Supplementary Information for

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

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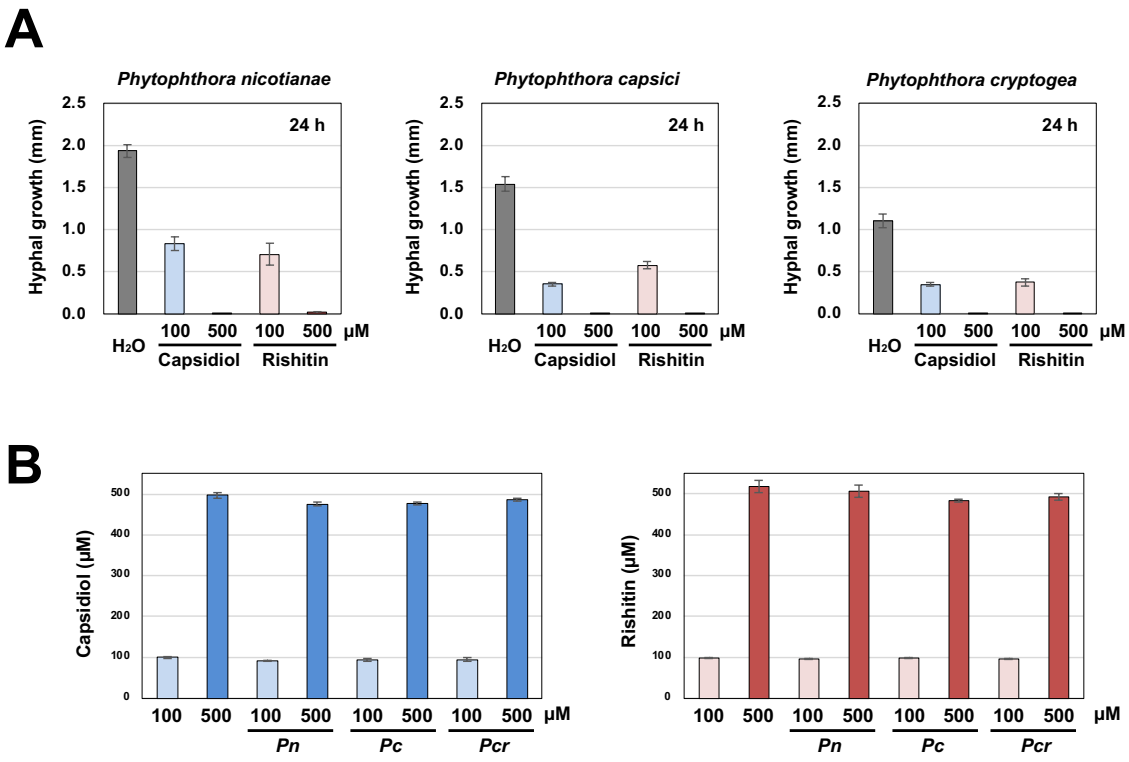

Fig. S1. Sensitivity and metabolic capacity of sesquiterpenoid phytoalexins in oomycete pathogens isolated from Solanaceae plants. **(A)** Mycelial blocks (approx. 1 mm^3) of the indicated pathogen were incubated in 50 µl water, 100 or 500 µM capsidiol or rishitin. Outgrowth of hyphae from the mycelial block was measured after 24 h of incubation (n = 6). **(B)** Residual capsidiol and rishitin was quantified after 48 h of incubation (n = 3). *Pn*, *Phytophthora nicatianae* (strain Pn96 isolated from tobacco); *Pc*, *P. capsici* (strain CH01CMP1 isolated from green pepper); *Pcr*, *P. cryptogea* (strain CH88-18 isolated from nipplefruit).

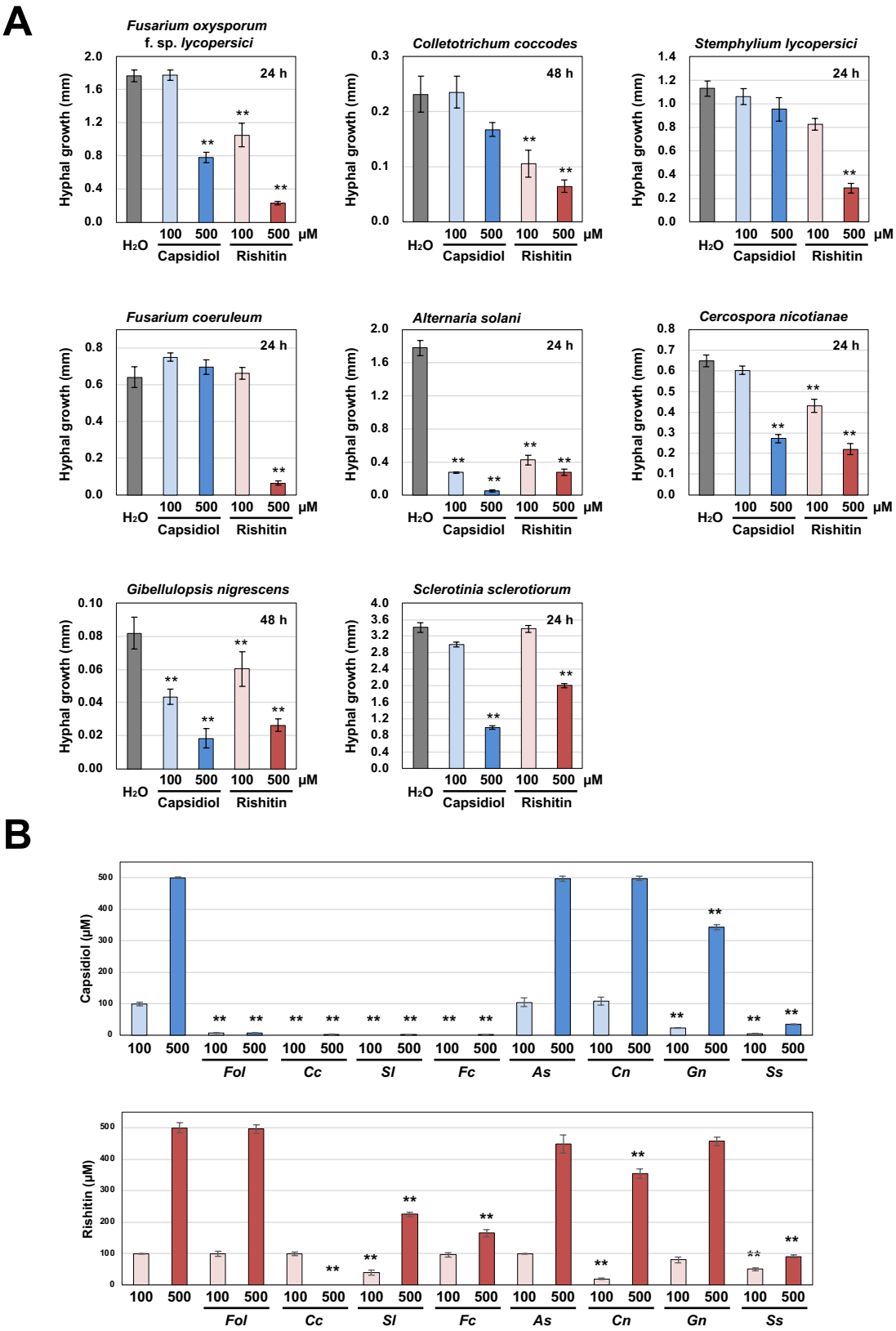

Fig. S2. Sensitivity and metabolic capacity of sesquiterpenoid phytoalexins in fungal pathogens isolated from Solanaceae plants. **(A)** Mycelial blocks (approx. 1 mm^3) of the indicated pathogen were incubated in 50 µl water, 100 µM or 500 µM capsidiol or rishitin. Outgrowth of hyphae from the mycelial block was measured after 24 h or 48 h of incubation (n = 6). **(B)** Residual

capsidiol and rishitin was quantified by GC/MS after 48 h of incubation. Data marked with asterisks are significantly different from control as assessed by the two-tailed Student's *t*-test: $^{**}P < 0.01$. *Fol*, *Fusarium oxysporum* f. sp. *lycopersici* (strain 9855-1 isolated from tomato); *Cc*, *Colletotrichum coccodes* (strain 9855-1 isolated from potato); *Sl*, *Stemphylium lycopersici* (strain KuNBY1 isolated from tobacco); Fc, *F. coeruleum* (strain K. Kita 37 isolated from potato); As, *Alternaria solani* (KL1 isolated from potato); *Cn*, *Cercospora nicotianae* (strain CTC5 isolated from tobacco); *Gn*, *Gibellulopsis nigrescens* (strain Kita44 isolated from potato); *Ss*, *Sclerotinia sclerotiorum* (isolate SU-1 isolated from eggplant).

Supplementary Note 1

Several Fungal Pathogens Isolated from Solanaceae plants can metabolize capsidiol or rishitin.

Fusarium oxysporum f. sp. *lycopersici* (*Fol*), a soilborne plant pathogen causes Fusarium wilt on tomato. *Fol* strain 9855-1 can metabolize capsidiol and showed tolerance to 100 μM capsidiol, while it cannot metabolize rishitin, which is produced by its host plant tomato.

Colletotrichum coccodes (*Cc*) is known to have a wide host range that causes anthracnose on tomato and onion, and black dot disease on potato. *Cc* strain PTK1 (isolated from potato) can metabolize both capsidiol and rishitin, and shows tolerance to 100 μM capsidiol. Metabolism of rishitin was not observed when treated with 100 μM but was induced when 500 μM were used. Thus, rishitin metabolism in *Cc* may be activated when *Cc* is exposed to high concentrations of rishitin.

Stemphylium lycopersici (*Sl*) has been isolated from a broad range of host plants, including tobacco and tomato. *Sl* strain KuNBY1 isolated from tobacco metabolizes both capsidiol and rishitin, and showed tolerance to 100 and 500 μM capsidiol and 100 μM rishitin.

Fusarium coeruleum (*Fc*) is the causal agent of potato dry rot. *Fc* strain K. Kita 37 can metabolize both capsidiol and rishitin, and showed tolerance to 500 μM capsidiol and 100 μM rishitin. Metabolism of rishitin was not observed when treated with 100 μM but induced when 500 μM were used. Similar to *Cc*, the metabolism of rishitin was induced when *Fc* was incubated in 500 μM rishitin.

Alternaria solani (*As*) is the causal pathogen of tomato and potato early blight. *As* strain KL1 isolated from potato can metabolize neither capsidiol nor rishitin, and is sensitive to both phytoalexins.

Cercospora nicotianae (*Cn*) is the pathogen causing tobacco frog-eye leaf spot. Although *Cn* strain CTC5 cannot metabolize capsidiol, it is tolerant to 100 μM capsidiol. *Cn* can partially metabolize rishitin.

Gibellulopsis nigrescens (*Gn*, former *Verticillium nigrescens*) is the causal agent of Verticillium wilt of potato. *Gn* strain kita44 can partially metabolize capsidiol, but didn't show tolerance to capsidiol.

Ss, *Sclerotinia sclerotiorum* (*Ss*) is a polyxenous pathogen causing white mold on a wide range of plant species. *Ss* isolate SU-1 (isolated from eggplant) can metabolize both capsidiol and rishitin, and showed tolerance to 100 μM capsidiol and 100 μM rishitin.

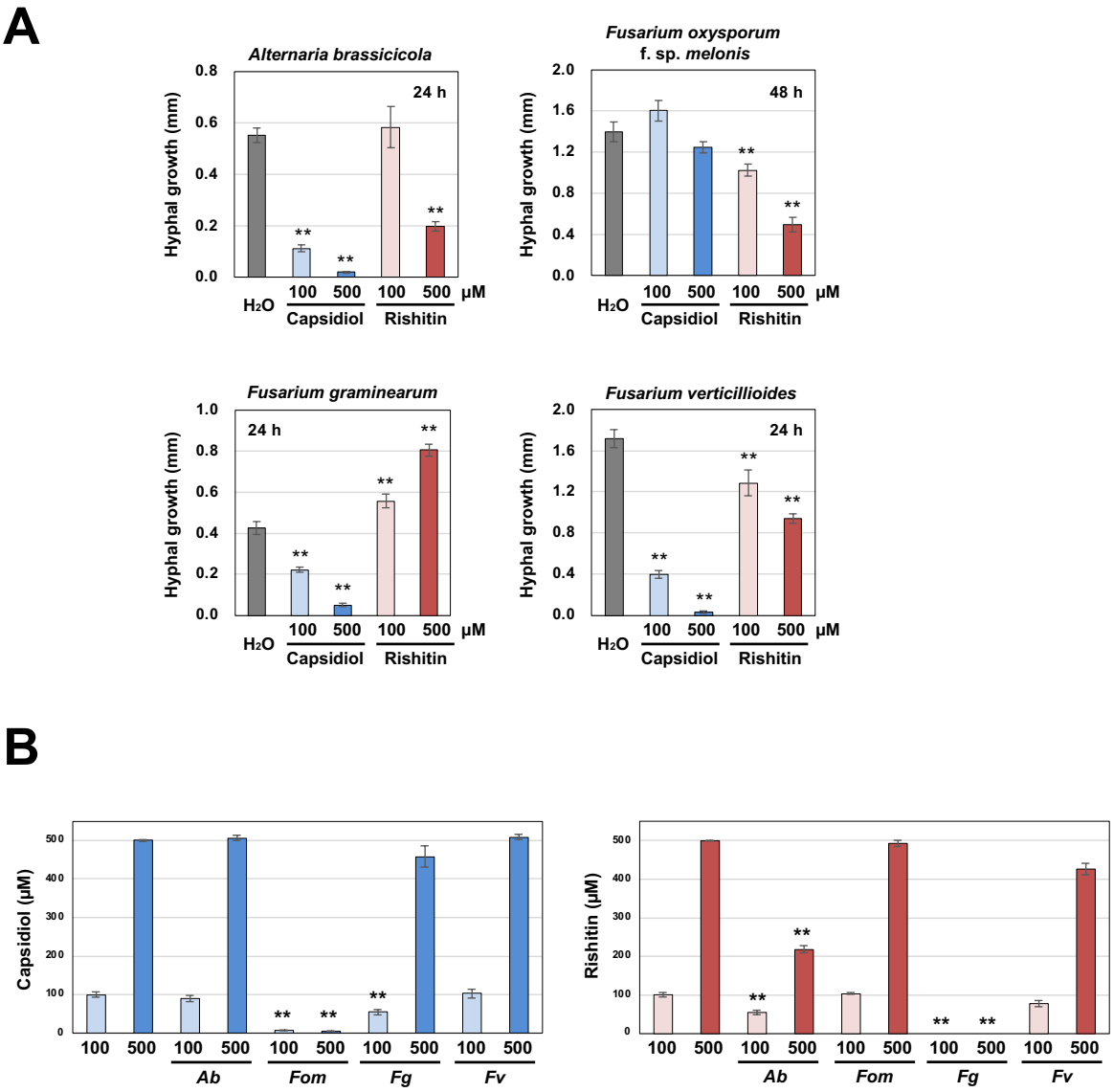

Fig. S3. Sensitivity and metabolic capacity of sesquiterpenoid phytoalexins in fungal pathogens. **(A)** Mycelial blocks (approx. 1 mm^3) of the indicated pathogen were incubated in 50 µl water, 100 µM or 500 µM capsidiol or rishitin. Outgrowth of hyphae from the mycelial block was measured after 24 h incubation (n = 6). **(B)** Residual capsidiol and rishitin was quantified by GC/MS after 48 h of incubation. Data marked with asterisks are significantly different from control as assessed by the two-tailed Student's *t*-test: $^{**}P < 0.01$. *Ab*, *Alternaria brassicicola* (strain BA31 isolated from Broccoli); *Fom*, *Fusarium oxysporum* f. sp. *melonis* (strain Mel02010 isolated from melon); *Fg*, *F. graminearum* sensu stricto (strain 407011 isolated from wheat); Fv, *F. verticillioides* (strain Maize L-2 isolated from maize).

Supplementary Note 2

Several Fungal Pathogens Isolated from Non-Solanaceae Plants Can also Metabolize Capsidiol or Rishitin.

Alternaria brassicicola (*Ab*) is a necrotrophic pathogen that causes black spot disease, particularly on *Brassica* species. *Ab* strain BA31 (isolated from broccoli) can metabolize rishitin and showed tolerance to 100 μM rishitin.

Fusarium oxysporum f. sp. *melonis* (*Fom*) is pathogenic on melon, causing Fusarium wilt. *Fom* strain Mel02010 (isolated from melon, Namiki *et al.* 1994) can metabolize capsidiol and showed tolerance to 100 and 500 μM capsidiol.

Fusarium graminearum sensu stricto (*Fg*) is the causal agent of Fusarium head blight of cereals including barley and wheat. *Fg* strain 407011 (isolated from wheat, Suga *et al.* 2016) cannot metabolize capsidiol, and its growth was inhibited by capsidiol. Notably, in contrast, the growth of *Fg* is significantly enhanced in 100 and 500 μM rishitin and *Fg* strain 407011 can metabolize rishitin, indicating that *Fg* strain 407011 can metabolize and assimilate rishitin.

F. verticillioides (*Fv*) is a major fungal pathogen of cereals, such as wheat, sorghum and maize. Asymptomatic endophytic infection of this fungus in maize is also reported. *Fv* strain Maize L-2 (isolated from maize) cannot metabolize capsidiol and rishitin, and is sensitive to both phytoalexins.

