the reactions detected in *B.*
281 *cinerea* (Supplemental Figs. S4 and S14B). Structural analysis of oxidized
282 capsenone indicated that the end product of these reactions is capsenone 11,12-
283 epoxide (Fig. 1 and Supplemental Fig. S15-17, Supplementary Notes 5).
284

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

285 **BcCPDH is required for the pathogenicity of *B. cinerea* on plant species**
286 **producing capsidiol**
287 Pathogenicity of *B. cinerea Δbccpdh* transformants was tested for several plant
288 species. On plant species that produce capsidiol, such as *N. benthamiana*, *N.*
289 *tabacum* and *C. annuum*, the development of disease symptoms by *Δbccpdh* was
290 significantly reduced compared with those caused by wild type *B. cinerea*. In
291 contrast, both wild type and *Δbccpdh* strains caused comparable symptoms in
292 potato, tomato, grape and eggplant, which is consistent with the finding that
293 expression of *Bccpdh* is not induced during the infection in these plant species
294 (Fig. 5C, Supplemental Fig. S18). In the complementation strain, the ability to
295 metabolize capsidiol to capsenone was restored, as was virulence against
296 capsidiol producing plants (Supplemental Fig. S19). These results indicated that
297 BcCPDH is a dedicated enzyme for the virulence of *B. cinerea* on capsidiol-
298 producing plant species.
299
300 **Distribution of *Bccpdh* homologues in the fungal kingdom**
301 The distribution of *Bccpdh* homologs in taxonomically related fungal species
302 (Ascomycota, Leotiomycetes) was investigated. A search for *Bccpdh* homologs in
303 the genome sequences of 6 host-specialized phytopathogenic *Botrytis* species,
304 such as *B. tulipae* (pathogen of tulip), *B. hyacinthi* (hyacinth) and *B. paeoniae*
305 (peony), indicated that among *Botrytis* species, *Bccpdh* is a unique gene only
306 found in *B. cinerea* (Supplemental Fig. S20). Consistently, 4 *Botrytis* species, not
307 including *B. cinerea*, were incubated with capsidiol, but no CPDH activity was
308 detected for the tested strains (Supplemental Fig. S21). Search in 11 draft
309 genomes of Leotiomycete fungi also indicated the absence of *Bccpdh* homologs
310 in these species. Comparison of the corresponding genome regions between
311 *Botrytis* species and *S. sclerotiorum* (another polyxenous pathogen belonging to
312 Leotiomycetes) revealed that the approx. 4.9 kb sequence surrounding *Bccpdh* is
313 unique to *B. cinerea* (Fig. 6). This unique sequence shows a lower GC content
314 compared to the surrounding region (Supplemental Fig. S22), suggesting that
315 *Bccpdh* might have been obtained via horizontal gene transfer (35). A blast search
316 using BcCPDH as query sequence revealed that probable orthologs can be found
317 only in some Pezizomycotina fungi belonging to Ascomycota (Supplemental Fig.
318 S20). Orthologs were found from a taxonomically diverse range of fungal species,
319 including animal and insect pathogens, and there was no correlation between their
320 homology and taxonomic relationship, which might indicate multiple horizontal
321 gene transfer events of the *cpdh* gene in the diversification of Ascomycota fungi
322 (Supplemental Figs. 20, 23-25, Supplementary Notes 6). Among plant pathogenic
323 fungi, *Bccpdh* homologs were found in *Fusarium* species, all of which are
324 pathogens that infect plants that do not produce capsidiol. This result is consistent
325 with a previous report showing that some *Fusarium* species can metabolize
326 capsidiol to capsenone (36).
327

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

CPDH activity is conserved among *Botrytis cinerea* strains

Although *B. cinerea* is a polyxenous fungus, it has been reported that different strains of *B. cinerea* exhibit various degrees of virulence on different host plants (37, 38). Hence, we investigated whether BcCPDH is conserved among strains isolated from diverse plant species. Twenty-four *B. cinerea* strains isolated from 14 different plant species were incubated with capsidiol and resultant capsenone was detected by GC/MS. All tested *B. cinerea* strains can metabolize capsidiol to capsenone, indicating that BcCPDH is highly conserved among *B. cinerea* strains even though their (most recent) host was not a producer of capsidiol (Fig. 7A, Supplemental Table S5). Similarly, 17 strains of *F. oxysporum* isolated from 7 plant species were subjected to the analysis for CPDH activity. Eight out of 17 *F. oxysporum* strains showed CPDH activity, and the activity was not related to the natural host of the strains (Fig. 7B, Supplemental Table S6). This result is consistent with the finding that *Bccpdh* orthologues can be found in 5 out of 14 available genomes of *F. oxysporum* (Supplemental Fig. S25, data not shown). These results indicate that the cpdh gene is randomly distributed in *F. oxysporum* strains, whereas it is highly conserved among *B. cinerea* strains.

Discussion

To survive the exposure to microorganisms in the environment, different plant species have developed diverse resistance mechanisms over the course of evolution. The structural variety of phytoalexins is a prime example of such diversity: different plant families produce phytoalexins with relatively similar basic structures, but their side-chain structures often differ from species to species (4). These differences in structure between species may have contributed to the determination of host specificity between plants and pathogens, as in some cases a pathogen may have acquired resistance to a particular phytoalexin, but an analogous substance is not overcome by the same resistance mechanism (39). However, plant pathogens with a broad host range such as *B. cinerea* must employ strategies to counter a multitude of diverse phytoalexins. The prompt killing of plant cells, or the presence of efflux pumps of extremely low specificity may present effective strategies for such pathogens. Indeed, *B. cinerea* produces host-nonspecific phytotoxins (such as botrydial and botcinic acid, 40, 41) upon infection, and activates transporters capable of effluxing diverse substances such as PDR-type ABC transporter BcatrB and MFS transporter Bcmfs1 (26, 27, 42). More recently, it has been shown that *B. cinerea* suppresses the immune response of different plant species via gene silencing by delivering various small RNAs into host cells (43, 44).

In addition to effective infection mechanisms which generally facilitate infection of a wide range of plants, this study revealed that *B. cinerea* responds to chemical cues from the host plant to adapt its infection strategy to overcome specific plant resistance mechanisms. *B. cinerea* strictly distinguishes structurally similar phytoalexins, such as capsidiol and rishitin, and activates appropriate detoxification responses. Interestingly, treatment of *B. cinerea* with phytoalexins

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

373 activated not only genes involved in detoxification and efflux of toxic compounds,
374 but also genes predicted to be involved in pathogenicity to plants. These results
375 support the notion that *B. cinerea* utilizes phytoalexins as a means to identify a
376 given host and adjust the method of infection.
377 Expression of the *Bccpdh* gene is activated specifically during infection of
378 capsidiol-producing plants, and the pathogenicity of the *bccpdh* mutant strains is
379 compromised on plants producing capsidiol, but does not suffer any disadvantages
380 on plants that do not produce capsidiol. These results suggest that BcCPDH is a
381 critical component that specifically enables *B. cinerea* to infect capsidiol producing
382 plants. Despite capsidiol producing plants representing only a small fraction of the
383 over 400 host plants of *B. cinerea*, CPDH activity was maintained in all investigated
384 *B. cinerea* strains isolated from plants that do not produce capsidiol. This may hint
385 at the presence of a selection pressure against the loss of CPDH, despite it only
386 affecting a limited number of host plants. This poses the question of whether *B.*
387 *cinerea* is able to maintain acquired resistances for a prolonged time, which may
388 explain how it evolved and establish itself as the polyxenous pathogen that it is.
389