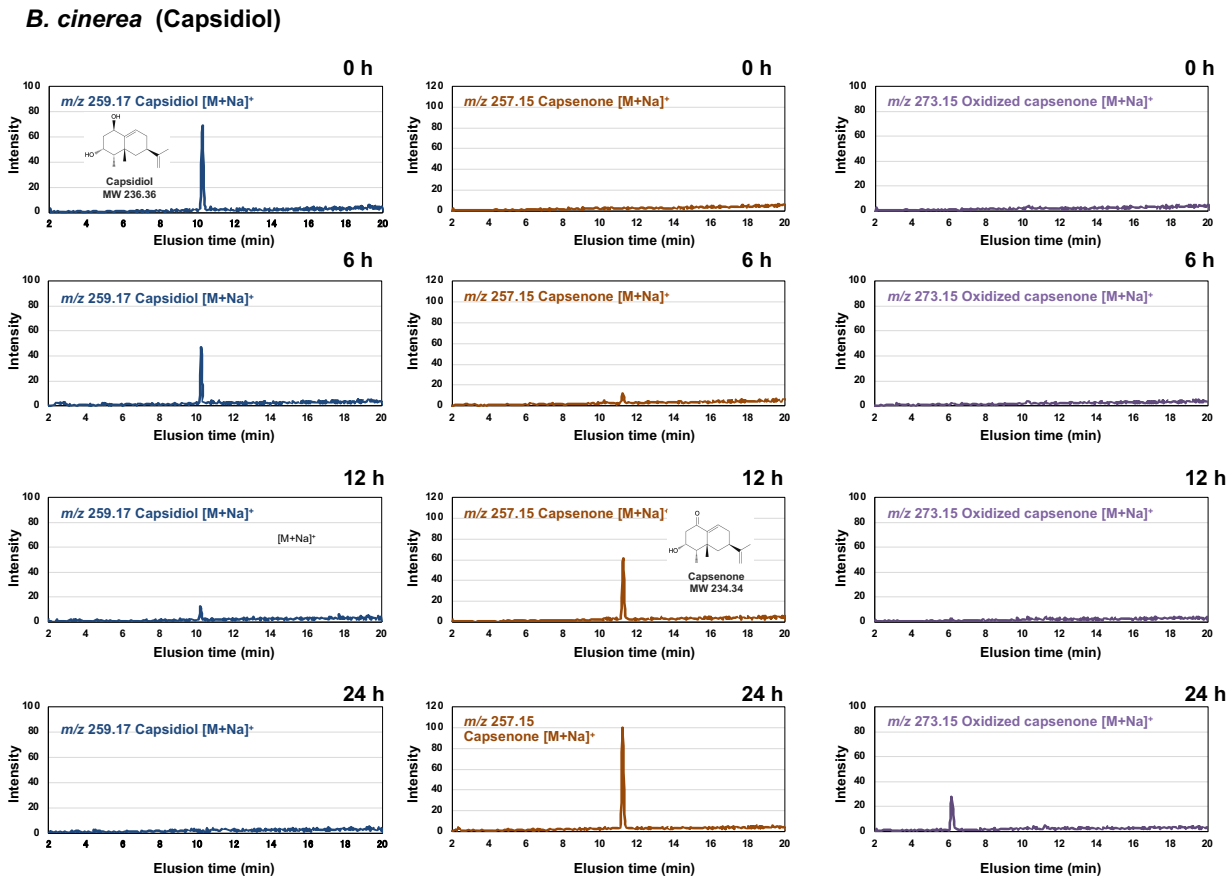

Fig. S4. Metabolism of capsidiol by *Botrytis cinerea*.

Mycelial blocks (approx. 1 mm^3) of *B. cinerea* were incubated in 50 µl of 100 µM capsidiol, and the residual capsidiol and its metabolites were detected by LC/MS.

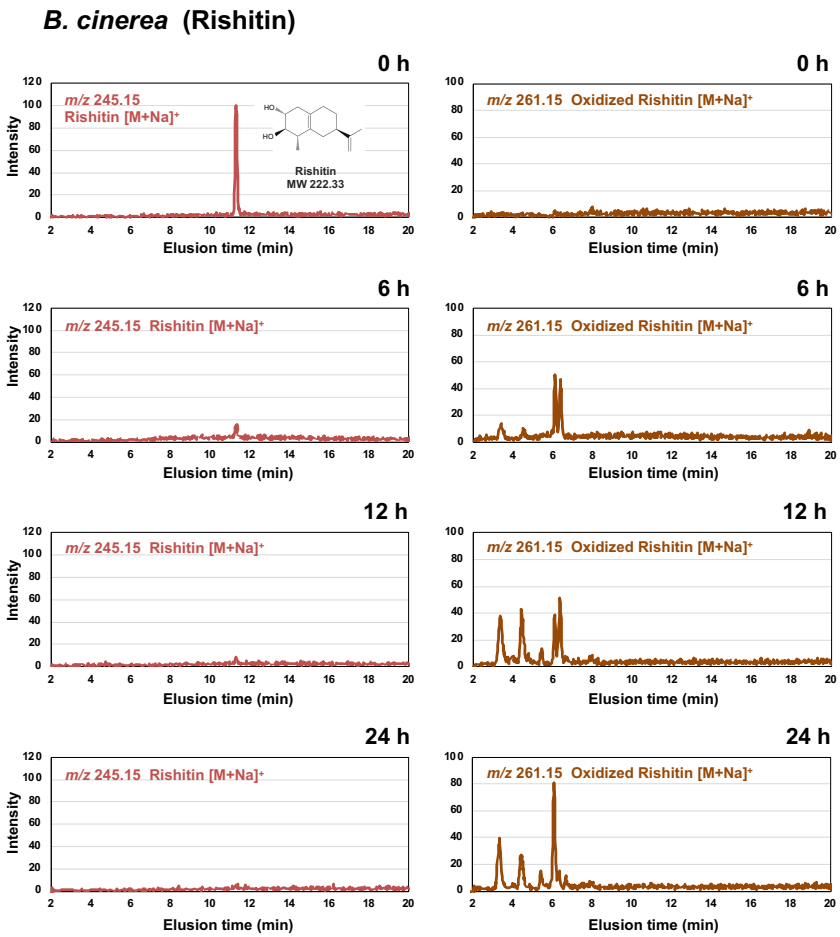

Fig. S5. Metabolism of rishitin by *Botrytis cinerea*.

Mycelial blocks (approx. 1 mm^3) of *B. cinerea* were incubated in 50 µl of 100 µM rishitin, and residual rishitin and its metabolites were detected by LC/MS.

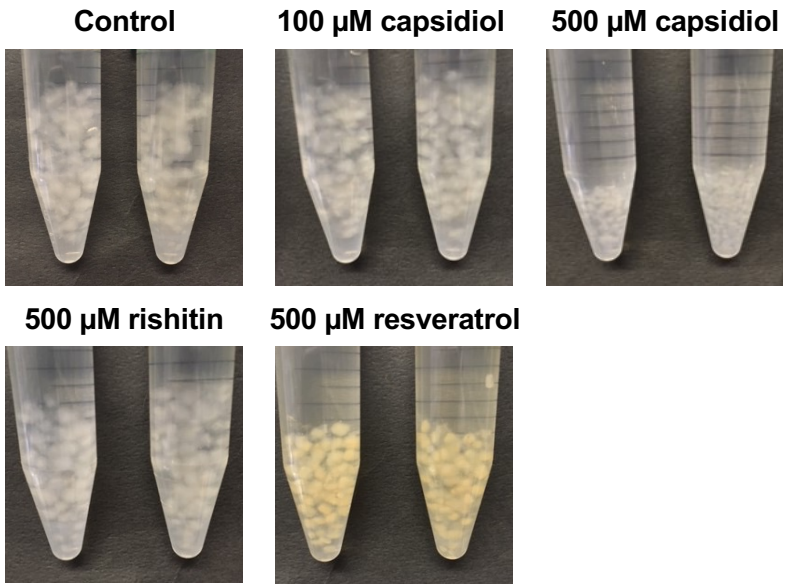

Fig. S6. Mycelial blocks of *B. cinerea* were incubated in CM medium or CM medium containing 100 μM or 500 μM capsidiol, 500 μM rishitin, or 500 μM resveratrol. Images were taken after 24 h of incubation.

Table S1. *Botrytis cinerea* genes significantly upregulated by treatment with capsidiol.

Gene ID	FPKM value (Average)				Log2 fold change (relative to control)						Annotation	Motif
	Control	100 µM Capsidiol	500 µM Rishitin	500 µM Resveratrol	100 µM Capsidiol	P value	500 µM Rishitin	P value	500 µM Resveratrol	P value		
Bcin08g00930.1	0.64	826.91	1.05	0.73	10.34	0.00	0.71	0.29	0.18	0.82	Hypothetical protein	Short chain dehydrogenase; cl27753
Bcin15g00050.1	1.12	398.16	0.87	0.89	8.47	0.00	-0.37	0.13	-0.33	0.39	Bcayt1	Transferase family; cl23789
Bcin15g00040.1	0.47	147.83	0.31	0.38	8.30	0.00	-0.61	0.44	-0.31	0.63	Hypothetical protein	Major Facilitator Superfamily; cd06174
Bcin15g00030.1	0.12	25.49	0.28	0.08	7.72	0.01	1.22	0.27	-0.58	0.72	Hypothetical protein	Protein of unknown function (DUF3237); cl07905
Bcin15g00020.1	0.13	17.32	0.35	0.70	7.04	0.00	1.42	0.42	2.42	0.01	Hypothetical protein	Domain of unknown function (DUF1330); cl22966
Bcin10g03040.1	2.64	225.97	3.19	2.76	6.42	0.00	0.27	0.51	0.06	0.92	Hypothetical protein	Capsular polysaccharide synthesis protein; cl26275
Bcin12g01750.1	1.48	73.95	2.42	1.06	5.64	0.00	0.71	0.18	-0.48	0.53	Hypothetical protein	Classical short-chain dehydrogenases/reductases (SDR); cd05233
Bcin12g01130.1	3.43	76.66	2.80	3.16	4.48	0.00	-0.29	0.41	-0.12	0.78	Hypothetical protein	Cytochrome P450; cl12078
Bcin12g01740.1	0.59	11.39	0.59	0.69	4.27	0.00	0.00	1.00	0.23	0.88	Hypothetical protein	ND
Bcin06g00510.1	0.59	8.89	0.74	2.27	3.91	0.04	0.32	0.29	1.94	0.19	Bhp3	Fungal hydrophobin; pfam06766
Bcin01g01350.1	15.23	208.34	17.06	15.61	3.77	0.00	0.16	0.77	0.04	0.95	Hypothetical protein	Short-chain dehydrogenases/reductases (SDR); cl25409
Bcin13g05160.1	8.28	110.88	4.06	8.00	3.74	0.00	-1.03	0.10	-0.05	0.90	Hypothetical protein	ND
Bcin14g02870.1	2.98	34.21	3.12	2.34	3.52	0.00	0.07	0.85	-0.35	0.41	Bcmfs1	Fungal trichothecene efflux pump (TRI12); cl27908
Bcin01g05890.1	13.67	155.81	51.04	16.72	3.51	0.00	1.90	0.00	0.29	0.57	Bmr1	Macrolide transporter ATP-binding /permease protein; cl28180
Bcin02g07070.1	3.71	30.45	11.52	4.45	3.04	0.01	1.64	0.38	0.26	0.61	Hypothetical protein	ND
Bcin01g03450.1	0.21	1.62	2.63	1.83	2.95	0.00	3.65	0.14	3.13	0.15	Hypothetical protein	Domain of unknown function (DUF4185); cl16414
Bcin13g05150.1	1013.70	7444.87	808.85	590.14	2.88	0.00	-0.33	0.30	-0.78	0.04	Hypothetical protein	Basic leucine zipper (bZIP), DNA-binding and dimerization domain; cd12193
Bcin12g06760.1	20.11	134.82	3.86	8.10	2.75	0.04	-2.38	0.11	-1.31	0.23	Hypothetical protein	ND
Bcin04g05650.1	3.77	21.76	17.45	5.47	2.53	0.02	2.21	0.32	0.54	0.12	Hypothetical protein	Bicupin, oxalate decarboxylase family; TIGR03404
Bcin08g02330.1	4.13	21.83	5.38	4.27	2.40	0.00	0.38	0.31	0.05	0.92	Bcria1	Elongation factor Tu GTP binding domain; cl27769
Bcin10g01350.1	1.56	8.11	1.44	1.07	2.38	0.00	-0.12	0.81	-0.54	0.41	Hypothetical protein	Short-chain dehydrogenases/reductases (SDR); cl25409
Bcin16g00810.1	0.98	4.29	2.59	0.92	2.13	0.02	1.40	0.39	-0.09	0.94	Hypothetical protein	ND
Bcin16g01490.1	0.42	1.83	23.66	3.09	2.12	0.02	5.81	0.03	2.87	0.38	Hypothetical protein	Cytochrome P450; cl12078
Bcin15g00060.1	1.02	4.43	1.80	1.38	2.11	0.01	0.81	0.00	0.43	0.19	Hypothetical protein	Glutathione S-transferase; cl25459
Bcin10g05150.1	61.52	260.33	90.00	85.38	2.08	0.00	0.55	0.02	0.47	0.07	Bctif6	Eukaryotic translation initiation factor 6-like protein; PTZ00136

ND, not detected.

Table S2. *Botrytis cinerea* genes significantly upregulated by treatment with rishitin

Gene ID	FPKM value (Average)				Log2 fold change (relative to control)						Annotation	Motif
	Control	100 µM Capsidiol	500 µM Rishitin	500 µM Resveratrol	100 µM Capsidiol	P value	500 µM Rishitin	P value	500 µM Resveratrol	P value		
Bcin07g05430.1	0.21	0.04	24.89	2.48	-2.32	0.41	6.86	0.01	3.54	0.02	Hypothetical protein	Cytochrome P450; cl12078
Bcin13g00710.1	0.80	0.51	69.45	61.88	-0.64	0.10	6.44	0.01	6.28	0.03	BcatrB	Macrolide transporter ATP-binding /permease protein; cl28180
Bcin08g04910.1	0.50	0.18	29.52	1.50	-1.50	0.05	5.89	0.02	1.58	0.00	Hypothetical protein	Phenylcoumaran benzylic ether reductase like, atypical SDRs; cd05259
Bcin16g01490.1	0.42	1.83	23.66	3.09	2.12	0.02	5.81	0.03	2.87	0.38	Hypothetical protein	Cytochrome P450; cl12078
Bcin06g00650.1	0.09	0.11	3.65	3.52	0.26	0.84	5.28	0.00	5.22	0.01	Hypothetical protein	Cytochrome P450; cl12078
Bcin07g02220.1	1.07	2.90	17.52	1.35	1.43	0.02	4.03	0.01	0.34	0.53	Bmr3	Macrolide transporter ATP-binding /permease protein; cl28180
Bcin03g04480.1	2.45	2.77	32.63	7.67	0.17	0.67	3.73	0.02	1.65	0.04	Hypothetical protein	Classical short-chain dehydrogenases/reductases; cd05233
Bcin08g04920.1	0.62	0.59	6.84	1.10	-0.08	0.47	3.45	0.03	0.82	0.10	Hypothetical protein	GAL4-like Zn2Cys6 binuclear cluster DNA-binding domain; cd00067
Bcin04g03050.1	0.55	0.71	5.74	1.26	0.37	0.61	3.39	0.00	1.20	0.11	Bcgpr5	Seven-transmembrane G protein-coupled receptor superfamily; cl28897
Bcin13g02720.1	11.26	26.69	105.31	14.45	1.25	0.01	3.23	0.00	0.36	0.30	BcatrD	Pleiotropic Drug Resistance (PDR) Family protein; TIGR00956
Bcin06g07120.1	5.21	5.26	43.77	8.22	0.02	0.94	3.07	0.01	0.66	0.32	Hypothetical protein	Hypothetical protein; Provisional; cl27550
Bcin14g01070.1	48.96	53.91	386.41	123.65	0.14	0.51	2.98	0.01	1.34	0.00	Hypothetical protein	Medium chain reductase/dehydrogenase/zinc-dependent alcohol dehydrogenase-like family; cl16912
Bcin05g05130.1	1.49	1.90	11.27	1.39	0.35	0.28	2.92	0.02	-0.10	0.72	Hypothetical protein	ND
Bcin05g05120.1	1.25	1.82	9.36	1.65	0.54	0.16	2.90	0.01	0.39	0.39	Hypothetical protein	Short chain dehydrogenase; cl27753
Bcin16g02850.1	2.59	3.08	18.78	6.32	0.25	0.16	2.86	0.00	1.29	0.01	Hypothetical protein	FAD/FMN-containing dehydrogenase; COG0277
Bcin03g07770.1	4.39	9.82	28.87	106.82	1.16	0.03	2.72	0.04	4.60	0.00	Bcalo4	NAD(P)H-nitrite reductase, large subunit; cl26176
Bcin07g04570.1	8.17	7.47	44.67	11.76	-0.13	0.45	2.45	0.01	0.53	0.14	Hypothetical protein	UDP-glucoronosyl and UDP-glucosyl transferase; cl26154
Bcin08g04720.1	0.89	2.16	4.33	1.72	1.27	0.08	2.27	0.02	0.94	0.20	Hypothetical protein	ND
Bcin15g01970.1	2.25	4.02	10.77	0.48	0.84	0.17	2.26	0.00	-2.23	0.04	Hypothetical protein	ND
Bcin01g00080.1	2.72	3.52	12.75	5.38	0.37	0.21	2.23	0.00	0.98	0.04	Bcboa8	2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases; COG0654
Bcin11g01310.1	2.34	1.12	10.85	3.17	-1.06	0.01	2.21	0.00	0.44	0.26	Hypothetical protein	Alpha/beta hydrolases; cl21494
Bcin09g00450.1	1.75	3.54	7.65	3.66	1.02	0.00	2.13	0.02	1.07	0.00	Hypothetical protein	Alpha/beta hydrolases; cl21494
Bcin08g04610.1	18.99	24.39	81.98	21.31	0.36	0.26	2.11	0.00	0.17	0.70	Hypothetical protein	Short chain dehydrogenase; cl27753
Bcin15g04480.1	9.56	17.27	40.58	16.64	0.85	0.01	2.08	0.00	0.80	0.16	Hypothetical protein	Short chain dehydrogenase; cl27753
Bcin11g02050.1	5.51	8.15	23.26	5.98	0.56	0.04	2.08	0.01	0.12	0.74	Hypothetical protein	17-beta-hydroxysteroid dehydrogenase XI-like, short-chain dehydrogenases/reductases; cd05339
Bcin06g01060.1	1.23	1.20	5.12	3.73	-0.04	0.95	2.06	0.00	1.60	0.10	Hypothetical protein	ND
Bcin04g06990.1	22.62	26.67	93.16	7.50	0.24	0.24	2.04	0.00	-1.59	0.00	Hypothetical protein	Glycosyl hydrolase family 43; cd08998

ND, not detected.