390 **How does *B. cinerea* recognize phytoalexins with similar structures?**

391 Although *B. cinerea* is known to have the ability to metabolize a wide variety of
392 phytoalexins (18), only a small portion of these detoxification mechanisms are
393 required for the infection of any particular plant. Thus, *B. cinerea* needs to strictly
394 control those various detoxification mechanisms, since maintaining sufficient
395 levels of all these detoxification mechanisms represents a considerable waste of
396 resources. Elucidating the mechanism by which *B. cinerea* recognizes
397 phytoalexins and activates a specific set of genes is one of the important subjects
398 for further research. Two major scenarios can be envisioned: first, *B. cinerea* may
399 possess receptors that can identify the chemical structures of phytoalexins. In
400 plants, various terpenes with diverse structures are employed as hormones
401 recognized by specific receptors, such as gibberellins, cytokinins, and abscisic
402 acid (39). However, the possibility that *B. cinerea* maintains receptors to perceive
403 all of the myriad of phytoalexins may not be realistic. The second scenario would
404 be that *B. cinerea* recognizes the damage caused by phytoalexins. However, as
405 for capsidiol and rishitin, both are presumed to have cell membranes as their
406 targets, and these phytoalexins are relatively unspecific toxicants that cause
407 damage even to plant cells (5, 13, 17, 45), so it is not certain whether there exists
408 a specific target by which these two compounds can be distinguished. Given that
409 some genes, such as *BcatrB* encoding a multidrug resistance pump, are commonly
410 induced by structurally unrelated phytoalexins and fungicides treatments (26, 27),
411 *B. cinerea* may possess both induction mechanisms. Using the reporter system
412 developed in this study, mutant strains defective in capsidiol response could be
413 isolated to elucidate the mechanism by which *B. cinerea* distinctly identifies
414 phytoalexins.
415

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

416 **Does *B. cinerea* possess other capsidiol resistance mechanism besides**
417 **detoxification by BcCPDH?**
418 Although the *bccpdh* knockout mutants showed reduced virulence on plant
419 species producing capsidiol, the mutant can develop the disease symptom on
420 these plants and showed tolerance to 100 µM capsidiol, same as the wild type. In
421 contrast, *Phytophthora* spp., *Alternaria* spp. and *E. festucae* are sensitive to the
422 same concentration of capsidiol. Although we also isolated Bcin16g01490, which
423 we found to oxidize capsidiol, its activity is fairly limited, indicating that it is unlikely
424 to confer sufficient protection against capsidiol. We therefore presumed that *B.*
425 *cinerea* has another mechanism for capsidiol tolerance other than detoxification.
426 RNAseq analysis of *B. cinerea* genes upregulated by capsidiol treatment identified
427 2 genes (Bcin15g00040.1 and Bcin14g02870.1/Bcmfs1) encoding MFS
428 transporters and a gene (Bcin01g05890.1/Bcbmr1) encoding an ABC transporter
429 (Supplemental Table S1). Bcmfs1 has been reported to be involved in the
430 resistance of *B. cinerea* to structurally different natural toxicants (camptothecin
431 produced by the plant *Camptotheca acuminata* and cercosporin produced by the
432 plant pathogenic fungus *Cercospora kikuchii*) and fungicides (sterol demethylation
433 inhibitors, DMIs) (42). The *bcbmr1* mutants showed an increased sensitivity to
434 fungicides, polyoxin (a chitin synthetase inhibitor) and iprobenfos (a choline
435 biosynthesis inhibitor) (46). The expression of one or more of these transporters
436 may be involved in capsidiol efflux from *B. cinerea* cells, and regulated by signaling
437 that is common to *Bccpdh*.
438
439 **Why and how can *B. cinerea* strains stably maintain BcCPDH?**
440 CPDH activity was detected in all tested *B. cinerea* strains isolated from plants
441 that do not produce capsidiol, and the *Bccpdh* gene is conserved in the genomes
442 of published *B. cinerea* strains. In *F. oxysporum*, in contrast, there were strains
443 with and without CPDH activity regardless of the host plant, and consistently, some
444 publicly available *F. oxysporum* genomes contain *cpdh* homologs and others do
445 not. As shown in this study, metabolic capacity (and tolerance) to capsidiol and
446 rishitin can be detected in some fungal strains regardless of the natural host of
447 these pathogens, and perhaps unused detoxification activities are maintained in
448 the population of phytopathogenic filamentous fungi for future use.
449 It is theoretically implausible that all *B. cinerea* strains maintain an enzyme
450 required only upon infection of capsidiol-producing plants, even if said gene is
451 completely repressed in its expression under normal circumstances. Given that *B.*
452 *cinerea* has an extremely wide host range, some strains will go for long periods
453 where capsidiol detoxification ability is not a selection pressure. One possibility is
454 that BcCPDH has functions other than capsidiol degradation. For example,
455 BcCPDH might be involved in the detoxification of antimicrobial substances
456 produced by insects, because it has been reported that spores of *B. cinerea* are
457 transmitted between plants via insects such as thrips (47). Since homologous
458 genes for *Bccpdh* are found in several insect-infecting fungi, it is expected that
459 CPDH-like enzymes in these species can metabolize insect-derived substances.
460 We, therefore, performed preliminary experiments to determine whether *Bccpdh*

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

461 expression can be induced by inoculation of *B. cinerea* P_*Bccpdh:GFP*
462 transformant with several insects, but to date, no induction of GFP expression has
463 been detected (data not shown). Alternatively, *B. cinerea* may have acquired the
464 trait of having many hosts because of its ability to maintain rarely used virulence
465 factors. Future clarification of the phytoalexin recognition mechanisms and
466 comparative analysis of the genome with that of closely related *Botrytis* species
467 are anticipated to elucidate unknown features of *B. cinerea* that have led to its
468 evolution as a polyxenous fungi.
469

470 **Materials and Methods**
471

472 Comprehensive descriptions of the materials and methods used in this study,
473 including, biological materials, vector construction, transformation of *E. festucae*
474 and *B. cinerea*, confocal microscopy, pathogenicity tests and detection and
475 structural analysis of phytoalexins and their derivatives, are available in
476 Supplemental data.
477