Table S3. *Botrytis cinerea* genes significantly upregulated by treatment with resveratrol

Gene ID	FPKM value (Average)				Log2 fold change (relative to control)						Annotation	Motif
	Control	100 μM Capsidiol	500 μM Rishitin	500 μM Resveratrol	100 μM Capsidiol	P value	500 μM Rishitin	P value	500 μM Resveratrol	P value		
Bcin14g05330.1	0.60	0.56	1.37	102.69	-0.12	0.89	1.18	0.53	7.41	0.00	Hypothetical protein	Intradiol_dioxygenase_like; cd03457
Bcin13g00710.1	0.80	0.51	69.45	61.88	-0.64	0.10	6.44	0.01	6.28	0.03	BcatrB	Macrolide transporter ATP-binding /permease protein; cl28180
Bcin06g00650.1	0.09	0.11	3.65	3.52	0.26	0.84	5.28	0.00	5.22	0.01	Hypothetical protein	Cytochrome P450; cl12078
Bcin03g07770.1	4.39	9.82	28.87	106.82	1.16	0.03	2.72	0.04	4.60	0.00	Bcalo4	NAD(P)H-nitrite reductase, large subunit; cl26176
Bcin16g04210.1	26.92	16.27	18.72	556.50	-0.73	0.04	-0.52	0.33	4.37	0.00	Bcidl1	Intradiol_dioxygenase_like; cd03457
Bcin14g02510.1	44.07	12.30	211.14	816.50	-1.84	0.00	2.26	0.09	4.21	0.00	Bclcc2	Multicopper oxidase with three cupredoxin domains; cl28276
Bcin07g04100.1	2.47	3.02	2.12	45.11	0.29	0.56	-0.22	0.78	4.19	0.01	Hypothetical protein	S-adenosylmethionine-dependent methyltransferases class I; cl17173
Bcin04g02650.1	2.54	4.34	8.37	31.25	0.77	0.04	1.72	0.04	3.62	0.00	Hypothetical protein	FAD binding domain; cl27552
Bcin07g05430.1	0.21	0.04	24.89	2.48	-2.32	0.41	6.86	0.01	3.54	0.02	Hypothetical protein	Cytochrome P450; cl12078
Bcin06g06350.1	38.12	41.34	51.62	413.20	0.12	0.69	0.44	0.49	3.44	0.00	Bcnqo1	NADPH-dependent FMN reductase; cl00438
Bcin05g07100.1	67.10	93.43	151.28	711.57	0.48	0.39	1.17	0.26	3.41	0.04	Hypothetical protein	Redox-sensitive bicupin YhaK, pirin superfamily; COG1741
Bcin15g05080.1	4.05	1.47	2.17	27.75	-1.46	0.15	-0.90	0.25	2.78	0.02	Bcgas5	Glycosyl hydrolase family 1; cl23725
Bcin06g05110.1	22.06	33.15	63.56	148.92	0.59	0.00	1.53	0.05	2.76	0.02	Hypothetical protein	Redox-sensitive bicupin YhaK, pirin superfamily; COG1741
Bcin04g06040.1	4.29	1.80	8.56	28.76	-1.25	0.25	1.00	0.14	2.75	0.01	Hypothetical protein	CESA_CelA_like; cd06421
Bcin07g03750.1	14.49	15.26	16.26	89.34	0.07	0.79	0.17	0.38	2.62	0.00	Hypothetical protein	ND
Bcin15g00020.1	0.13	17.32	0.35	0.70	7.04	0.00	1.42	0.42	2.42	0.01	Hypothetical protein	Domain of unknown function (DUF1330); cl22966
Bcin02g04490.1	41.96	44.10	101.46	221.26	0.07	0.40	1.27	0.00	2.40	0.00	Hypothetical protein	The Class III extradiol dioxygenase, 4,5-DOPA dioxygenase, cd07363
Bcin03g05130.1	1.06	0.87	0.95	5.44	-0.27	0.43	-0.15	0.67	2.37	0.00	Bcalo3	NAD(P)H-nitrite reductase, large subunit; cl26176
Bcin03g06320.1	96.23	81.03	77.15	433.90	-0.25	0.41	-0.32	0.19	2.17	0.00	Hypothetical protein	Methyltransferase domain; pfam13649
Bcin04g06920.1	3.39	2.82	4.41	15.01	-0.26	0.57	0.38	0.47	2.15	0.01	Hypothetical protein	alpha/beta hydrolases; cl21494
Bcin11g06310.2	0.22	0.34	2.74	0.98	0.58	0.12	3.61	0.13	2.13	0.02	Hypothetical protein	Phenylcoumaran benzylic ether reductase like, atypical SDRs; cd05259
Bcin11g02620.1	4.91	6.13	12.84	20.03	0.32	0.37	1.39	0.10	2.03	0.00	Bchol1	Major Facilitator Superfamily; cd06174
Bcin02g03510.1	30.05	22.31	44.17	120.32	-0.43	0.22	0.56	0.06	2.00	0.00	Hypothetical protein	ND

ND, not detected.

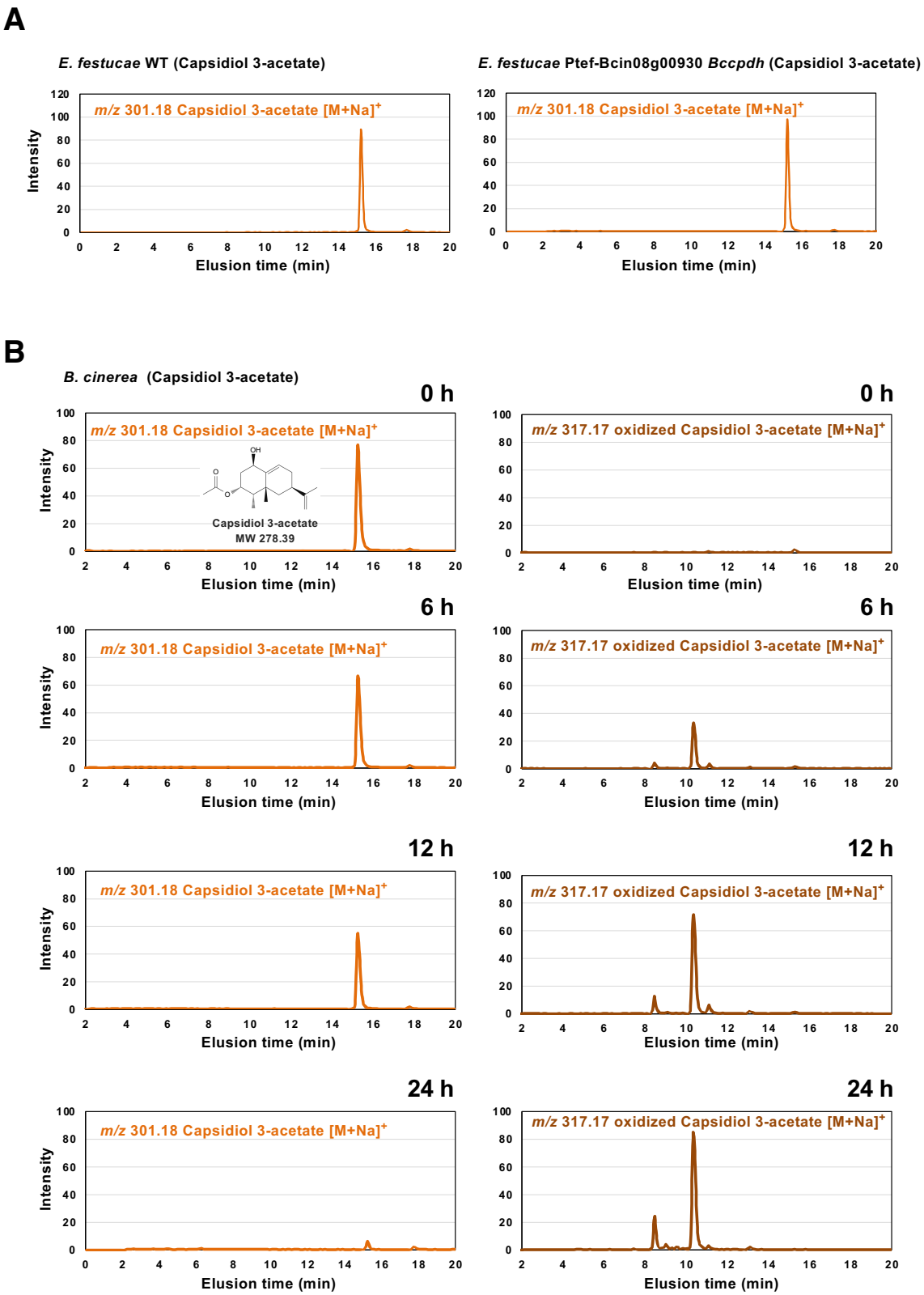

Fig. S7. (A) Mycelia of *E. festucae* wild type (WT) or transformants expressing Bcin08g00930 (*BcCPDH*) were incubated in 100 µM capsidiol acetate for 48 h. Capsidiol acetate was detected by LC/MS. **(B)** Mycelia of *B. cinerea* was incubated in 100 µM capsidiol acetate for indicated time and capsidiol acetate and their oxidized metabolites were detected by LC/MS.

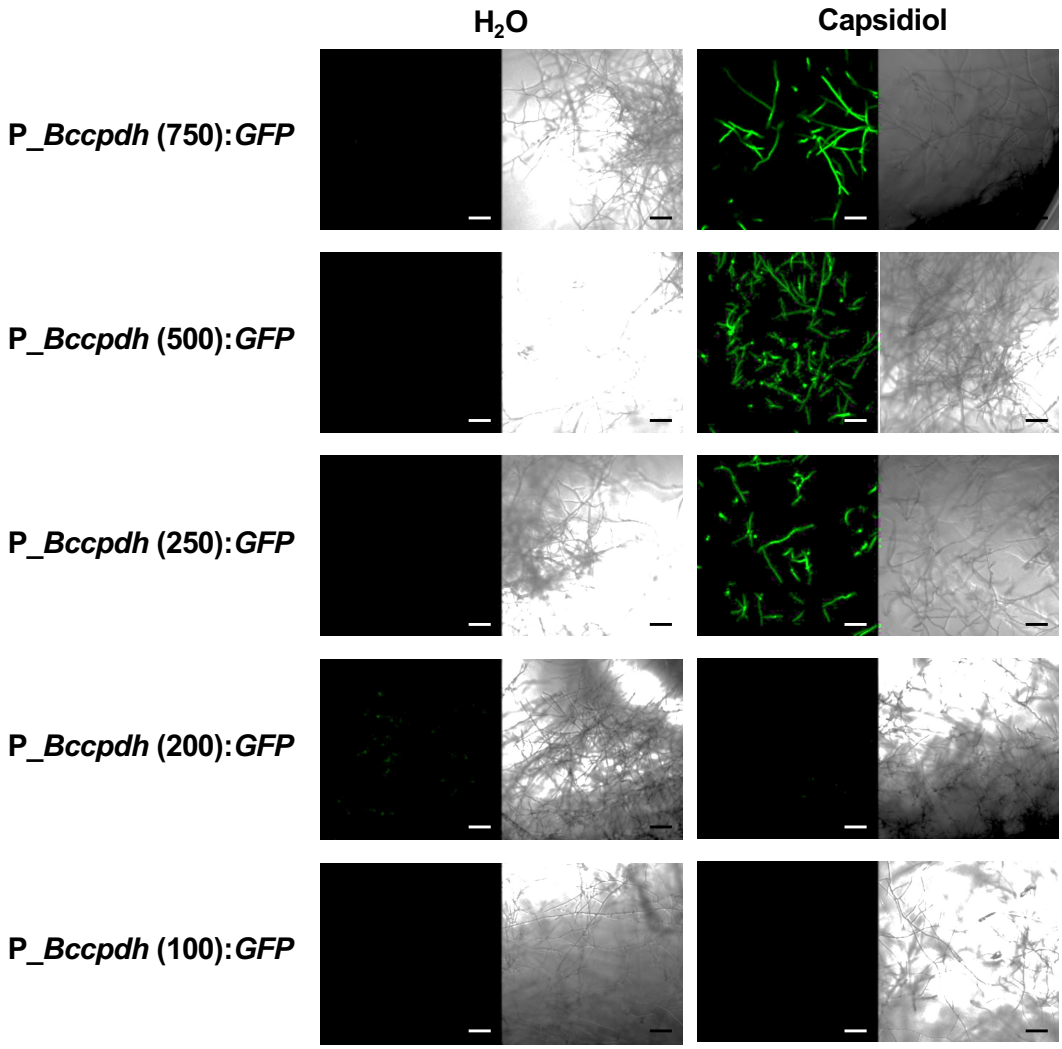

Fig. S8. *B. cinerea* transformants expressing GFP under the control of different lengths (upstream from the start codon of the gene) of *Bccpdh* promoter were incubated in water or 500 µM capsidiol. Expression of GFP was monitored by confocal laser microscopy 1 day after the treatment. Bars = 150 µm.

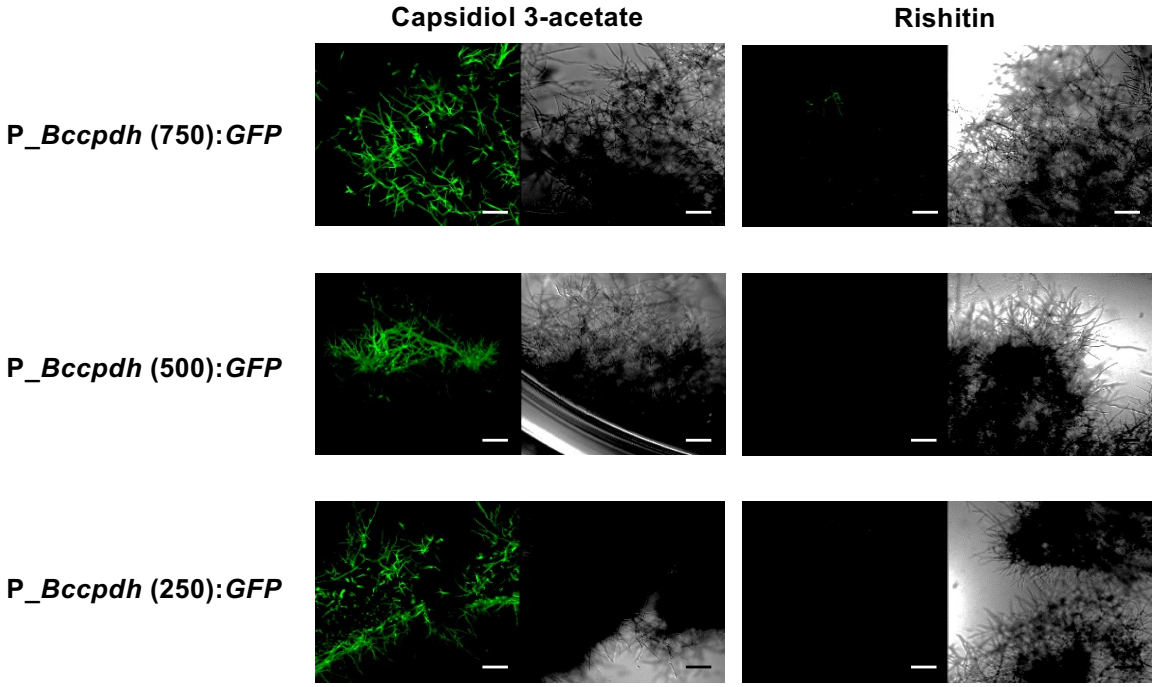

Fig. S9. *B. cinerea* transformants expressing GFP under the control of different lengths (upstream from the start codon of the gene) of *Bccpdh* promoter were incubated in 500 µM capsidiol 3-acetate or rishitin. Expression of GFP was monitored by confocal laser microscopy 1 day after the treatment. Bars = 150 µm.

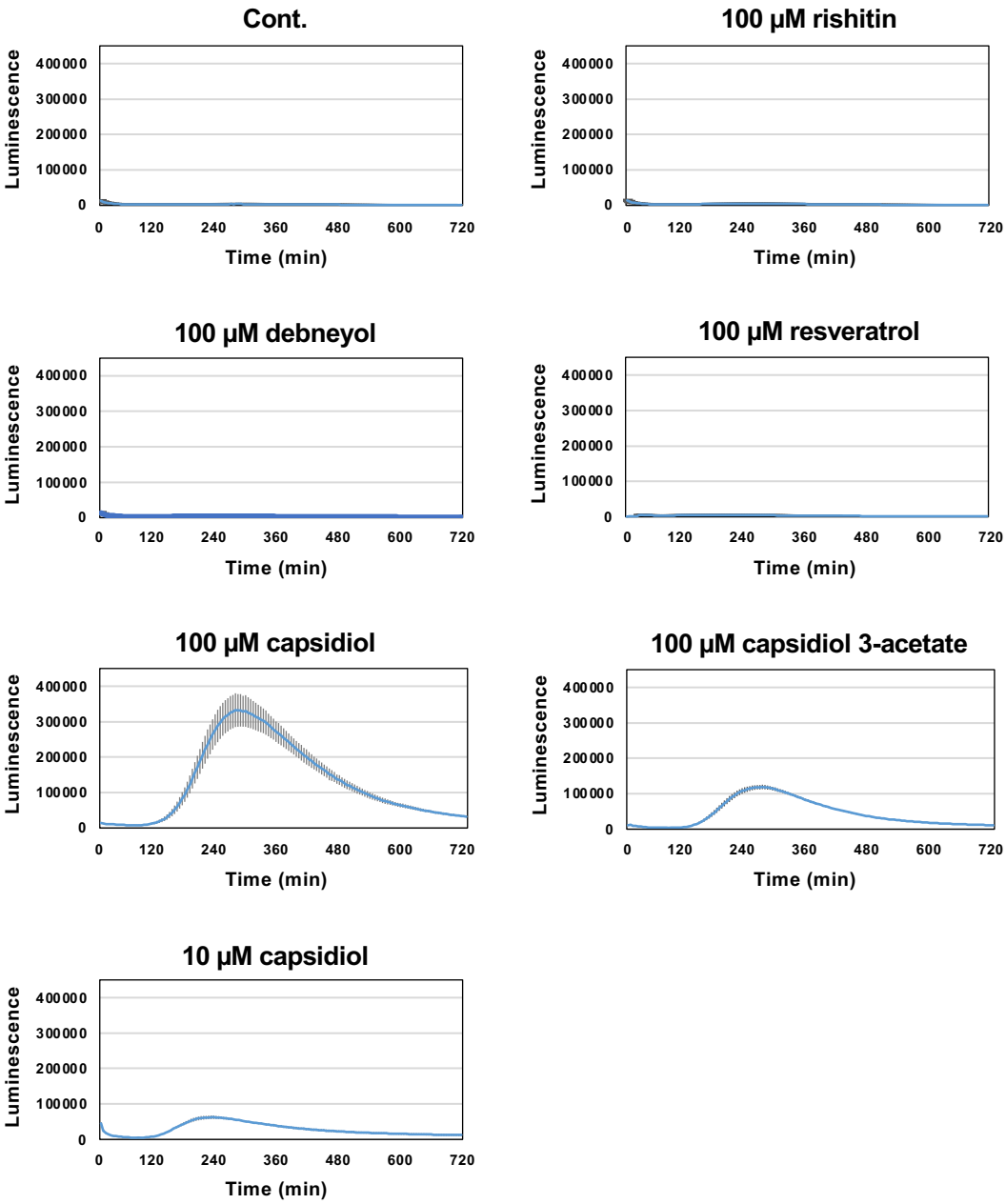

Fig. S10. Luminescence intensity of *B. cinerea* transformant P_*Bccpdh:Luc* containing the *Luciferase* gene under the control of 250 bp *Bccpdh* promoter. The transformant was incubated in water, 100 μM capsidiol, capsidiol 3-acetate, rishitin, resveratrol or debneyol or 10 μM capsidiol. 50 μM D-luciferin was used as the substrate of luciferase. Data are means ±SE (n = 4).