478 **Data Availability**
479

480 RNA-seq data reported in this work are available in GenBank under the accession
481 number DRA013980.
482

483 **Acknowledgments**
484

485 We thank Prof. Barry Scott (Massey University, New Zealand) for providing *E.*
486 *festucae* strain Fl1 and critical reading of the manuscript. We also thank Dr. David
487 Jones (The Australian National University, Australia) for *N. benthamiana* seeds,
488 Ms. Kayo Shirai (Hokkaido Central Agricultural Experiment Station, Japan), and
489 Dr. Seishi Akino (Hokkaido University, Japan) for providing *P. infestans* isolate
490 08YD1, Prof. Takashi Tsuge (Chubu University, Japan) for providing *Fusarium*
491 strains, Dr. Haruhisa Suga for providing *Fusarium graminearum* strain, Mr.
492 Masashi Matsusaki (Aichi Prefectural Agricultural Research Center, Japan) for
493 providing *Fusarium oxysporum* f. sp. *lycopersici* strain, and Mr. Taku Kawakami
494 (Mie Prefecture Agricultural Research Institute, Japan) for providing *B. cinerea*
495 strains. We are also grateful to Prof. Kazuhito Kawakita for valuable suggestions,
496 and Dr. Kenji Asano and Mr. Seiji Tamiya (National Agricultural Research Center
497 for Hokkaido Region, Japan) and Mr. Yasuki Tahara (Nagoya University, Japan)
498 for providing tubers of potato cultivars.
499
500

501 **References**
502

503 1. K. O. Müller, H. Börger, Experimentelle Untersuchungen über die
504 Phytophthora: Resistenz der Kartoffel. *Arb. Biol. Anst. Reichsanst.* **23,** 189–231
505 (1940).

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

506 2. J. Kuc, Phytoalexins, stress metabolism, and disease resistance in plants.
507 *Annu. Rev. Phytopathol.* **33,** 275–297 (1995).
508 3. C. J. Brooks, D. G. Watson, Phytoalexins. *Nat. Prod. Rep.* **2,** 427–459 (1985).
509 4. R. Hammerschmidt, Phytoalexins: What have we learned after 60 years?
510 *Annu. Rev. Phytopathol.* **37,** 285–306 (1999).
511 5. M. Turelli, C. Coulomb, P. J. Coulomb, J. P. Roggero, M. Bounias, Effects of
512 capsidiol on the lipid and protein content of isolated membranes of *Phytophthora*
513 *capsici*. *Physiol. Plant Pathol.* **24,** 211–221 (1984).
514 6. E. E. Rogers, J. Glazebrook, F. M. Ausubel, Mode of action of the *Arabidopsis*
515 *thaliana* phytoalexin camalexin and its role in *Arabidopsis*-pathogen interactions.
516 *Mol. Plant-Microbe Interact.* **9,** 748–757 (1996).
517 7. N. Katsui, *et al.,* The structure of rishitin, a new antifungal compound from
518 diseased potato tubers. *Chem. Commun.* **1,** 43–44 (1968).
519 8. J. A. Bailey, R. S. Burden, G. G. Vincent, Capsidiol: An antifungal compound
520 produced in *Nicotiana tabacum* and *Nicotiana clevelandii* following infection with
521 tobacco necrosis virus. *Phytochemistry* **14,** 597 (1975).
522 9. U. Vögeli, J. Chappell, Induction of sesquiterpene cyclase and suppression of
523 squalene synthetase activities in plant cell cultures treated with fungal elicitor.
524 *Plant Physiol.* **88,** 1291–1296 (1988).
525 10. Y. Shibata, K. Kawakita, D. Takemoto, Age-related resistance of *Nicotiana*
526 *benthamiana* against hemibiotrophic pathogen *Phytophthora infestans* requires
527 both ethylene- and salicylic acid-mediated signaling pathways. *Mol. Plant-Microbe*
528 *Interact.* **23,** 1130–1142 (2010).
529 11. S. Rin, *et al.,* Expression profiles of genes for enzymes involved in capsidiol
530 production in *Nicotiana benthamiana*. *J. Gen. Plant Pathol.* **86,** 340–349 (2020).
531 12. S. Imano, *et al.,* AP2/ERF transcription factor NbERF-IX-33 is involved in the
532 regulation of phytoalexin production for the resistance of *Nicotiana benthamiana*
533 to *Phytophthora infestans*. *Front. Plant Sci.* **12,** 821574 (2021).
534 13. Y. Shibata, *et al.,* The full-size ABCG transporters Nb-ABCG1 and Nb-ABCG2
535 function in pre- and postinvasion defense against *Phytophthora infestans* in
536 *Nicotiana benthamiana*. *Plant Cell* **28,** 1163–1181 (2016).
537 14. Y. He, et al., The Arabidopsis pleiotropic drug resistance transporters PEN3
538 and PDR12 mediate camalexin secretion for resistance to *Botrytis cinerea*. *Plant*
539 *Cell* **31,** 2206–2222 (2019).
540 15. T. Shiraishi, H. Oku, M. Isono, S. Ouchi, The injurious effect of pisatin on the
541 plasma membrane of pea. *Plant Cell Physiol.* **16,** 939–942 (1975).
542 16. DA. Smith, Toxicity of phytoalexins. In Phytoalexins, J.A. Bailey and J.W.
543 Mansfield, eds. (Glasgow/London: Blackie.), 218–252 (1982).
544 17. G. D. Lyon, Evidence that the toxic effect of rishitin may be due to membrane
545 damage. *J. Exp. Bot.* **31,** 957–966 (1980).
546 18. M. S. C. Pedras, P. W. K. Ahiahonu, Metabolism and detoxification of
547 phytoalexins and analogs by phytopathogenic fungi. *Phytochemistry* **66,** 391–411
548 (2005).

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

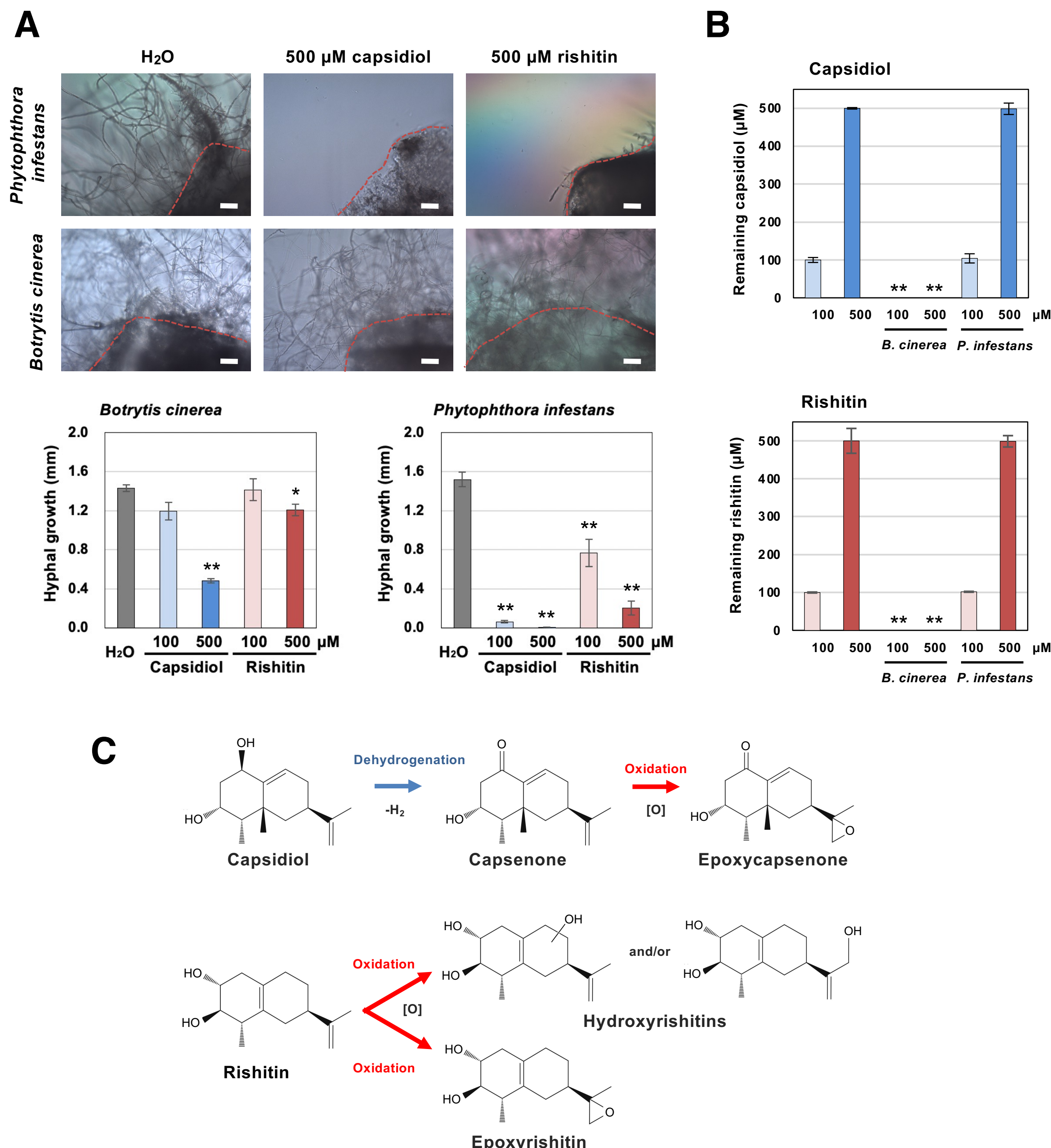
549 19. M. Sbaghi, P. Jeandet, R. Bessis, P. Leroux, Degradation of stilbene-type
550 phytoalexins in relation to the pathogenicity of *Botrytis cinerea* to grapevines. *Plant*
551 *Pathol.* **45,** 139–144 (1996).
552 20. P. Suleman, A. M. Tohamy, A. A. Saleh, M. A. Madkour, D. C. Straney,
553 Variation in sensitivity to tomatine and rishitin among isolates of *Fusarium*
554 *oxysporum* f. sp. *lycopersici*, and strains not pathogenic on tomato. *Physiol. Mol.*
555 *Plant Pathol.* **48,** 131–144 (1996).
556 21. K. M. Weltring, K. Loser, J. Weimer, Genetic instability of rishitin metabolism
557 and tolerance and virulence on potato tubers of a strain of *Gibberella pulicaris*. *J.*
558 *Phytopathol.* **146,** 393–398 (1998).
559 22. C. C. Wasmann, H. D. VanEtten, Transformation-mediated chromosome loss
560 and disruption of a gene for pisatin demethylase decrease the virulence of *Nectria*
561 *haematococca* on pea. *Mol*. *Plant-Microbe Interact.* **9,** 793–803 (1996).
562 23. E. W. B. Ward, A. Stoessl, Postinfectional inhibitors from plants. III.
563 Detoxification of capsidiol, an antifungal compound from peppers. *Phytopathology*
564 **62,** 1186 (1972).
565 24. H. W. Gardner, A. E. Desjardins, S. P. McCormick, D. Weisleder,
566 Detoxification of the potato phytoalexin rishitin by *Gibberella pulicaris.*
567 *Phytochemistry* **37,** 1001–1005 (1994).
568 25. J. A. L. van Kan, et al., A gapless genome sequence of the fungus *Botrytis*
569 *cinerea*. *Mol. Plant Pathol.* **18,** 75–89 (2017).
570 26. H. Schoonbeek, G. del Sorbo, M. A. de Waard, The ABC transporter BcatrB
571 affects the sensitivity of *Botrytis cinerea* to the phytoalexin resveratrol and the
572 fungicide fenpiclonil. *Mol. Plant-Microbe Interact.* **14,** 562–571 (2001).
573 27. T. Vermeulen, H. Schoonbeek, M. A. de Waard, The ABC transporter BcatrB
574 from *Botrytis cinerea* is a determinant of the activity of the phenylpyrrole fungicide
575 fludioxonil. *Pest Manag. Sci.* **57,** 393–402 (2001).
576 28. A. J. vanden Wymelenberg, D. Cullen, R. N. Spear, B. Schoenike, J. H.
577 Andrews, Expression of green fluorescent protein in *Aureobasidium pullulans* and
578 quantification of the fungus on leaf surfaces. *Biotechniques* **23,** 686–690 (1997).
579 29. R. S. Burden, et al., Debneyol, a fungicidal sesquiterpene from TNV infected
580 *Nicotiana debneyi*. *Phytochemistry* **24,** 2191–2194 (1985).
581 30. Z. Guo, G. J. Wagner, Biosynthesis of labdenediol and sclareol in cell-free
582 extracts from trichomes of *Nicotiana glutinosa*. *Planta* **197,** 627–632 (1995).
583 31. R. Uegaki, S. Kubo, T. Fujimori, Stress compounds in the leaves of *Nicotiana*
584 *undulata* induced by TMV inoculation. *Phytochemistry* **27,** 365–368 (1988).
585 32. P.-M. Molot, P. Mas, M. Conus, H. Ferrière, P. Ricci, Relations between
586 capsidiol concentration, speed of fungal invasion and level of induced resistance
587 in cultivars of pepper (*Capsicum annuum*) susceptible or resistant to *Phytophthora*
588 *capsici*. *Physiol. Plant Pathol.* **18,** 379–389 (1981).
589 33. J. Bohlmann, et al., Gene expression of 5-*epi*-aristolochene synthase and
590 formation of capsidiol in roots of *Nicotiana attenuata* and *N. sylvestris*.
591 *Phytochemistry* **60,** 109–116 (2002).
592 34. M. Matsukawa, et al., *Nicotiana benthamiana* calreticulin 3a is required for the
593 ethylene-mediated production of phytoalexins and disease resistance against