Supplementary Note 3

***Bccpdh* promoter is activated by capsidiol in a concentration-dependent manner.**

B. cinerea transformant P_*Bccpdh:Luc* was produced for the expression of *Luciferase* (*Luc*) under the control of 250 bp *Bccpdh* promoter (250 bp upstream from the start codon of the gene). The *Bccpdh* promoter was activated within the first 2 h after incubation with either 10 or 100 μM capsidiol. The peak of promoter activation was approx. at 5 h for 100 μM capsidiol and within 4 h for 10 μM capsidiol, and the degree and duration of promoter activation was concentration dependent. This result indicates that the activity of the *Bccpdh* promoter immediately decreases once capsidiol is metabolized.

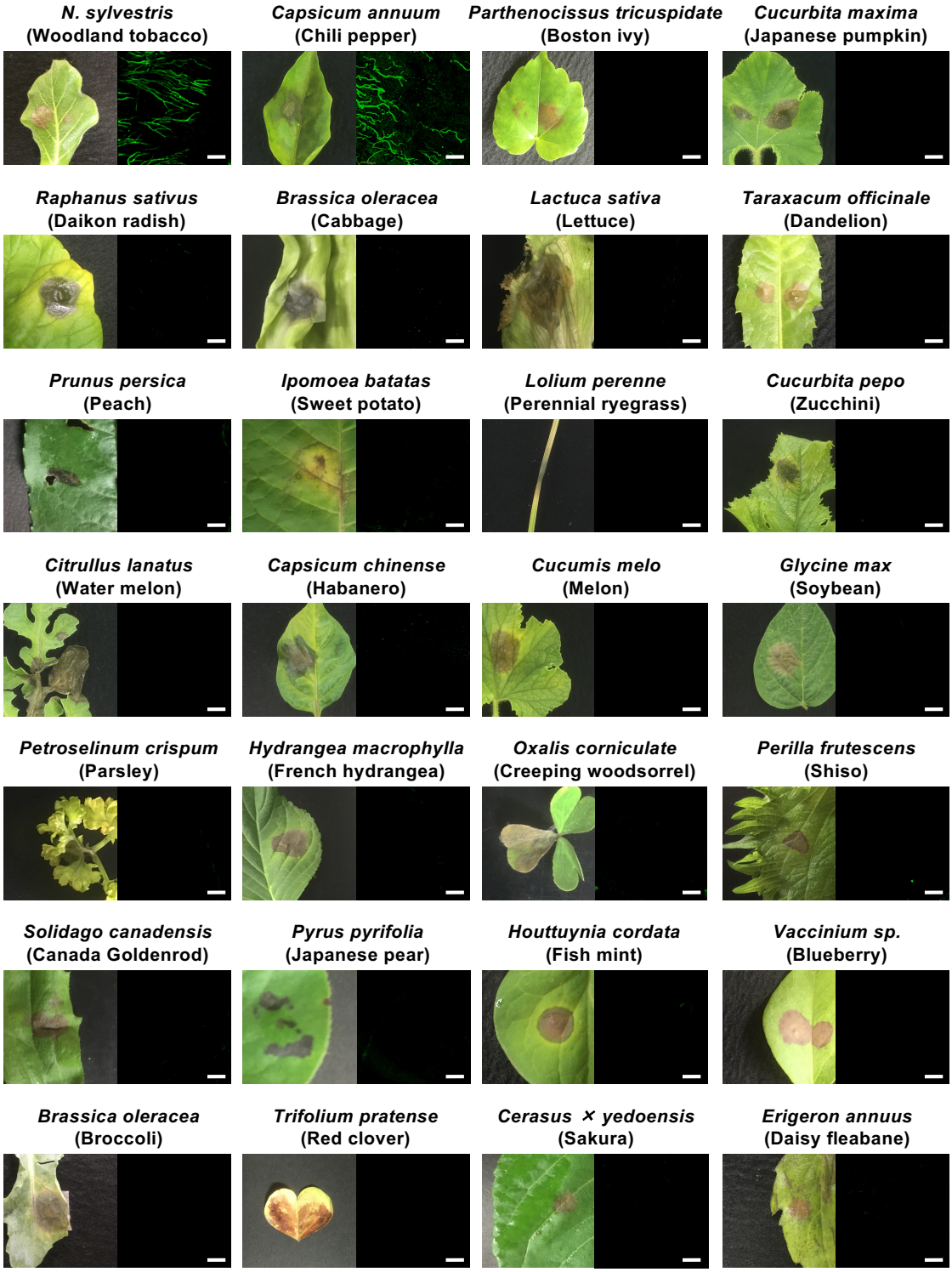

Fig. S11. *B. cinerea Bccpdh* promoter is activated during the infection in plants producing capsidiol. Leaves of indicated plants were inoculated with the mycelia of *B. cinerea* P_*Bccpdh*:*GFP* transformant and hyphae at the edge of the lesion was observed by confocal laser microscopy 2 or 3 d after the inoculation. Bars = 100 μm.

Table S4. Expression of GFP in *Botrytis cinerea* P_*Bccpdh*:*GFP* transformant on different plant species.

Family	Host plant	Common name	Disease symptom	Expression of GFP in *B. cinerea* P_*Bccpdh*:*GFP*	Production of capsidiol (Reference)
Solanaceae	*Nicotiana benthamiana*	Benth	+	+	Matsukawa *et al.* (2013)
Solanaceae	*Nicotiana tabacum*	Tobacco	+	+	Bailey *et al.* (1975)
Solanaceae	*Nicotiana sylvestris*	Woodland tobacco	+	+	Bohlmann *et al.* (2002)
Solanaceae	*Capsicum annuum*	Bell pepper	+	+	Molot *et al.* (1981)
Solanaceae	*Capsicum annuum*	Chilli pepper	+	+	Molot *et al.* (1981)
Solanaceae	*Solunum lycopercicum*	Tomato	+	-	n.r
Solanaceae	*Solunum tuberosum*	Potato	+	-	n.r
Solanaceae	*Solunum melongena*	Eggplant	+	-	n.r
Rosaceae	*Fragaria* × *ananassa*	Strawberry	+	-	n.r
Rosaceae	*Pyrus pyrifolia*	Japanese pear	+	-	n.r
Rosaceae	*Prunus persica*	Peach	+	-	n.r
Rosaceae	*Cerasus* × *yedoensis*	Sakura	+	-	n.r
Rosaceae	*Malus domestica*	Apple	+	-	n.r
Rosaceae	*Rosa hybrida*	Miniature rose	+	-	n.r
Brassicaceae	*Brassica oleracea* var. *capitata*	Cabbage	+	-	n.r
Brassicaceae	*Arabidopsis thaliana*	Thale cress	+	-	n.r
Brassicaceae	*Brassica oleracea* var. *italica*	Broccoli	+	-	n.r
Brassicaceae	*Raphanus sativus* var. *longipinnatus*	Daikon radish	+	-	n.r
Brassicaceae	*Brassica rapa* var. *pekinensis*	Chinese cabbage	+	-	n.r
Brassicaceae	*Brassica rapa* var. *rapa*	Turnip	+	-	n.r
Fabaceae	*Phaseolus vulgaris*	Common bean	+	-	n.r
Fabaceae	*Pisum sativum*	Pea	+	-	n.r
Fabaceae	Glycine max	Soybean	+	-	n.r
Fabaceae	*Trifolium repens*	White clover	+	-	n.r
Fabaceae	*Trifolium pratense*	Red clover	+	-	n.r
Asteraceae	*Taraxacum officinale*	Dandelion	+	-	n.r
Asteraceae	*Erigeron annuus*	Annual fleabane	+	-	n.r
Asteraceae	*Lactuca sativa*	Lettuce	+	-	n.r
Asteraceae	*Solidago altissima*	Tall goldenrod	+	-	n.r
Asteraceae	*Glebionis coronaria*	Crown daisy	+	-	n.r
Asteraceae	*Chrysanthemum* × *morifolium*	Florist's daisy	+	-	n.r
Cucurbitaceae	*Cucurbita pepo*	Zucchini	+	-	n.r
Cucurbitaceae	*Cucurbita maxima*	Winter squash	+	-	n.r
Cucurbitaceae	*Cucumis sativus L.*	Cucumber	+	-	n.r
Cucurbitaceae	*Citrullus lanatus*	Watermelon	+	-	n.r
Amaryllidaceae	*Allium fistulosum*	Welsh onion	+	-	n.r
Amaryllidaceae	*Allium cepa*	Onion	+	-	n.r
Amaryllidaceae	*Allium tuberosum*	Chinese chives	+	-	n.r
Vitaceae	*Vitis* × *labruscana*	Grape	+	-	n.r
Vitaceae	*Parthenocissus tricuspidata*	Boston Ivy	+	-	n.r
Lamiaceae	*Perilla frutescens* var. *crispa*	Shiso	+	-	n.r
Lamiaceae	*Ocimum basilicum*	Basil	+	-	n.r
Poaceae	*Lolium perenne*	Perennial ryegrass	+	-	n.r
Saururaceae	*Houttuynia cordata*	Fish mint	+	-	n.r
Moraceae	*Morus australis*	Mulberry	+	-	n.r
Malvaceae	Abelmoschus esculentus	Okra	+	-	n.r
Ebenaceae	*Diospyros kaki*	Persimmon	+	-	n.r
Asparagaceae	*Asparagus officinalis*	Asparagus	+	-	n.r
Ericaceae	*Vaccinium* ssp.	Blueberry	+	-	n.r
Hydrangeaceae	Hydrangea macrophylla	Hydrangea	+	-	n.r
Convolvulaceae	*Ipomoea batatas*	Sweet potato	+	-	n.r
Caryophyllaceae	*Dianthus caryophyllus*	Carnation	+	-	n.r
Oxalidaceae	*Oxalis corniculata*	Creeping woodsorrel	+	-	n.r

n.r. not reported.

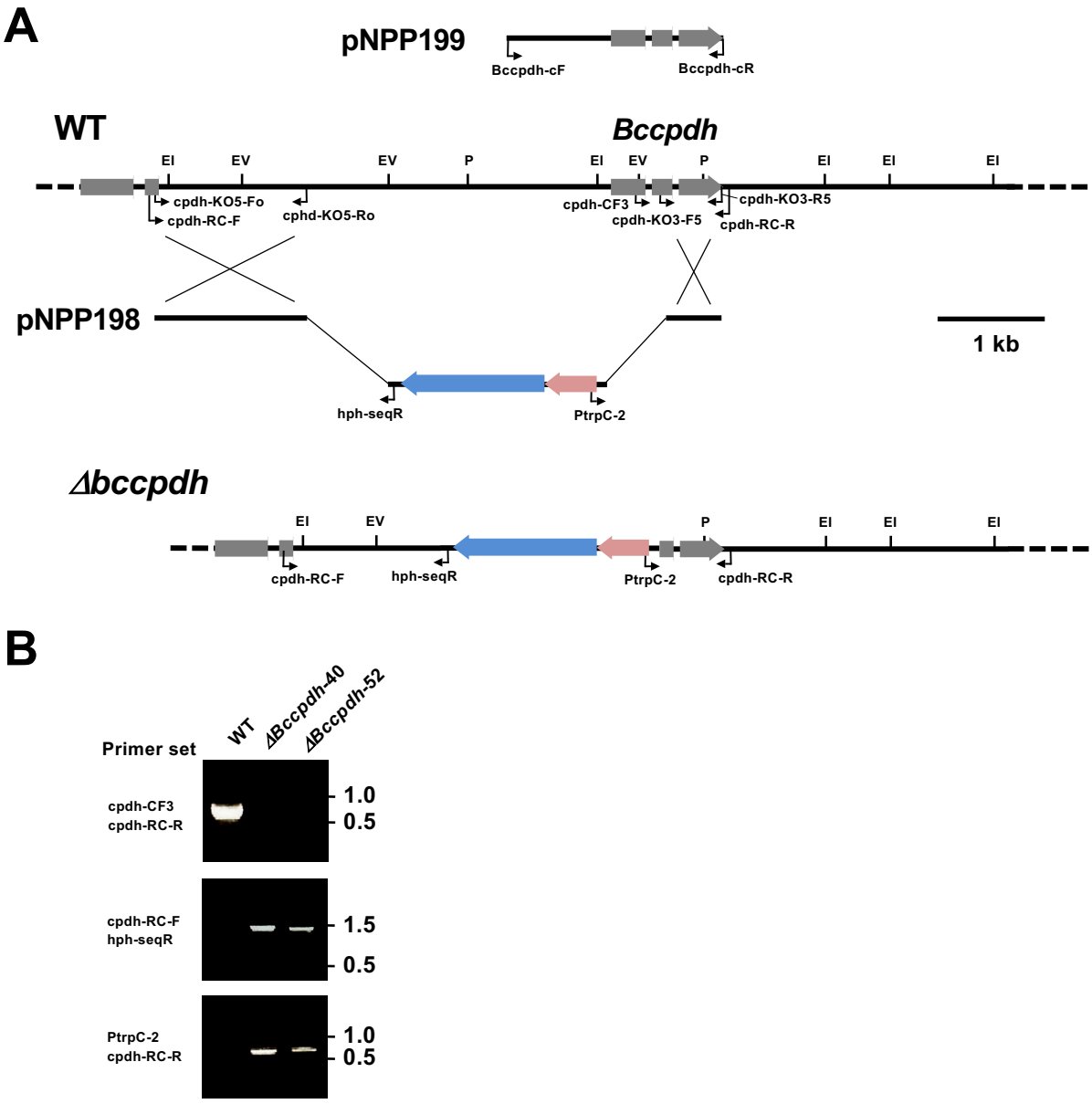

Fig. S12. Targeted gene replacement of the *B. cinerea Bccpdh* locus.

(A) Physical map of the *Bccpdh* wild-type (WT) genomic region, linear insert of *Bccpdh* replacement construct pNPP198 and complementation construct pNPP199, showing restriction enzyme sites for *Eco*RV (EV), *Eco*RI (EI) and *Pst*I (P). The mutated genomic locus of *Bccpdh* deletion mutant (*Δbccpdh*) is depicted to show homologous recombination of the *hph* cassette. Primers used for the construction of deletion vector and screening for the replacement event are indicated by arrowheads. **(B)** Confirmation of gene disruption in isolated *Δbccpdh* strains by PCR. Genomic DNA from *B. cinerea* wild type and *Δbccpdh* strains were used for PCR with indicated primers.

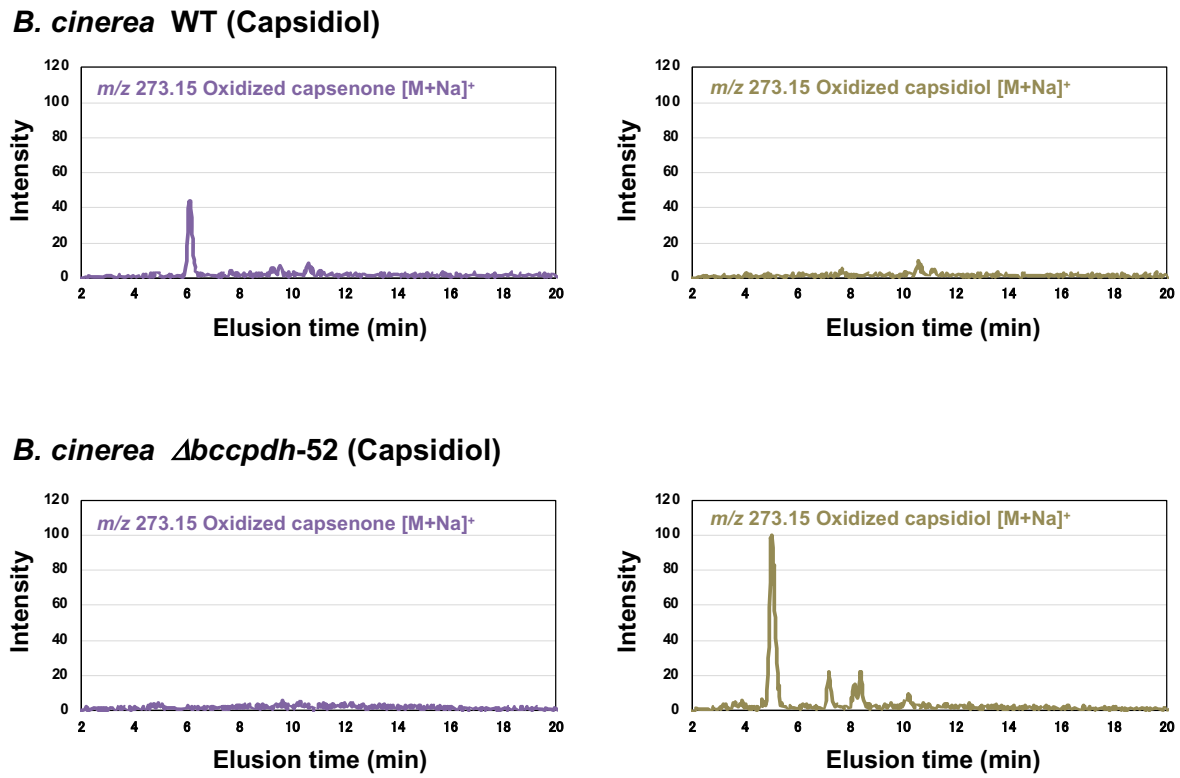

Fig. S13. Mycelial blocks (approx. 1 mm^3) of *B. cinerea* wild type (WT) or *Bccpdh* KO mutant strain (*Δbccpdh*-52) were incubated in 50 µl of 100 µM capsidiol for 4 days. Oxidized capsenone and oxidized capsidiol were detected by LC/MS.

Supplementary Note 4

Capsidiol is oxidized in *Δbccpdh* by a cytochrome P450 encoded by Bcin16g01490.

After the incubation of capsidiol with *B. cinerea Δbccpdh*, oxidized capsidiol (one major and at least two minor peaks) were detected, while oxidized capsidiol was not detected in the metabolites after the incubation with wild type *B. cinerea* (Fig. S13), probably because capsidiol is quickly metabolized to capsenone (Fig. S4). The major oxidized capsidiol was detected after the incubation of capsidiol with *E. festucae* expressing Bcin16g01490 encoding a cytochrome P450 (Fig. S14B), indicating that the lack of BcCPDH alters the pathway in *Δbccpdh* towards a direct oxidation of capsidiol by Bcin16g01490 (Fig. S14C). It should be noted, however, that a substantial amount of capsidiol is remaining after the incubation with *B. cinerea Δbccpdh* or *E. festucae* expressing Bcin16g01490, indicating that this cytochrome P450 does not play a major role in capsidiol detoxification.

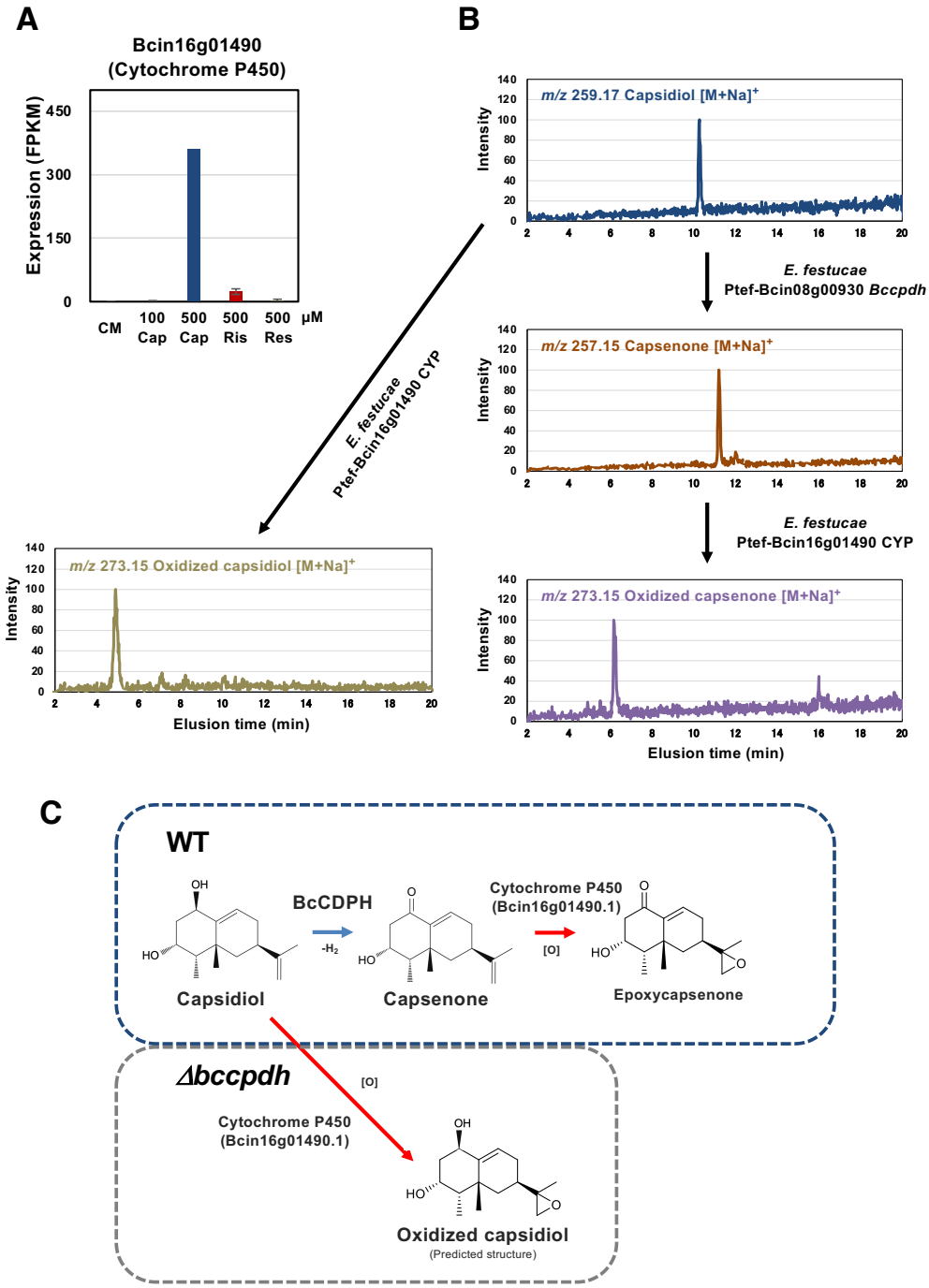

Fig. S14. (A) Expression profiles of Bcin16g01490. The gene expression (FPKM value) was determined by RNA-seq analysis of *B. cinerea* cultured in CM media containing 100 μM capsidiol, 500 μM rishitin or 500 μM resveratrol (n = 3) or 500 μM Capsidiol (n = 1) for 24 h. Data are mean ± SE. **(B)** Mycelia of *E. festucae* transformant expressing *Bccpdh* was incubated in CM media containing 500 μM capsidiol for 70 h and collected culture filtrate was then incubated with *E. festucae* expressing Bcin16g01490 gene for 5 days. The resultant metabolite was subjected to the structural analysis as shown in Figs. S15-S17. Alternatively, the mycelia of *E. festucae* expressing Bcin16g01490 was incubated in 100 μM capsidiol for 4 days. The metabolite was detected by LC/MS. **(C)** Predicted metabolism of capsidiol in *B. cinerea* wild type (WT) and *Δbccpdh*.

Supplementary Note 5

Chemical analysis of oxidized capsenone.

E. festucae transformant expressing Bcin08g00930 (*Bccpdh*) under the control of constitutive TEF prompter (Vanden Wymelenberg *et al.* 1997) was cultured in 10 ml CM media containing 100 μM capsidiol for 70 h and the culture filtrate was collected. The filtrate was sterilized using a syringe filter (pore size 0.45 μm, Millipore) and further incubated with *E. festucae* transformant expressing Bcin16g01490 (encoding a cytochrome P450) for 5 days.

The resultant supernatant was extracted with EtOAc and the extract was analyzed by LC/MS. The major peak appearing at 8.1 min showed the ion peaks of *m/z* 251.1638 (calcd for $C_{15}H_{23}O_3$ $[M+H]^+$: 251.1642) and 273.1455 (calcd for $C_{15}H_{22}O_3Na$ $[M+Na]^+$: 273.1461) (Fig. S15), suggesting the molecular formula to be $C_{15}H_{22}O_3$.

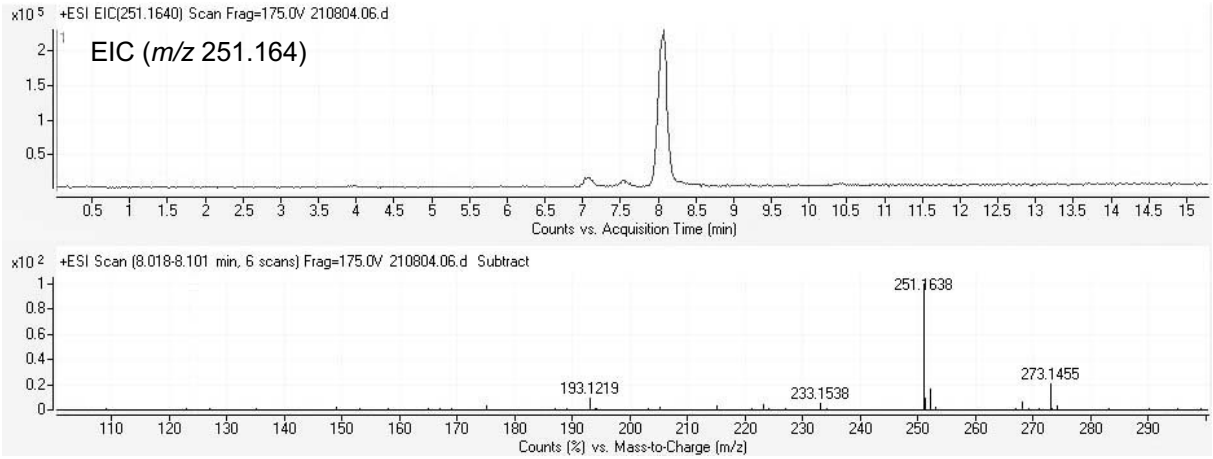

Fig. S15. LC/MS analysis of supernatant of *Epichloë* transformant cultured with capsidiol

The extract was further purified by HPLC (Fig. S16) to give the product that possesses the molecular formula of $C_{15}H_{22}O_3$ mentioned above. The molecular formula suggested that this product was formed from capsidiol ($C_{15}H_{24}O_2$) by dehydration (-2H) and oxygen insertion (+O).

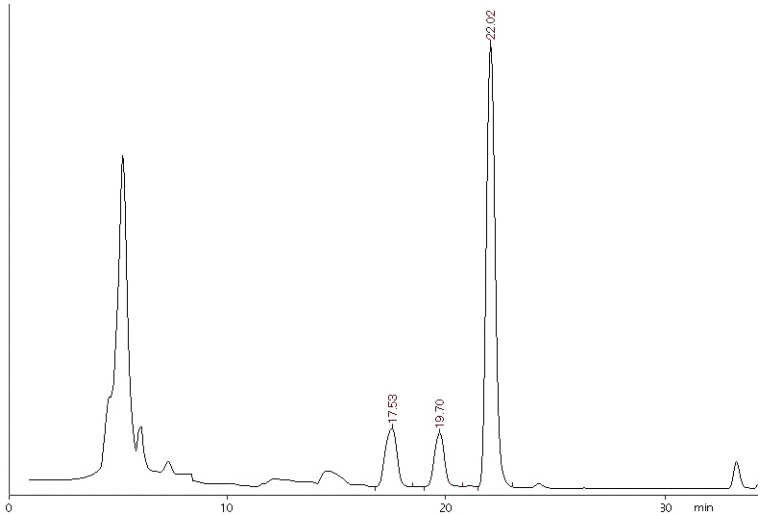

Fig. S16. Preparative HPLC of capsidiol metabolites.
The peak at 22 min was found to be capsenone 11,12-epoxide

The structure of the metabolite was determined by two-dimensional NMR analyses. COSY and TOCSY experiments revealed two partial frameworks corresponding to C2-C15 and C6-C9 of capsidiol (Fig. S17). Other components are two singlet methyls (C13 and C14) and a methylene group (C12) as suggested by ^{1}H NMR. The lack of the oxy-methine proton (H1) of capsidiol suggests that this position is oxidized to ketone like capsenone, which was supported by the absorption maximum at 250 nm (photodiode array detection in HPLC). The singlet methyl at C14 corresponds to the C14 position of capsidiol due to similar chemical shifts (1.29 and 1.36, respectively). The singlet methyl at C13 (d 1.73) of capsidiol was shifted to the high-field area at d 1.13, and olefinic protons at C12 (d 4.68 and 4.82) of capsidiol largely shifted to the high field area (d 2.58 and 2.68). These facts strongly suggested that the 1,1-disubstituted olefin at C11-C12 in capsidiol is oxidized to epoxide. Therefore, in the light of the molecular formula, we concluded that the metabolite is capsenone 11,12-epoxide as shown in Fig. S17.

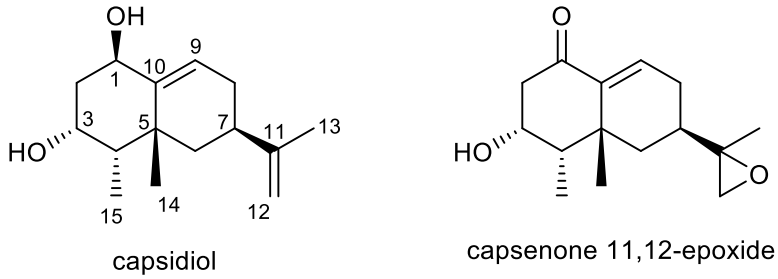

Fig. S17. Structures of capsidiol and capsenone 11,12-epoxide (Epoxycapsenone)

Methods for the structural analysis of oxidized capsenone.

General procedure

NMR spectra were investigated on an Avance ARX400 spectrometer (Bruker Bio Spin, Yokohama, Japan). The chemical shifts (ppm) were referenced to the solvent residual peak at d_H 7.26 ppm ($CDCl_3$). LC/MS was measured by a 1100 High-Performance Liquid Chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) connected to an Agilent 6520 Accurate-Mass Q-TOF spectrometer.

Extraction and LC/MS analysis

The supernatant of the culture broth (10 mL) with capsidiol (100 µM) was extracted with EtOAc (10 mL, twice). The organic layers were concentrated and the residual oil was dissolved in MeCN (0.5 mL) to give a stock solution (2 mM equivalent to capsidiol). A portion (2 µl) of the solution was diluted to 1 mL with 50% MeCN and 5 µl was used for LC/MS analysis.

Purification of capsenone 11,12-epoxide

The stock solution of the EtOAc extract was concentrated and re-dissolved in 30% MeCN (0.5 mL) and subjected to preparative HPLC [Develosil ODS-UG-5 (10 x 250 mm), 20-50% MeCN (45 min), 3 mL/min, detected at 230 nm] to give capsenone 11,12-epoxide (0.13 mg).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.68 (d, J=6.0 Hz, 1H, H-9), 4.47 (m, 1H, H-3), 2.72 (dd, J=16.4, 5.6 Hz, H-2), 2.68 (d, J=4.6 Hz, 1H, H-12), 2.58 (d, J=4.6 Hz, 1H, H-12), 2.35 (dd, J=16.4, 11.6 Hz, 1H, H-2), 2.33 (m, 1H, H-8), 1.99 (brd, J=14.4 Hz, 1H, H-6), 1.92 (m, 1H, H-4), 1.85 (ddd, J=17.0, 11.8, 2.2 Hz, 1H, H-8), 1.57 (m, 1H, H-7), 1.31 (t, J=14.4 Hz, 1H, H-6), 1.29 (s, 3H, H-13), 1.13 (s, 3H, H-14), 1.00 (d, J=7.2 Hz, H-15). ESI-TOF-MS(+) *m/z* 251.1638 (calcd for $C_{15}H_{23}O_3$ $[M+H]^+$: 251.1642), 273.1455 (calcd for $C_{15}H_{22}O_3Na$ $[M+Na]^+$: 273.1461).

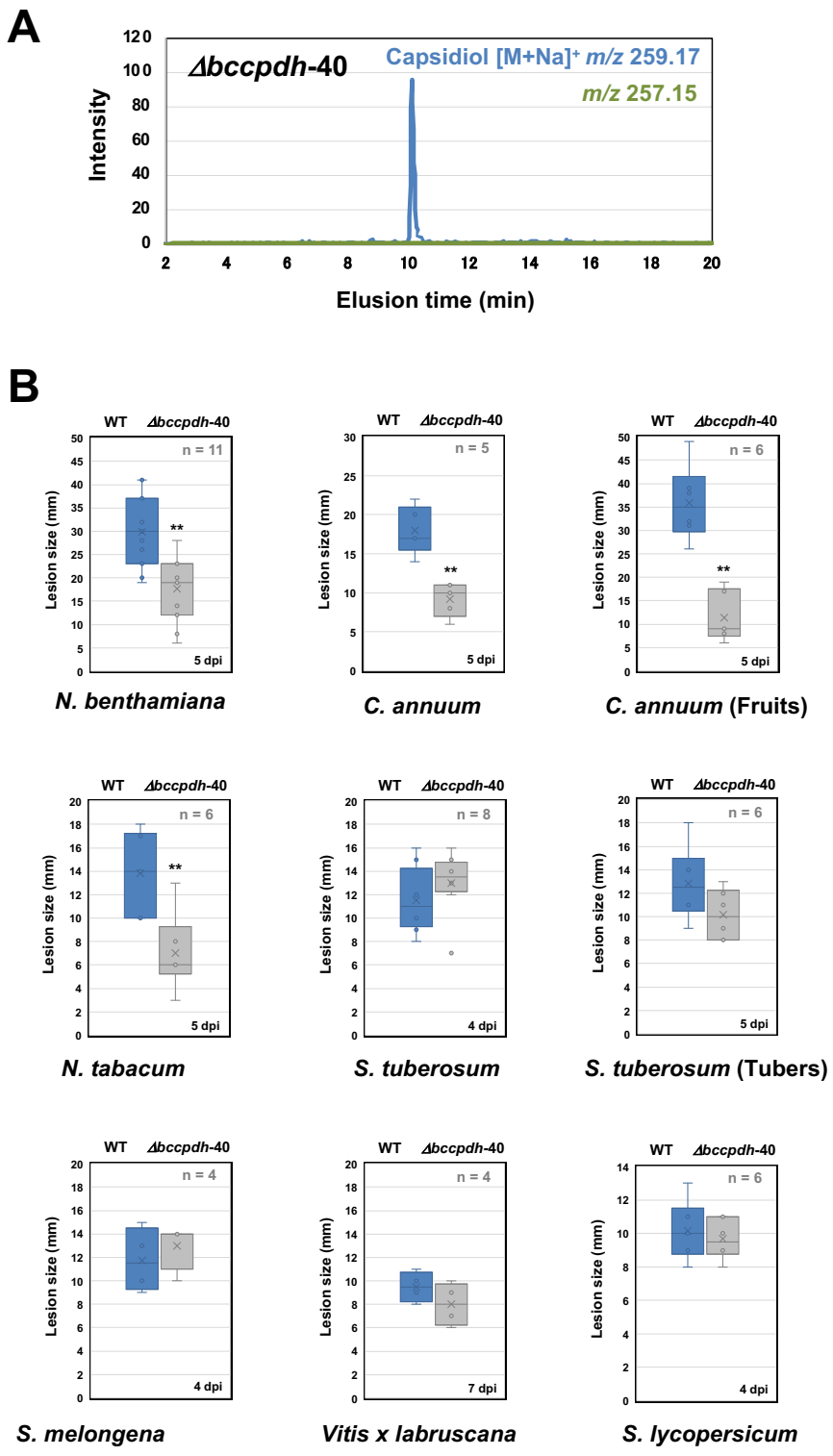

Fig. S18. (A) Mycelial blocks (approx. 1 mm^3) of *B. cinerea Bccpdh* KO strain (*Δbccpdh*-40) were incubated in 50 µl of 100 µM capsidiol for 4 days and capsidiol, but not capsenone, was detected by LC/MS. **(B)** Indicated plants were inoculated with a mycelial block (5 mm^3) of wild type (WT) or *Δbccpdh*-40 and lesion size was measured at 4 to 7 days after the inoculation (dpi). Asterisks indicate a significant difference from WT as assessed by two-tailed Student's *t*-test. $^{**}P < 0.01$. Lines and crosses (x) in the columns indicate the median and mean values, respectively.