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

594 oomycete pathogen *Phytophthora infestans*. *Mol. Plant-Microbe Interact.* **26,** 880–
595 892 (2013).
596 35. M. Ravenhall, N. Škunca, F. Lassalle, C. Dessimoz, Inferring horizontal gene
597 transfer. *PLoS Comput. Biol.* **11,** e1004095 (2015).
598 36. A. Stoessl, C. H. Unwin, E. W. B. Ward, Postinfectional fungus inhibitors from
599 plants: Fungal oxidation of capsidiol in pepper fruit. *Phytopathology* **63,** 1225–1231
600 (1973).
601 37. A. Mercier, et al., The polyphagous plant pathogenic fungus *Botrytis cinerea*
602 encompasses host-specialized and generalist populations. *Environ. Microbiol.* **21,**
603 4808–4821 (2019).
604 38. C. Plesken, et al., Genetic diversity of *Botrytis cinerea* revealed by multilocus
605 sequencing, and identification of *B. cinerea* populations showing genetic isolation
606 and distinct host adaptation. *Front. Plant Sci.* **12,** 663027 (2021).
607 39. E. Pichersky, R. A. Raguso, Why do plants produce so many terpenoid
608 compounds? *New Phytol.* **220,** 692–702 (2018).
609 40. N. Deighton, I. Muckenschnabel, A. J. Colmenares, I. G. Collado, B.
610 Williamson, Botrydial is produced in plant tissues infected by *Botrytis cinerea*.
611 *Phytochemistry* **57,** 689–692 (2001).
612 41. J. L. Reino, R. Hernández-Galán, R. Durán-Patrón, I. G. Collado, Virulence–
613 toxin production relationship in isolates of the plant pathogenic fungus *Botrytis*
614 *cinerea. J. Phytopathol.* **152,** 563–566 (2004).
615 42. K. Hayashi, H. jan Schoonbeek, M. A. de Waard, Bcmfs1, a novel major
616 facilitator superfamily transporter from *Botrytis cinerea*, provides tolerance towards
617 the natural toxic compounds camptothecin and cercosporin and towards
618 fungicides. *Appl. Environ. Microbiol.* **68,** 4996–5004 (2002).
619 43. D. Baulcombe, Plant science. Small RNA - The secret of noble rot. *Science*
620 **342,** 45–46 (2013).
621 44. M. Wang, A. Weiberg, E. Dellota, D. Yamane, H. Jin, Botrytis small RNA *Bc-*
622 *siR37* suppresses plant defense genes by cross-kingdom RNAi. *RNA Biol.* **14,** 421
623 (2017).
624 45. M. Camagna, M. Ojika, D. Takemoto, Detoxification of the solanaceous
625 phytoalexins rishitin, lubimin, oxylubimin and solavetivone via a cytochrome P450
626 oxygenase. *Plant Signal. Behav.* **15,** 1707348 (2020).
627 46. M. Nakajima, J. Suzuki, T. Hosaka, T. Hibi, K. Akutsu, Functional analysis of
628 an ATP-binding cassette transporter gene in *Botrytis cinerea* by gene disruption.
629 *J. Gen. Plant Pathol.* **67,** 212–214 (2001).
630 47. M. Fermaud, R. E. Gaunt, Thrips obscuratus as a potential vector of *Botrytis*
631 *cinerea* in kiwifruit. *Mycol. Res.* **99,** 267–273 (1995).
632

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

633 ## Figures

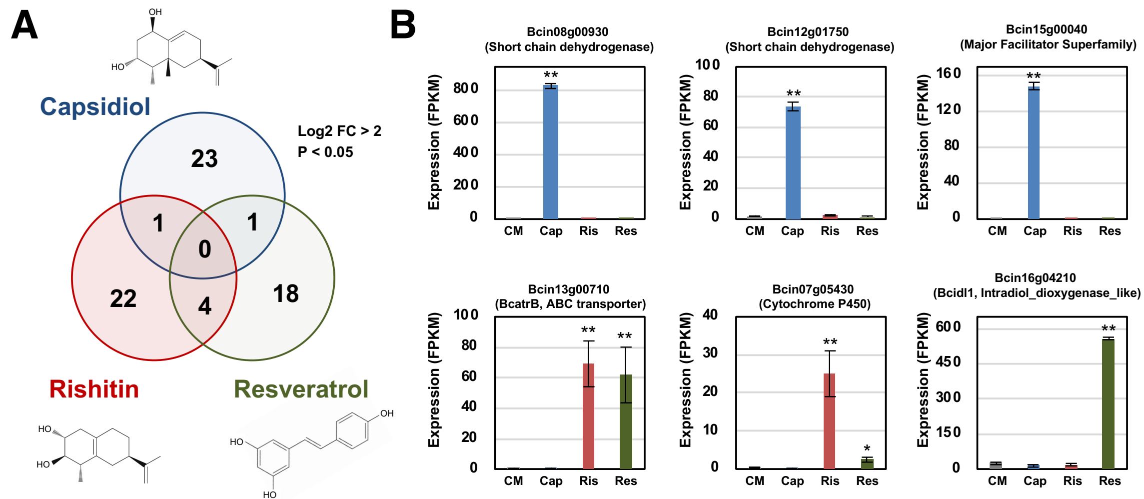


634

635 **Fig. 1** Sensitivities and metabolic capacities of *Botrytis cinerea* and *Phytophthora*
636 *infestans* to sesquiterpenoid phytoalexins.
637 **(A)** Mycelial blocks (approx. 1 mm^3) of indicated pathogen were incubated in 50 μl
638 water, 500 μM capsidiol or 500 μM rishitin. Outgrowth of hyphae from the mycelial
639 block (outlined by dotted red lines) was measured after 24 h of incubation (n = 6).
640 Bars = 100 μm. **(B)** Residual capsidiol and rishitin was quantified by GC/MS 2 days
641 after the incubation. **(C)** Predicted metabolism of capsidiol and rishitin by *B.*
642 *cinerea*. Note that the structure of oxidized capsenone was determined in this

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

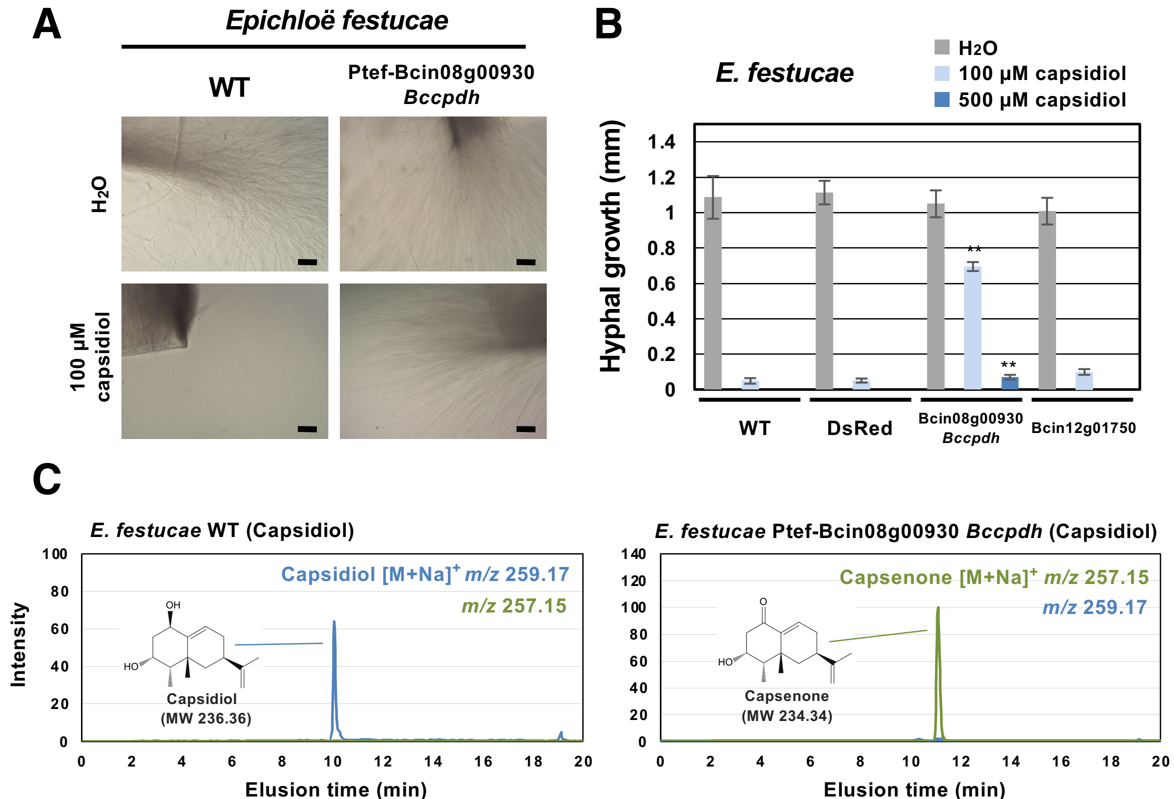
643 study. Data marked with asterisks are significantly different from control as
644 assessed by the two-tailed Student's *t*-test: **P < 0.01, *P < 0.05.