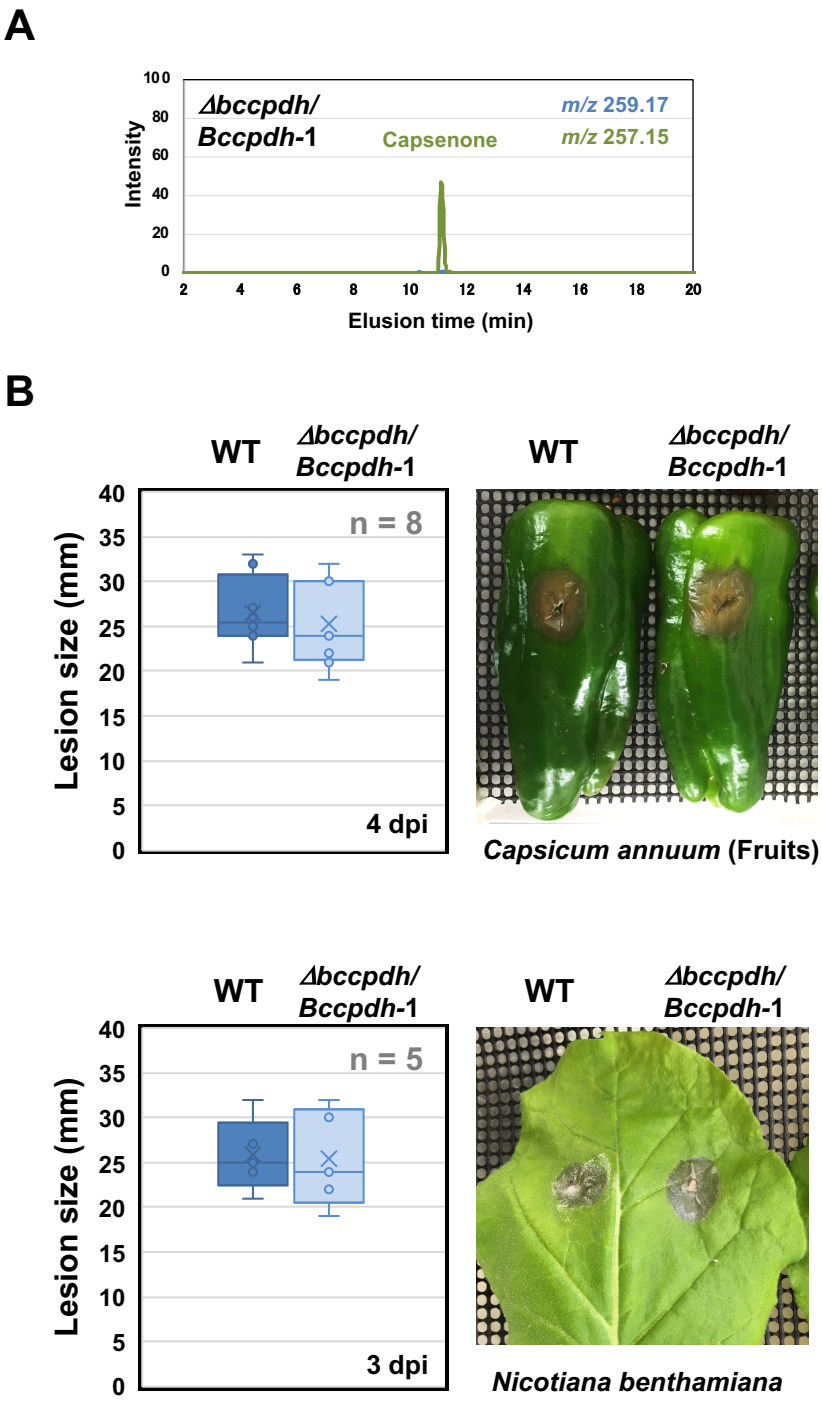

Fig. S19. (A) Mycelial blocks (approx. 1 mm^3) of *B. cinerea Bccpdh* complemented strain (*Δbccpdh/Bccpdh*-1) were incubated in 50 µl of 100 µM capsidiol for 4 days and capsenone was detected by LC/MS. **(B)** *C. annuum* fruits and *N. benthamiana* leaves were inoculated with a mycelial block (5 mm^3) of *B. cinerea* wild type (WT) or *Δbccpdh/Bccpdh*-1 and lesion size was measured at 4 or 3 days after the inoculation (dpi). Lines and crosses (x) in the columns indicate the median and mean values, respectively.

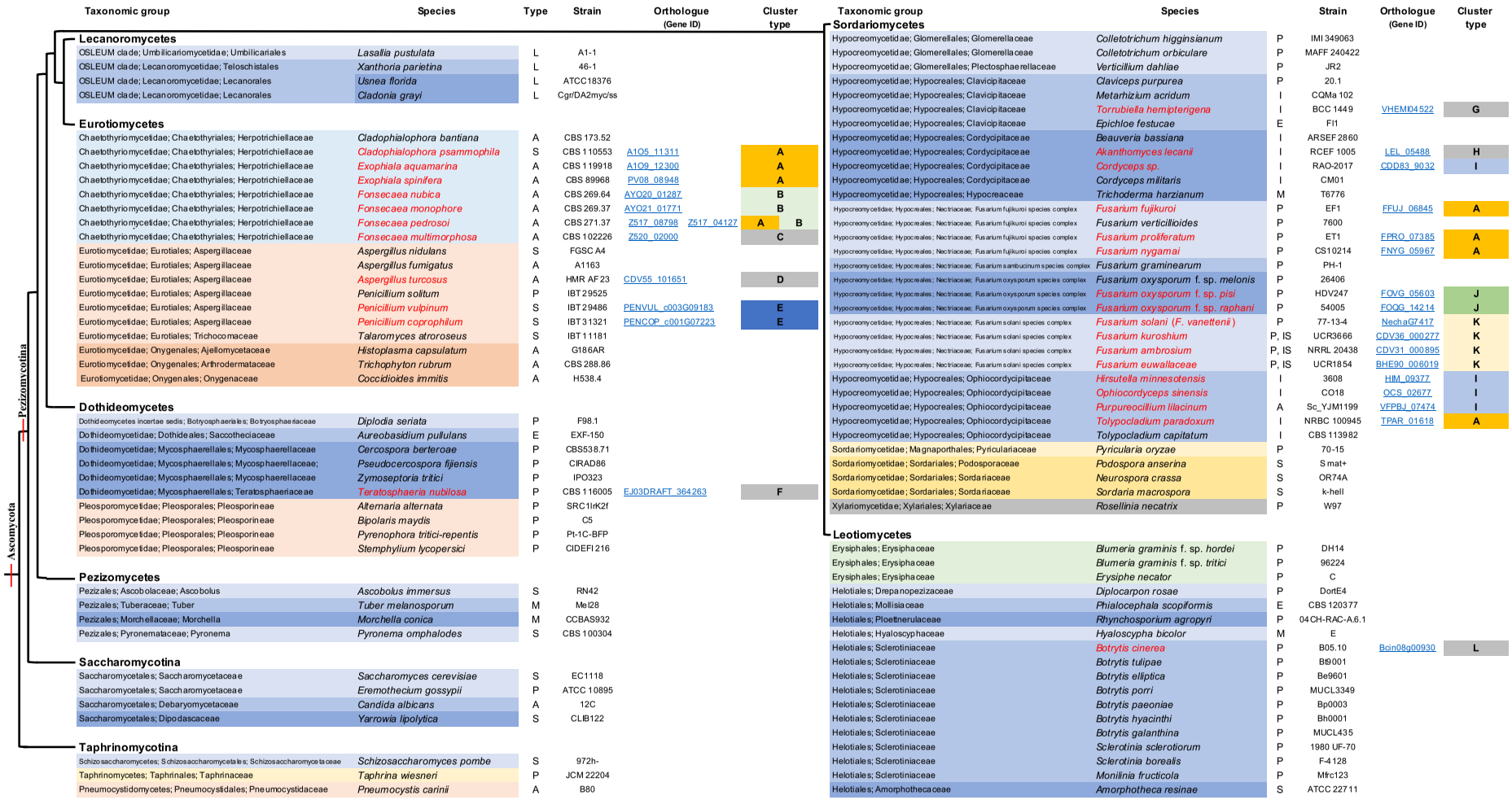

Fig. S20 Distribution of *B. cinerea* CPDH orthologues in Ascomycota fungi. Cluster types were classified based on the conservation of genes around CPDH orthologs in the genome (See Figs. S24 and 25). The types of fungi were categorized as follows. L, Lichen; A, Animal pathogen; S, Saprophyte; P, Plant pathogen, E, Endophyte; M, Mycorrhiza; I, Insect pathogen, IS, Insect symbiont.

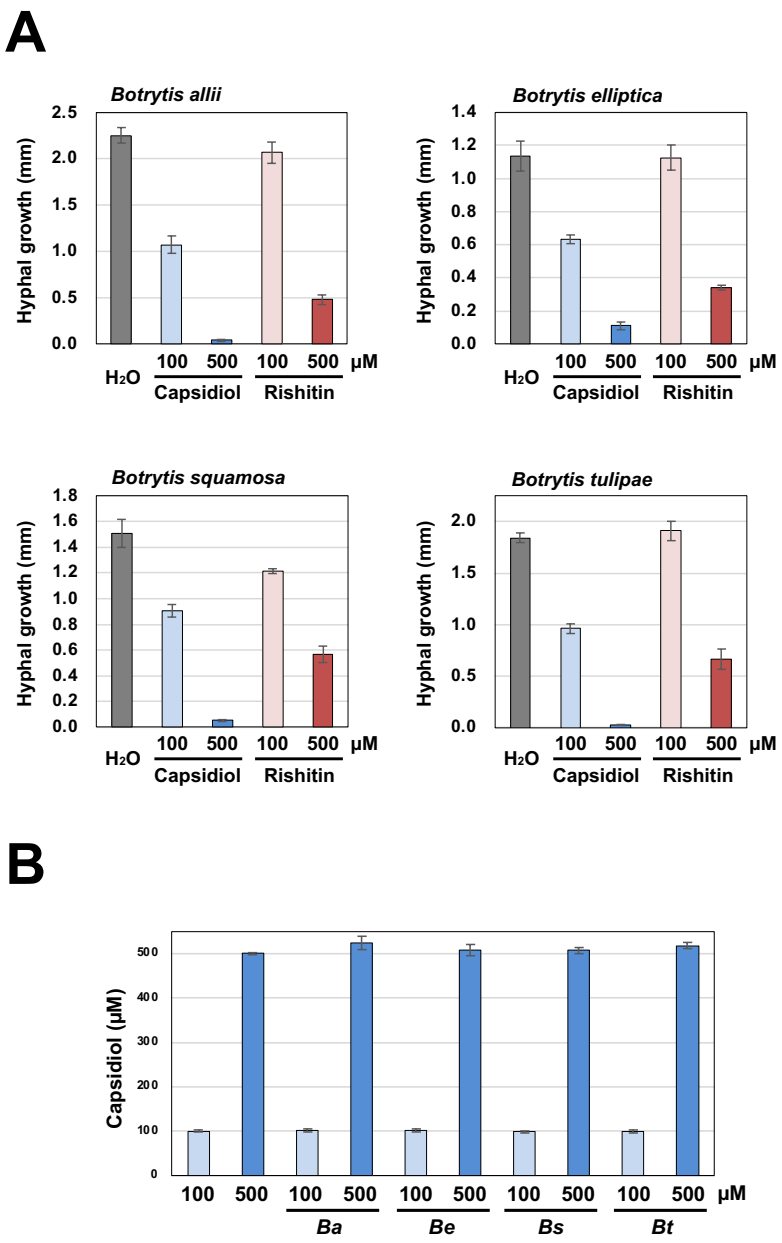

Fig. S21. Sensitivity and metabolic capacity of sesquiterpenoid phytoalexins in *Botrytis* species. **(A)** Mycelial blocks (approx. 1 mm^3) of the indicated pathogen were incubated in 50 µl water, 100 or 500 µM capsidiol or rishitin. Growth of hyphae from the mycelial block was measured after 24 h incubation (n = 6). **(B)** Residual capsidiol was quantified after 48 h incubation (n = 3). *Ba*, *B. allii* (isolated from onion); *Be*, *B. elliptica* (*Lilium* sp.); *Bs*, *Botrytis squamosa* (Chinese chive); *Bt, B. tulipae* (tulip).

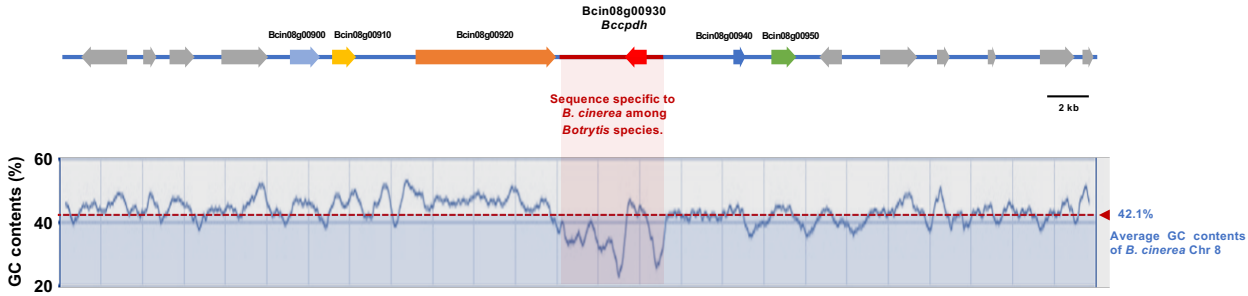

Fig. S22 GC content plot (window size 500 bp) of *B. cinerea* genomic region surrounding *Bccpdh* gene (Bcin08g00930) in chromosome 8. The average GC content of chromosome 8 (42.1%) is indicated by a dotted red line.

Supplementary Note 6

CPDH orthologs in the fungal kingdom

CPDH orthologs were found in some Ascomycota fungi. Based on the genes surrounding the *CPDH* orthologs, conserved synteny of the loci was found among different species. Phylogenetic analysis of *CPDH* orthologs indicates that sequence similarity did not necessarily correlate with the taxonomic relationship. Rather, *CPDH* orthologs of the same cluster type tend to form a clade in the phylogenetic tree, which might indicate *CPDH* orthologs (and surrounding genes) were transferred via multiple horizontal gene transfer (HGT) events. For *Fusarium* species, *CPDH* orthologs were detected in species of three species complexes (*F. fujikuroi*, *F. oxysporum* and *F. solani* species complexes), consistent with Stoessl et al. (1973) that reported *F. oxysporum* and *F. solani* can metabolize capsidiol to capsenone. However, cluster types of three species complexes are different, which may indicate that these *Fusarium* species complexes obtained *CPDH* orthologs by independent HGT events. *Bccpdh* locus in *B. cinerea* doesn't show similarity with other *cpdh* clusters, suggesting that *B. cinerea* might obtain ancestral *Bccpdh* independently from an unidentified organism.

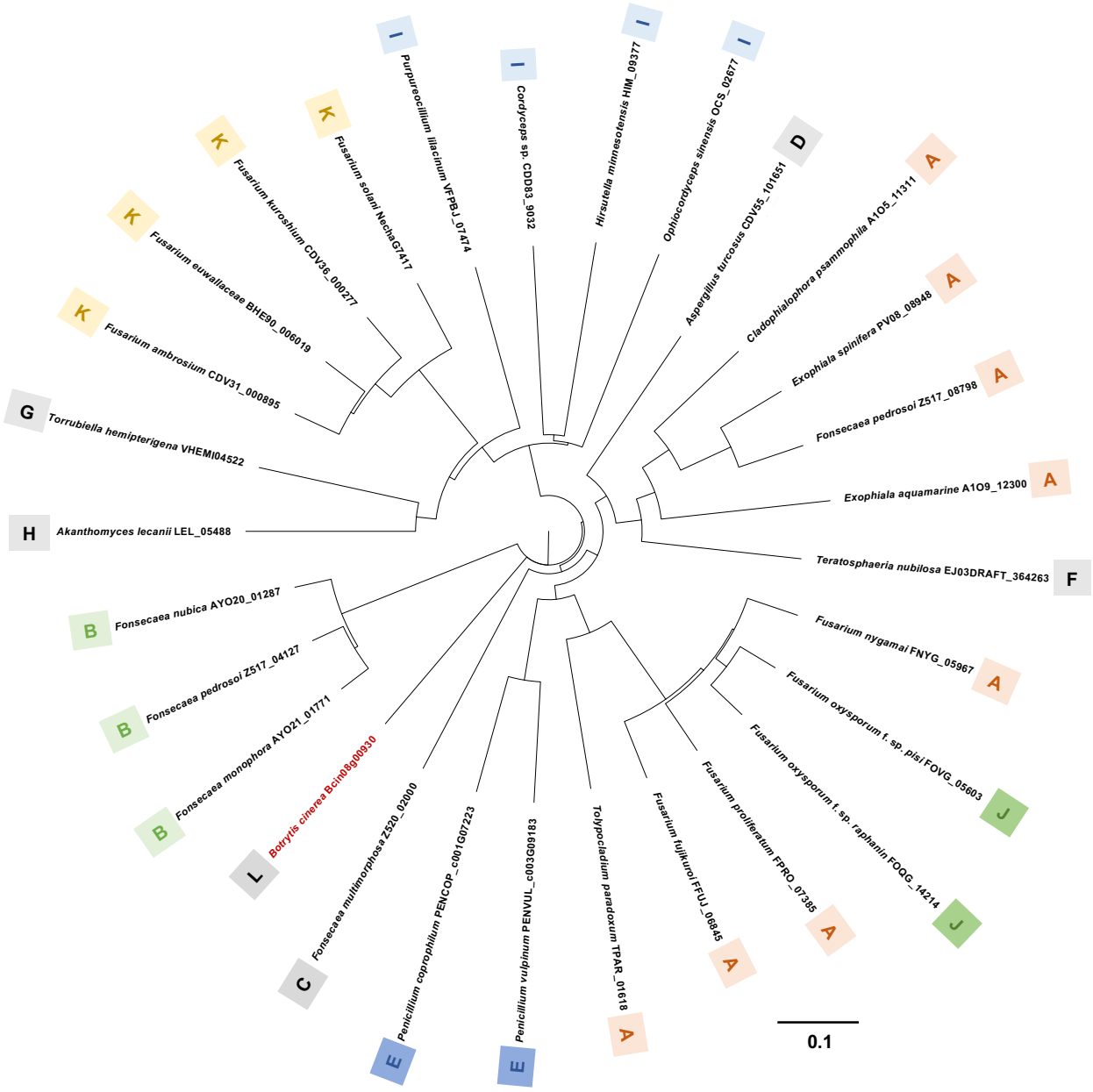

Fig. S23. A phylogenetic tree of BcCPDH orthologs from Ascomycota fungi. The deduced amino acid sequences of CPDH orthologs were aligned by ClustalW (Thompson et al., 1994), and the phylogenetic tree was constructed using the neighbor-joining (NJ) method (Saitou and Nei, 1987). The scale bar corresponds to 0.1 estimated amino acid substitutions per site. Cluster types (A to L) classified based on the conservation of genes around CPDH orthologs in the genome are indicated (See Figs. S20, 24 and 25).