645

646 **Fig. 2** Unique set of genes are upregulated in *Botrytis cinerea* treated with
647 capsidiol, rishitin and resveratrol. **(A)** Venn diagram showing genes upregulated in
648 *B. cinerea* cultured in CM media containing 100 μM capsidiol, 500 μM rishitin or
649 500 μM resveratrol for 24 h. The numbers of significantly upregulated genes by
650 phytoalexin treatment with Log2 FC > 2 compared with control (CM without
651 phytoalexin) and P value < 0.05 are presented. **(B)** Expression profiles of
652 representative genes upregulated by the treatment with capsidiol, rishitin or
653 resveratrol. The gene expression (FPKM value) was determined by RNA-seq
654 analysis of *B. cinerea* cultured in CM media containing 100 μM capsidiol, 500 μM
655 rishitin or 500 μM resveratrol for 24 h. Data are mean ± SE (n = 3). Asterisks
656 indicate a significant difference from the control (CM) as assessed by two-tailed
657 Student's *t*-test, **P < 0.01, *P < 0.05.

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

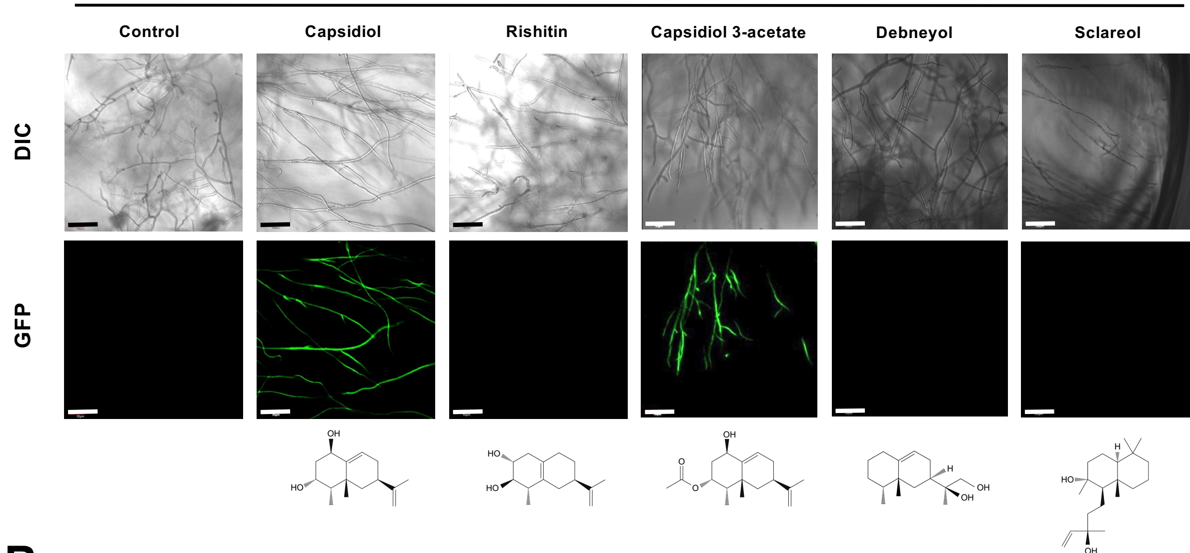


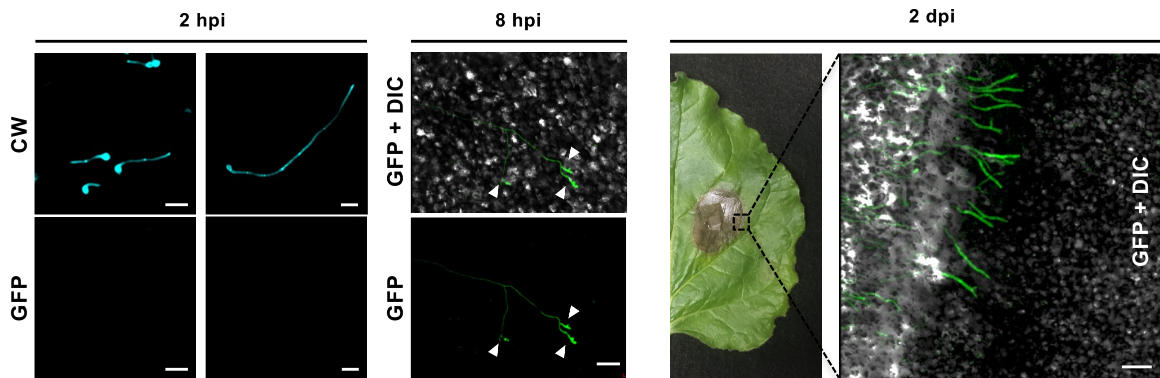
658

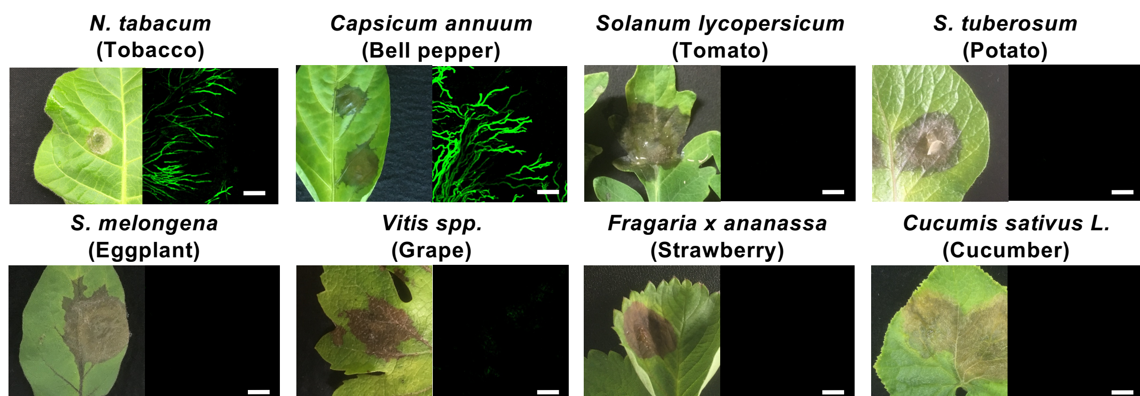
659

660 **Fig. 3** Bcin08g00930 encodes a capsidiol detoxification enzyme, capsidiol
661 dehydrogenase BcCPDH.
662 **(A)** Mycelia of *Epichloë festucae* wild type (WT) or a transformant expressing
663 Bcin08g00930 were incubated in water or 100 µM capsidiol and outgrowth of
664 mycelia was observed 7 days after inoculation. Bars = 100 µm. **(B)** Hyphal
665 outgrowth of *E. festucae* WT, transformants expressing DsRed, Bcin08g00930
666 (BcCPDH) or Bcin12g01750 in water, 100 µM or 500 µM capsidiol was measured