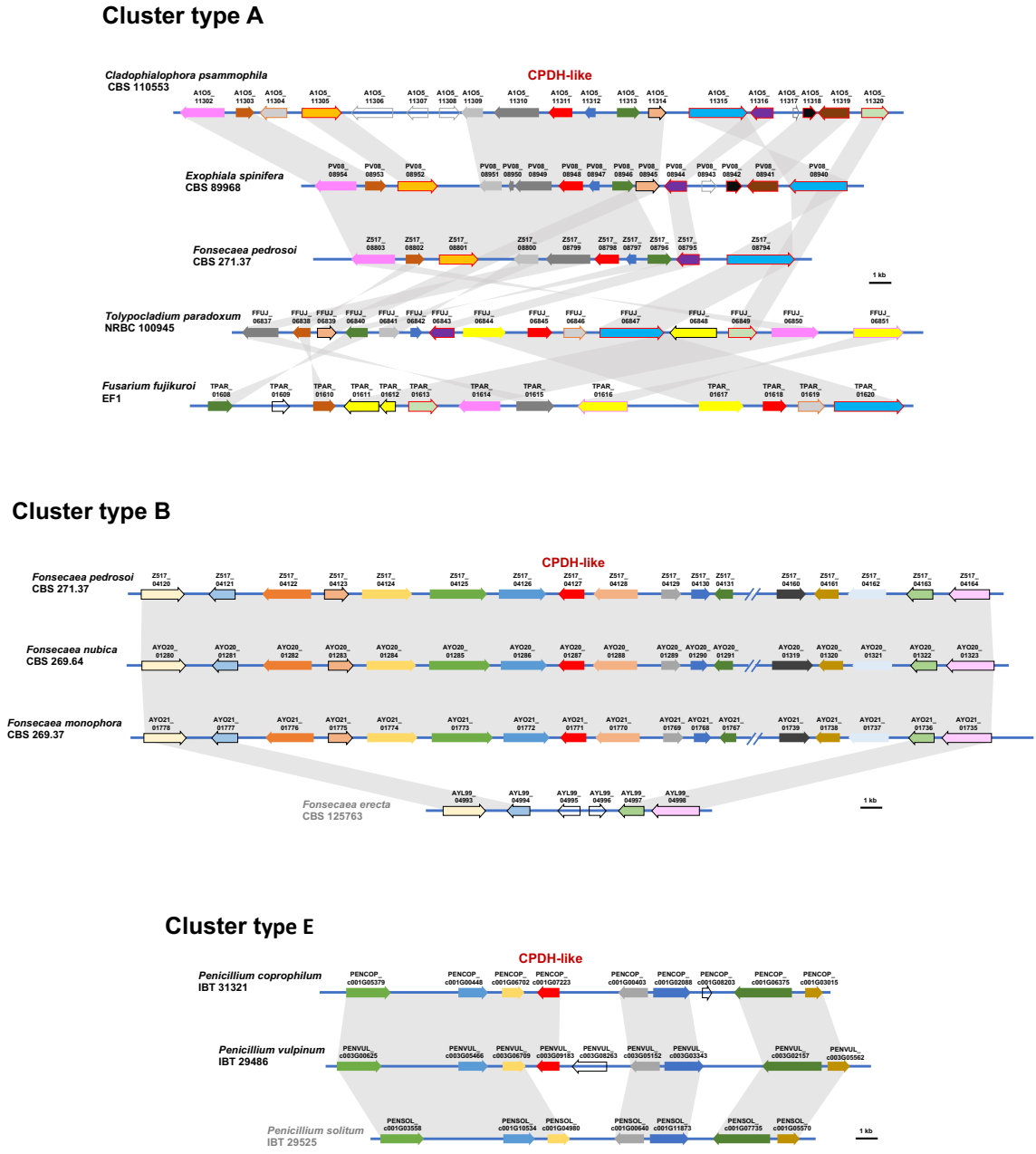

Fig. S24. Conserved synteny of the loci containing fugal CPDH orthologues in cluster types A, B and E (See Fig. S20). The matching colors in each cluster type indicate orthologous genes. Genes encoding CPDH orthologues are shown as red arrows. Scale bars = 1 kb.

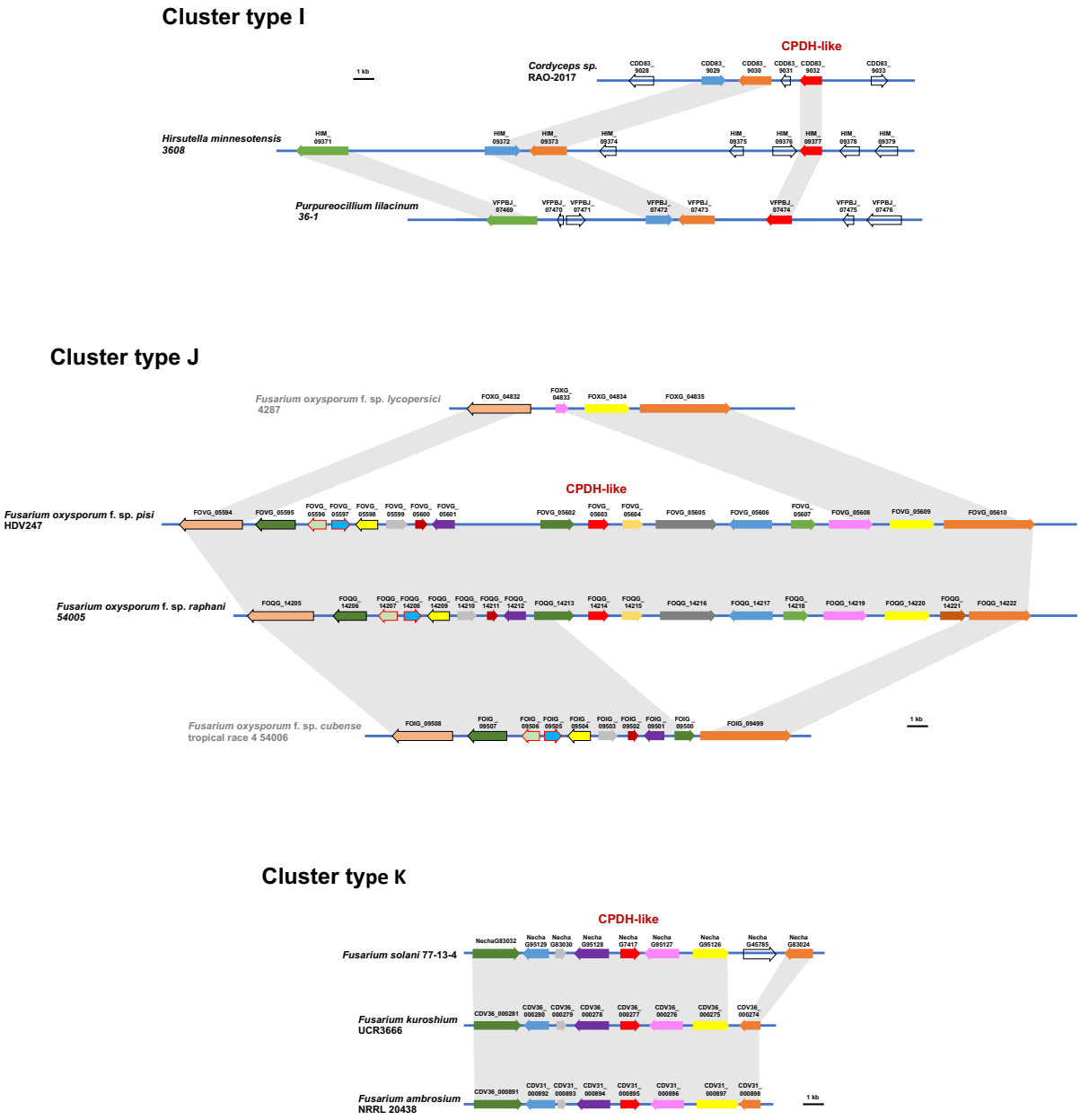

Fig. S25. Conserved synteny of the loci containing fugal CPDH orthologues in cluster types I, J and K (See Fig. S20). The matching colors in each cluster type indicate orthologous genes. Genes encoding CPDH orthologues are shown as red arrows. Scale bars = 1 kb.

Table S5. CPDH activity in *B. cinerea* strains isolated form diferent plant species.

Isolated from	Strain name	Isolated		CPDH activity
		Location*	Year	
Tomato	2019052	Mie	2019	+
Tomato	2018107	Mie	2018	+
Tomato	2017017	Mie	2017	+
Tomato	NBc1	Aichi	2009	+
Eggplant	2019001	Mie	2019	+
Eggplant	2018018	Mie	2018	+
Eggplant	2017017	Mie	2017	+
Strawberry	AI18	Mie	2018	+
Strawberry	2019086	Mie	2019	+
Strawberry	2018105	Mie	2018	+
Strawberry	2017100	Mie	2017	+
Cucumber	2019101	Mie	2019	+
Cucumber	2018152	Mie	2018	+
Cucumber	2017149	Mie	2017	+
Asparagus	HSB3	Hokkaido	2011	+
Lettuce	KBC-2	Kawaga	2012	+
Pea	T.K-26-1	Ibaraki	1995	+
Rose	T.K-31-3	Niigata	1996	+
Barley	TAC96-O1	Toyama	1996	+
Bitter orange	S-1-1	Wakayama	2003	+
Flowering dogwood	LFP-BB-6	Fukuoka	1982	+
Okra	Okurami-2	Mie	2007	+
Morus sp.	Y-1	Yamagata	1980	+
Vitis sp.	4519-1	Akita	1986	+

*Prefecture name in Japan.

Table S6. CPDH activity in *Fusarium oxysporum* strains isolated form diferent plant species.

forma specialis	Host plant	Strain name	Isolated		CPDH activity
			Location*	Year	
cucumerinum	Cucumber	Cu:8-1	Toyama	1990	+
cucumerinum	Cucumber	KF-13	Aomori	1981	+
cucumerinum	Cucumber	1-19	Kyoto	1988	-
cucumerinum	Cucumber	JPPAC 10	Fukuoka	1978	+
cucumerinum	Cucumber	MG1126	Miyagi	unknown	+
melonis	Melon	2-10	Nagasaki	1991	-
melonis	Melon	B-1	Kanagawa	1990	+
melonis	Melon	Mel02010	Nagasaki	unknown	+
melonis	Hami melon	2-32	Yamagata	unknown	-
lagenariae	Calabash	KF-01	Aomori	1978	+
lagenariae	Calabash	Lag:4-1	Wakayama	unknown	+
lagenariae	Calabash	Lag:6-1	Kumamoto	1979	-
lycopersici	Tomato	9859-1	Aichi	unknown	+
niveum	Watermelon	03-05543	Shizuoka	1963	-
niveum	Watermelon	Niv:1-0	Fukuoka	unknown	-
niveum	Watermelon	80WF-2	Kagoshima	1988	-
momordicae	Bitter melon	90NF1-2	Kagoshima	unknown	-
momordicae	Bitter melon	24-11	Kagoshima	1994	-
raphani	Daikon radish	03-05123	unknown	unknown	+

*Prefecture name in Japan.

Supplementary Note 7

Materials and Methods

Biological material, growth conditions and incubation in phytoalexins.

Fungal and oomycete strains used in this study were listed in Tables S7, S8 and S9. They were grown on potato dextrose agar (PDA), rye media or V8 agar as indicated in the Tables at 23°C. For the incubation of fungal or oomycete strains in phytoalexins, mycelia blocks (approx. 1 mm^3) were excised from the growing edge of the colony on indicated media using a dissection microscope (Stemi DV4 Stereo Microscope, Carl Zeiss, Oberkochen, Germany) and submerged in 50 µl of water or indicated phytoalexin in a sealed 96 well clear plate. The plate was incubated at 23˚C for the indicated time and outgrowth of hyphae was monitored under light microscope BX51 (Olympus, Tokyo, Japan) and measured using ImageJ software (Schneider et al., 2012). Capsidiol, capsidiol 3-acetate and debneyol were purified from *Nicotiana tabacum* as previously reported (Matsukawa *et al.*, 2013) and synthesized rishitin (Murai *et al.* 1975) was provided from former Prof. Akira Murai (Hokkaido University, Japan). Resveratrol and scraleol are obtained from Sigma-Aldrich (Burlington, MA, USA).

Table S7. Oomycete and fungal strains used in this study.

Fungal species	Strain	Origin	Media	References
Oomycete strains				
Phytophthora infestans	08YB1	Potato	Rye	Shibata *et al.* 2010
Phytophthora nicotianae	Pn96	Tobacco	V8	MAFF305940*
Phytophthora capsici	CH01CMP1	Green pepper	V8	MAFF242869*
Phytophthora cryptogea	CH88-18	Nipplefruit	V8	MAFF306435*
Fungal strains				
Alternaria solani	KL1	Potato	PDA	MAFF244036*
Colletotrichum coccodes	PTK1	Potato	PDA	MAFF243012*
Fusarium coeruleum	K. Kita 37	Potato	PDA	MAFF235977*
Gibellulopsis nigrescens	Kita44	Potato	PDA	MAFF235985*
Rhizoctonia solani	NR19	Potato	PDA	MAFF237435*
Sclerotinia sclerotiorum	SU-1	Eggplant	PDA	MAFF744080*
Stemphylium lycopersici	KuNBY1	Tobacco	PDA	MAFF306895*
Cercospora nicotianae	CTC5	Tobacco	PDA	MAFF243736*
Alternaria brassicicola	BA31	Broccoli	PDA	MAFF242993*
Fusarium graminearum s. str	407011	Wheat	PDA	Suga *et al.* 2016
Fusarium verticillioides	Maize L-2	Maize	PDA	MAFF240086*
Botrytis allii	Yuki11-1	Onion	PDA	MAFF307143*
Botrytis tulipae	4-3	Tulip	PDA	MAFF245230*
Botrytis squamosa	5ND4	Chinese chive	PDA	MAFF244973*
Botrytis elliptica	S0210	*Lilium* sp.	PDA	MAFF306626*

*MAFF No. of strains obtained from stock center of Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan.

Table S8. *Botrytis cinerea* strains used in this study.

Fungal species	Strain	Host plant	Media	References
Botrytis cinerea	NBc1	Tomato	PDA	Tsuge unpublished
	2019052	Tomato	PDA	Kawakami unpublished
	2018107	Tomato	PDA	Kawakami *et al.* 2019
	2017017	Tomato	PDA	Kawakami *et al.* 2019
	2019001	Eggplant	PDA	Kawakami unpublished
	2018018	Eggplant	PDA	Kawakami *et al.* 2019
	2017017	Eggplant	PDA	Kawakami *et al.* 2019
	AI18	Strawberry	PDA	This study
	2019086	Strawberry	PDA	Kawakami unpublished
	2018105	Strawberry	PDA	Kawakami *et al.* 2019
	2017100	Strawberry	PDA	Kawakami *et al.* 2019
	2019101	Cucumber	PDA	Kawakami unpublished
	2018152	Cucumber	PDA	Kawakami *et al.* 2019
	2017149	Cucumber	PDA	Kawakami *et al.* 2019
	HSB3	Asparagus	PDA	MAFF243107*
	KBC-2	Lettuce	PDA	MAFF307162*
	T.K-26-1	Pea	PDA	MAFF237249*
	T.K-31-3	Rose	PDA	MAFF237516*
	TAC96-O1	Barley	PDA	MAFF237696*
	S-1-1	Bitter orange	PDA	MAFF306809*
	LFP-BB-6	Flowering dogwood	PDA	MAFF410003*
	Okurami-2	Okra	PDA	MAFF731108*
	Y-1	*Morus* sp.	PDA	MAFF840049*
	4519-1	Vitis sp.	PDA	MAFF615005*

*MAFF No. of strains obtained from stock center of Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan.

Table S9. *Fusarium oxysporum* strains used in this study.

Fungal species	forma specialis	Strain	Host plant	Media	References
Fusarium oxysporum	*cucumerinum*	Cu:8-1	Cucumber	PDA	Namiki *et al.* 1994
	cucumerinum	KF-13	Cucumber	PDA	Kuwata unpublished
	cucumerinum	1-19	Cucumber	PDA	Fukunishi unpublished
	cucumerinum	JPPAC 10	Cucumber	PDA	Kiso unpublished
	cucumerinum	MG1126	Cucumber	PDA	Honkura unpublished
	melonis	2-10	Melon	PDA	Sakaguchi unpublished
	melonis	B-1	Melon	PDA	Namiki *et al.* 1994
	melonis	Mel02010	Melon	PDA	Namiki *et al.* 1994
	melonis	2-32	Hami melon	PDA	Yuki unpublished
	lagenariae	KF-01	Calabash	PDA	Kuwata unpublished
	lagenariae	Lag:4-1	Calabash	PDA	Kobayashi unpublished
	lagenariae	Lag:6-1	Calabash	PDA	Kobayashi unpublished
	lycopersici	9859-1	Tomato	PDA	Matsusaki unpublished
	niveum	03-05543	Watermelon	PDA	Namiki *et al.* 1994
	niveum	Niv:1-0	Watermelon	PDA	Namiki *et al.* 1994
	niveum	80WF-2	Watermelon	PDA	Namiki *et al.* 1994
	momordicae	90NF1-2	Bitter melon	PDA	Namiki *et al.* 1994
	momordicae	24-11	Bitter melon	PDA	Yamaguchi unpublished
	raphani	03-05123	Daikon radish	PDA	Namiki *et al.* 1994

Quantitative analysis of phytoalexins by GCMS.

For the quantification of phytoalexins after the incubation with pathogens, the supernatant (50 µl) was collected, mixed with 50 µl ethyl acetate by vortexing for 1 min, and phytoalexin extracted in the organic solvent were collected and quantified by GC/MS using an Agilent Technologies 7890A GC System with a DuraBond Ultra Inert column (length 30 m; diameter 0.25 mm; film 0.25 µm, Agilent Technologies, Santa Clara, CA, USA) as previously described (Camagna *et al.* 2020). Pure capsidiol and rishitin were used for quantitative standards.

Detection of phytoalexins and their metabolites using LC/MS.

For the detection of phytoalexins and their metabolites after the incubation with pathogens, the supernatant (50 µl) was collected, mixed with 50 µl acetonitrile and measured by LC/MS (Accurate-Mass Q-TOF LC/MS 6520, Agilent Technologies) with ODS column Cadenza CD-C18, 75 x 2 mm (Imtakt, Kyoto, Japan).

Extraction of RNA and RNAseq analysis.