667 after 24 h of incubation. Data are mean ± SE (n = 6). Asterisks indicate a significant
668 difference from WT as assessed by two-tailed Student's *t*-test, $^{**}P < 0.01$. **(C)**
669 Mycelia of *E. festucae* WT or transformant expressing Bcin08g00930 (*Bccpdh*)
670 were incubated in 100 µM capsidiol or for 48 h. Capsidiol and capsenone were
671 detected by LC/MS.
672

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.







673

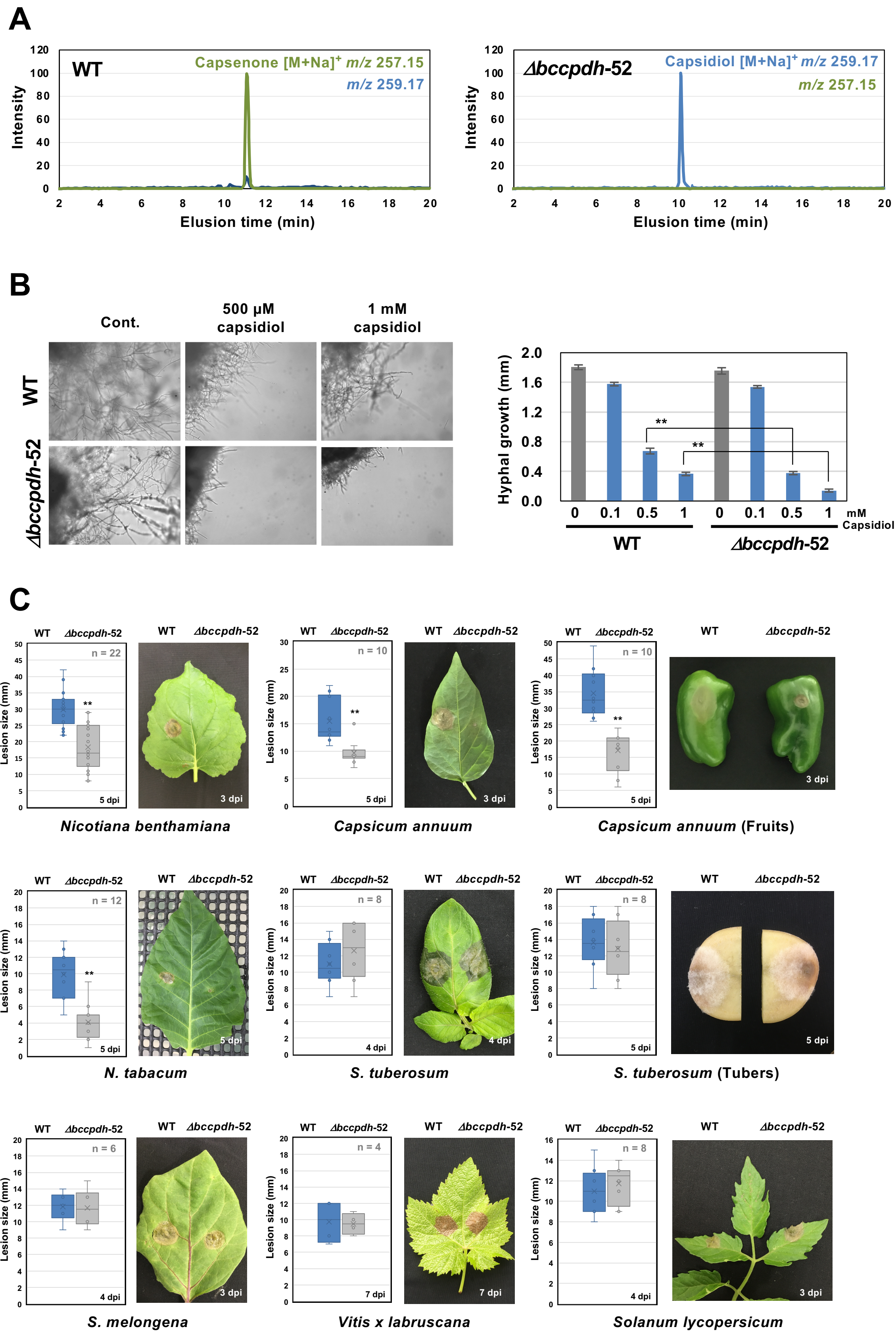
bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

674 **Fig. 4** Specific activation of the *B. cinerea Bccpdh* promoter by capsidiol and its
675 derivative.
676 **(A)** Mycelia of *B. cinerea* transformant containing *GFP* gene under the control of 1
677 kb *Bccpdh* promoter (P_*Bccpdh:GFP*) was incubated in CM media containing 500
678 µM of anti-microbial terpenoids. Bars = 50 µm. **(B)** (left and middle) Leaves of *N.*
679 *benthamiana* were inoculated with conidia of *B. cinerea* P_*Bccpdh:GFP*
680 transformant. Expression of GFP in germinating conidia on the leaf surface was
681 observed by confocal laser microscopy 2 or 8 h after inoculation (hpi). CW, stained
682 with calcofluor white. Arrowheads indicate the appressoria of *B. cinerea.* Bars =
683 20 µm. (right) Leaves of *N. benthamiana* were inoculated with mycelia of *B. cinerea*
684 P_*Bccpdh:GFP* transformant and the edge of the lesion was observed by confocal
685 laser microscopy 2 d after the inoculation (2 dpi). Bar = 100 µm. **(C)** Leaves of
686 indicated plant were inoculated with mycelia of *B. cinerea* P_*Bccpdh:GFP*
687 transformant and the edge of the lesion was observed by confocal laser
688 microscopy 2 d after the inoculation. Bars = 100 µm.

689

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

690

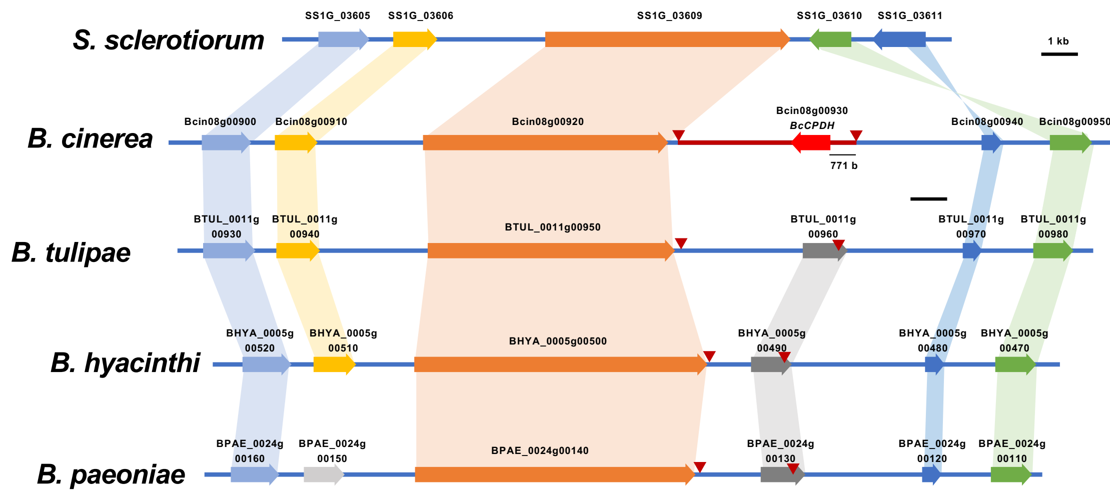


bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

691 **Fig. 5** *B. cinerea* BcCPDH is essential for the detoxification of capsidiol and
692 virulence in the plant species producing capsidiol.
693 **(A)** Mycelial block (approx. 1 mm^3) of *B. cinerea* wild type (WT) or *Bccpdh* KO
694 mutant strain (*Δbccpdh*-52) was incubated in 100 μM capsidiol for 4 d. Capsenone
695 and capsidiol was detected by LC/MS. **(B)** Mycelial blocks of *B. cinerea* WT or
696 *Δbccpdh*-52 were incubated in capsidiol and outgrowth of hyphae was measured
697 after 36 h of incubation. Data are mean ± SE (n = 10). Asterisks indicate a
698 significant difference from WT as assessed by two-tailed Student's *t*-test, **P <
699 0.01. **(C)** Leaves, tubers or fruits of indicated plant were inoculated with mycelial
700 block (5 mm^3) of *B. cinerea* WT or *Δbccpdh*-52 and lesion size was measured
701 between 4 and 7 days after inoculation (dpi). Asterisks indicate a significant
702 difference from WT as assessed by two-tailed Student's *t*-test. **P < 0.01. Lines
703 and crosses (x) in the columns indicate the median and mean values, respectively.

704

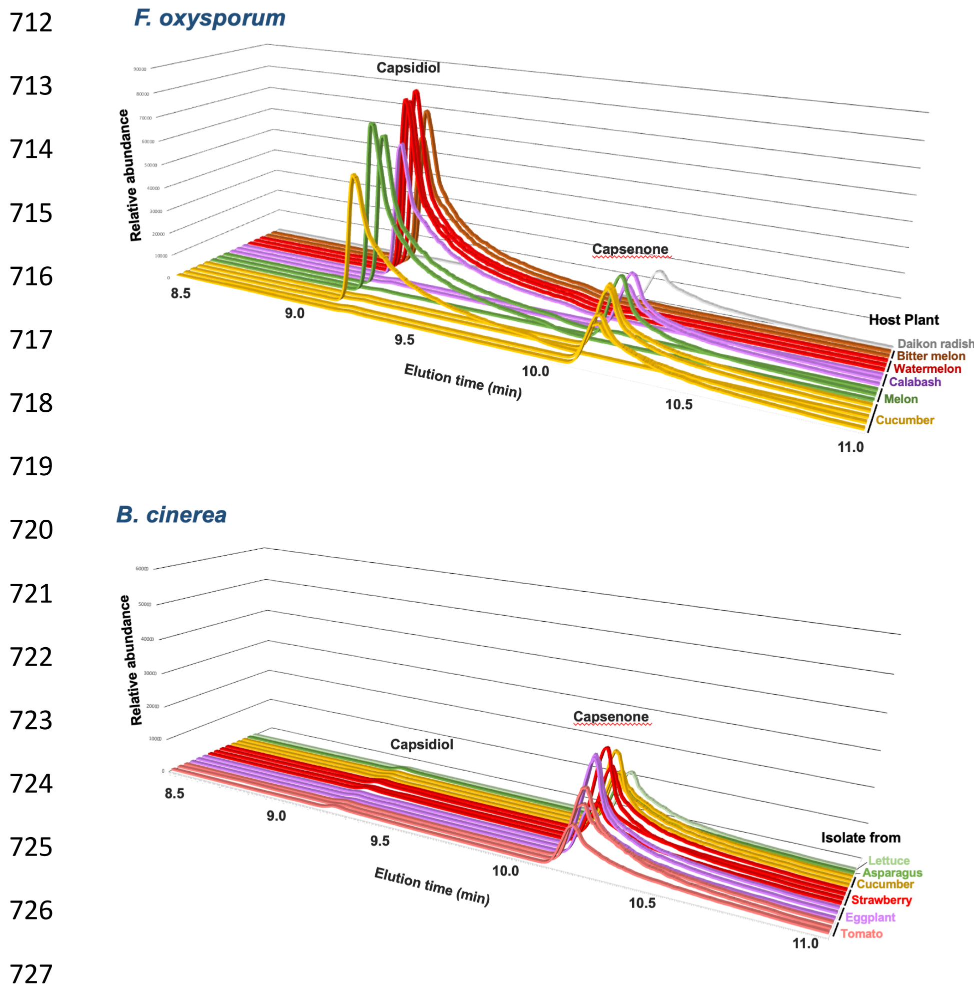
bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



705
706
707 **Fig. 6** Comparison of the *B. cinerea Bccpdh* gene locus with corresponding
708 genome region of other *Botrytis* species and *Sclerotinia sclerotiorum*. Edge of
709 conserved region among *Botrytis species* and specific region for *B. cinerea* (red
710 line) were indicated by red arrowheads.
711

bioRxiv preprint doi: https://doi.org/10.1101/2022.05.11.490027; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727



728 **Fig. 7** CPDH activity in *F. oxysporum* and *B. cinerea* strains isolated form a variety
729 of plants.
730 CPDH activity in *F. oxysporum* and *B. cinerea* strains isolated form a variety of
731 plants. Mycelial blocks (approx. 1 mm^3) were incubated in 500 μM capsidiol for 2
732 days and capsidiol or capsenone were detected by GC/MS. Each elution profile
733 describes the capsidiol and capsenone content in the solution after incubation of
734 the strain with capsidiol. Strains with a substantial peak for capsidiol indicate the
735 absence of CPDH activity.