Mycelial blocks (approx. 1 mm^3, 100 pieces) were incubated in 10 ml of CM media [1 g $Ca(NO_3)^2$, 0.2 g KH_2PO_4, 0.25 g $MgSO_4$, 0.15 g NaCl, 500 µl Micronutrient solution (Sanderson and Srb 1965), 1 g yeast extract, 1 g peptone /1L] with or without indicated concentration of phytoalexins at 23˚C for 24 h with gentle shaking (100 rpm) and frozen in liquid nitrogen. The frozen mycelia were ground using mortar and pestle, and the total RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The quality and quantity of isolated RNA were evaluated using Qubit RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The mRNA was purified with NEBNext Poly(A) mRNA magnetic isolation module (New England Biolabs, Ipswich, MA, USA) and used for the construction of cDNA libraries using the NEBNext Ultra II RNA library prep kit for Illumina and NEBNext Multiplex oligos for Illumina (New England Biolabs) according to the manufacturer's instructions. RNA-Seq libraries were sequenced using Illumina NextSeq 500 (Illumina, San Diego, CA, USA) with single-read mode. The nucleotides of each read with less than 13 quality value were masked and reads less than 50 bp in length were discarded before mapping. The filtered reads were mapped to annotated cDNA sequences for *B. cinerea* (Botrytis_cinerea.ASM83294v1.cdna.all.fa, http://fungi.ensembl.org/Botrytis_cinerea/Info/Index) using Bowtie software (Langmead *et al.* 2009) and the number of reads mapping to each annotated cDNA was counted. For each gene, the relative fragments per kilobase of transcript per million mapped reads (FPKM) values were calculated and significant

difference from the control was assessed by the two-tailed Student's *t*-test. RNA-seq data reported in this work are available in GenBank under the accession numbers DRA013980.

Extraction of genomic DNA, PCR and construction of vectors

Genomic DNA of *E. festucae* and *B. cinerea* was isolated from fungal mycelium grown in potato dextrose broth (PDB) as described previously (Byrd *et al.,* 1990) or using DNeasy Plant Mini Kit (QIAGEN). PCR amplification from genomic and plasmid DNA templates was performed using PrimeStar Max DNA polymerase (Takara Bio, Kusatsu, Japan) or GoTaq Master Mix (Promega, Madison, WI, USA). Vectors for heterologous expression, detection of promoter activity, gene knock out and complementation used in this study are listed in Table S10. Sequences of primers used for the construction of vectors and PCR to confirm the gene knockout are listed in Table S11.

Table S10. Plasmids used in this study

Vector name	Base vector	Restriction sites used	Insert	Primers used to amplify insert	References	Note
Base vectors						
pPN94	-	-	-	-	Takemoto *et al.* 2006	Base vector for gene expression under TEF promoter, Amp^R/Hyg^R
pNPP150	-	-	-	-	Niones and Takemoto 2015	Base vector for gene knockout (*HSVtk* marker), Amp^R/Gen^R
pNPP170	pNPP150	*Eco* RI/ *Sma* I	P_*BcactA* (Bcin16g02020)	IF-BcPact-F, IF-BcPact-R2	This study	Base vector for expression of gene under the control of *BcactA* promoter. Base vector for the construction of pNPP170-BcGFP, Amp^R/Hyg^R
pNPP170-BcGFP	pNPP170	*Not* I	BcGFP[1]	IF pNPP170-BcGFP-F, IF pNPP170-BcGFP-R	This study	Base vector for the promoter analysis using BcGFP marker, Amp^R/Hyg^R
pNPP210	pNPP170-BcGFP	*Eco* RI	OflucBK[2]	IF-OflucBK-F IF-OflucBK-R	This study	Base vector for promoter analysis using Luciferase marker, Amp^R/Hyg^R
pSF17.1	-		-	-	Tanaka *et al.* 2008	Base vector for complementation, Amp^R/Gen^R
Plasmids for heterologous expression of *B. cinerea* gene in *E. festucae*						
pNPP196 (pPN94-Bcin08g00930)	pPN94	*Bam* HI/ *Not* I	Bcin08g00930	pPN94-Bc08g00930-F, pPN94-Bc08g00930-R	This study	Constitutive expression of Bcin08g00930 (*Bccpdh*)
pNPP197 (pPN94-Bcin12g01750)	pPN94	*Bam* HI/ *Not* I	Bcin12g01750	pPN94-Bc12g01750-F, pPN94-Bc12g01750-R	This study	Constitutive expression of Bcin12g01750
pNPP201 (pPN94-Bcin16g01490)	pPN94	*Bam* HI/ *Not* I	Bcin16g01490	pPN94-p450-Ch16-F, pPN94-p450-Ch16-R	This study	Constitutive expression of Bcin16g01490
Plasmids for promoter analysis in *B. cinerea*						
pNPP202 (pNPP170-P_Bcin08g00930-BcGFP)	pNPP170-BcGFP	*Sac* I/ *Sma* I	P_*Bccpdh* (1 kb)	pNPP170-P_8g00930-F, pNPP170-P_8g00930-R	This study	Expression of GFP under the control of 1 kb *Bccpdh* promoter
pNPP203 (pNPP170-P_Bccpdh(750)-BcGFP)	pNPP170-BcGFP	*Sac* I/ *Sma* I	P_*Bccpdh* (750 bp)	pNPP170-P_8g00930-750F, pNPP170-P_8g00930-R	This study	Expression of GFP under the control of 750 bp *Bccpdh* promoter
pNPP204 (pNPP170-P_Bccpdh(500)-BcGFP)	pNPP170-BcGFP	*Sac* I/ *Sma* I	P_*Bccpdh* (500 bp)	pNPP170-P_8g00930-500F, pNPP170-P_8g00930-R	This study	Expression of GFP under the control of 500 bp *Bccpdh* promoter
pNPP205 (pNPP170-P_Bccpdh(250)-BcGFP)	pNPP170-BcGFP	*Sac* I/ *Sma* I	P_*Bccpdh* (250 bp)	pNPP170-P_8g00930-250F, pNPP170-P_8g00930-R	This study	Expression of GFP under the control of 250 bp *Bccpdh* promoter
pNPP206 (pNPP170-P_Bccpdh(200)-BcGFP)	pNPP170-BcGFP	*Sac* I/ *Sma* I	P_*Bccpdh* (200 bp)	pNPP170-P_8g00930-200F, pNPP170-P_8g00930-R	This study	Expression of GFP under the control of 200 bp *Bccpdh* promoter
pNPP207 (pNPP170-P_Bccpdh(100)-BcGFP)	pNPP170-BcGFP	*Sac* I/ *Sma* I	P_*Bccpdh* (100 bp)	pNPP170-P_8g00930-100F, pNPP170-P_8g00930-R	This study	Expression of GFP under the control of 100 bp *Bccpdh* promoter
pNPP211 (pNPP210-P_Bccpdh(250)-Luc)	pNPP210	*Eco* RI	P_*Bccpdh* (250 bp)	pNPP210-P_8g00930-250F, pNPP210-P_8g00930-R	This study	Expression of Luciferase under the control of 250 bp *Bccpdh* promoter
Plasmids for gene knockout and complimentation of *Bccpdh*						
pNPP198 (pNPP150-Bccpdh-KOver5)	pNPP150	*Sal* I/ EcoRI	5'*Bccpdh* -PtrpC-hph-3'*Bccpdh*	cpdh-KO5-Fo, cpdh-KO5-Ro, cpdh-KO3-F5, cpdh-KO3-R5	This study	Knockout vector for *Bccpdh*
pNPP199 (pSF17-*Bccpdh*)	pSF17.1	*Eco* RV	*Bccpdh* locus (2 kb)	pSF17-BcCPDH-F pSF17-BcCPDH-R	This study	Complimentation vector of *Bccpdh*

[1] *GFP* gene synthesized to optimize codon usage for *B. cinerea* (Leroch *et al.* 2011).

[2] *Luciferase* gene synthesized to optimize codon usage for fungi (Gooch *et al.* 2008). Modified to remove a restriction site (Murata *et al.* unpublished).

Table S11. Primers used in this study.

Primer name	Sequence 5'→3'
Primers for sequencing and comformation of gene knockout	
pII99-3	GGCTGGCTTAACTATGCG
PtrpC-2	CAAATTTTGTGCTCACCG
hph-seqR	ACTTCGAGCGGAGGCATC
Ptef-seq	TAACCTCTCTTCAGAAAG
TtrpC-seq	TCTGGAAGAGGTAAACCCG
BcGFP-seqR	CTTATGGCCATTGACGTCAC
cpdh-RC-F	CAGCACTTTGAGCTGATACG
cpdh-RC-R	AGTTCCTAAAGTTGTAAAGCC
cpdh-CF3	GGCTCTCATCAAGGATATCC
Primers for construction of Base vectors	
IF-BcPact-F	TACCGAGCTCGAATTCGATGTGCGTCCTCTTCTGC
IF-BcPact-R2	ACGTTAAGTGCGGCCGCGGTTGATAAATTAAGACG
IF pNPP170-BcGFP-F	TTATCAACCGCGGCCCCCGGGTTCACCATGGTTTC
IF pNPP170-BcGFP-R	ACGTTAAGTGCGGCCGAATTCCTATTTGTAAAGTT
IF-OflucBK-F	TACCGAGCTCGAATTCATGGAGGACGCCAAGAACA
IF-OflucBK-R	AAGTGCGGCCGAATTTCAGAGCTTGGACTTGCCGC
Primers for construction of vectors for heterologous expression	
pPN94-Bc08g00930-F	AACCTCTAGAGGATCATGGCAGCACTATCACTCAA
pPN94-Bc08g00930-R	ACGTTAAGTGCGGCCCTAAAGTTGTAAAGCCTGAA
pPN94-Bc12g01750-F	AACCTCTAGAGGATCCGATGAACTCCATTACAGCT
pPN94-Bc12g01750-R	ACGTTAAGTGCGGCCTCATGAAGTTCTCAACGTCC
pPN94-p450-Ch16-F	AACCTCTAGAGGATCATGTCGCCAGCACTCTTCGA
pPN94-p450-Ch16-R	ACGTTAAGTGCGGCCCTCCTCTCCAACTTTTAGGC
Primers for construction of vectors for promoter analysis	
pNPP170-P_8g00930-F	CCAAGCTGGGTACCGCTAGACACCTTCTTGGAACA
pNPP170-P_8g00930-R	AACCATGGTGAACCCTTCGATTGCTTTCAATAGTG
pNPP170-P_8g00930-750F	CCAAGCTGGGTACCGTATAAAAAAAGTATGAATTG
pNPP170-P_8g00930-500F	CCAAGCTGGGTACCGGCTTCCCTCTAAATGCTTCA
pNPP170-P_8g00930-250F	CCAAGCTGGGTACCGGTGATAACTTATGATTAAGT
pNPP170-P_8g00930-200F	CCAAGCTGGGTACCGGACCGCCAAGAAGTAGACAT
pNPP170-P_8g00930-100F	CCAAGCTGGGTACCGCCATAATATCTTATGAGTTT
pNPP210-P_8g00930-250F	TACCGAGCTCGAATTGTGATAACTTATGATTAAGT
pNPP210-P_8g00930-R	CGTCCTCCATGAATTTTCGATTGCTTTCAATAGTG
Primers for construction of knockout and complimentation vectors	
cpdh-KO5-Fo	ATGCCTGCAGGTCGAAGGATAATAGCGGCGTATGA
cpdh-KO5-Ro	ATCCTCTAGAGTCGAATCTAAGCCCCCAAGCTTCT
cpdh-KO3-F5	TACCGAGCTCGAATTGTCGTAACTTCCTCAAACTC
cpdh-KO3-R5	TATCATCGATGAATTTGTAAAGCCTGAACAGGAGC
pSF17-BcCPDH-F	GAATTCATCGATGATCTAGACACCTTCTTGGAACA
pSF17-BcCPDH-R	ACCGGCAGATCTGATTCCTAAAGTTGTAAAGCCTG

Extension sequence for in-fusion reaction are shown in red letters.

Fungal transformation

Protoplasts of *E. festucae* were prepared as follows. Mycelial blocks of *E. festucae* (approx. 1 mm^3, 100 pieces) were added to 50 ml PDB media in 100 ml Erlenmeyer flask and shaken for 3 to 4 days at 23˚C, 100 rpm. Mycelia from 3 flasks were collected by centrifugation at 3,000 x g for 10 min and suspended in 30 ml of OM buffer [1.2 mM $MgSO^4$, 10 mM phosphate buffer, pH5.8]. Mycelia were then collected by filtration using an 80-mesh nylon cloth, and suspended in 10 ml of enzyme solution [10 mg/ml lysing Enzymes (Sigma-Aldrich), 5 mg/ml Kitalase (Wako Pure Chemicals) in OM buffer] in 50 ml falcon tube and shaken at 28°C, 80 rpm for approx. 3 h. After removing undigested mycelia by filtration with a 200 mesh nylon cloth, 30 ml of 0.7 M NaCl was added and the protoplasts were precipitated by centrifugation at 3,000 x g for 5 min. The precipitated protoplasts were suspended in 20 ml of STC [1 M sorbitol, 50 mM Tris-HCl (pH 8.0), 50 mM $CaCl_2$] and the solution was centrifuged at 3,000 x g for 5 min. The precipitated protoplasts were resuspended in the STC solution to approx. 2.5 × 10^8 protoplasts/ml and mixed with 40% PEG solution [40% polyethylene glycol 4000 (Wako Pure Chemicals), 1 M sorbitol, 50 mM Tris-HCl (pH 8.0), 50 mM $CaCl_2$,] at 4:1. Aliquoted protoplast solution (100 µl, 2 x 10^8 /ml) was stored at -80°C until use.

Protoplasts of *B. cinerea* were prepared as follows. To induce the sporulation of *B. cinerea*, colonies grown on PDA in 90 mm Petri dishes were exposed to BLB blacklight (Peak wavelength 352 nm) for approx. 2 weeks. Sterile water (5-10 ml) was added to the Petri dishes and spores were released using a spreader from the mycelial surface. Spores (approx. 2 x 10^6) were added to 50 ml PDB media in 100 ml Erlenmeyer flask and shaken for 16 h at 23˚C, 100 rpm. Germinated hyphae were collected by centrifugation at 3,000 x g for 5 min, suspended in 20 ml of 0.7 M NaCl, and centrifuged at 3,000 x g for 5 min. The collected hyphae from 2 flasks were suspended in 5 ml of enzyme solution [10 mg/ml lysing Enzymes (Sigma-Aldrich), 5 mg/ml Kitalase (Wako Pure Chemicals) in 0.7 M NaCl] and shaken at 28°C, 80 rpm for approx. 3 h. After removing undigested mycelia by filtration with a 200 mesh nylon cloth, 15 ml of 0.7 M NaCl was added and the protoplasts were precipitated by centrifugation at 3,000 x g for 5 min. The protoplasts were suspended in 20 ml of STC and the solution was centrifuged at 3,000 x g for 5 min. The precipitated protoplasts were resuspended in the STC solution to approx. 2.5 × 10^8 (or lower) protoplasts/ml and mixed with 40% PEG solution at 4:1. Aliquoted protoplast solution (100 µl, 2 x 10^8 /ml or lower) was stored at -80°C until use.

Protoplasts of *E. festucae* or *B. cinerea* (100 µl) were mixed with 5 µg of either circular or linear (for gene KO) plasmids (<100 µl) and incubated on ice for 30 min. The mixture of protoplasts and plasmid DNA was gently mixed with 900 µl of PEG solution and further

incubated on ice for 20 min. Aliquots (100 µl) of the protoplast suspension were mixed with 3 ml of 0.8% YPSA media [0.1% yeast extract, 0.1% tryptone, 34.2% sucrose, 0.8% agar] melted and warmed to 50°C, and immediately poured into 90 mm Petri dishes containing approx. 10 ml of YPSA media (1.8% agar). Plates were incubated overnight at 23°C and overlaid with melted (and then cooled to 50°C) PDA containing 150 µg/ml (for *E. festucae*) or 75 µg/ml (for *B. cinerea*) hygromycin B or 400 µg geneticin (for *B. cinerea*). Plates were incubated at 23°C until colonies emerged, which were sub-cultured on PDA containing appropriate antibiotics.

For the isolation of *B. cinerea* knockout strains, candidate colonies were exposed to BLB blacklight for the induction of sporulation. Single spore isolation was performed to obtain purified knockout strains. Note that *Δbccpdh*-40 and -52 were isolated from separate transformation experiments. Transformants of *E. festucae* and *B. cinerea* used in this study are listed in Table S12.

Table S12. Transformants used in this study.

Strains	**Relevant characteristics**	**References**
Epichloë festucae		
WT-DsRed	Fl1/pNPP94-DsRed; Hyg^R	Kayano et al., 2013
Ef-Bcin08g00930	Fl1/pNPP196 ; Hyg^R	This study
Ef-Bcin12g01750	Fl1/pNPP197 ; Hyg^R	This study
Ef-Bcin16g01490	Fl1/pNPP201 ; Hyg^R	This study
Botrytis cinerea		
P_*Bccpdh:GFP*	AI18/pNPP202 ; Hyg^R	This study
P_*Bccpdh* (750)*:GFP*	AI18/pNPP203 ; Hyg^R	This study
P_*Bccpdh* (500)*:GFP*	AI18/pNPP204 ; Hyg^R	This study
P_*Bccpdh* (250)*:GFP*	AI18/pNPP205 ; Hyg^R	This study
P_*Bccpdh* (200)*:GFP*	AI18/pNPP206 ; Hyg^R	This study
P_*Bccpdh* (100)*:GFP*	AI18/pNPP207 ; Hyg^R	This study
P_*Bccpdh* (250)*:Luc*	AI18/pNPP211 ; Hyg^R	This study
Δbccpdh-52	Fl1/*Δbccpdh*::P*trpC*-*hph* ; Hyg^R	This study
Δbccpdh-40	Fl1/*Δbccpdh*::P*trpC*-*hph* ; Hyg^R	This study
Δbccpdh/Bccpdh-1	*Δbccpdh*-52/pNPP199; Hyg^R, Gen^R	This study

Pathogen inoculation

Leaves, fruits (bell pepper) or tuber (potato) of plant species were kept in moistened and sealed in a plastic chamber. Leaves detached from the plant were covered with a wet Kimwipes at the cut end of the stem. Mycelial blocks (approx. 5 mm^3) of *B. cinerea* were excised from the growing edge of the colony grown on PDA and placed on the downside of the leaf or on the fruit and tuber and covered with wet lens paper. For the inoculation on bell pepper fruits, the surface of the fruits was injured by a needle beneath the placed mycelial block. For the inoculation on *N. benthamiana*, mycelial blocks of *B. cinerea* were placed on the upside of leaves attached to the plant body and the plant was kept at high humidity at 23 ˚C for 1 day after the inoculation, and then moved to a growth room at 23 ˚C.

For the inoculation of *B. cinerea* spores (Fig. 4), *B. cinerea* spore suspension (1 x 10^4/ml) in glucose-phosphate solution (10 mM glucose, 10 mM NaH_2PO_4) was placed on the downside of *N. benthamiana* leaves in sealed plastic chambers, covered with wet lens paper, and incubated at 23 ˚C for indicated time.

Microscopy

Images of *B. cinerea* strains expressing GFP or hyphae stained with Calcofluor white (Sigma-Aldrich) were captured using a confocal laser scanning microscope FV1000-D (Olympus, Japan). The laser for detection of GFP was used as the excitation source at 488 nm, and GFP fluorescence was recorded between 515 and 545 nm. The laser for detection of Calcofluor white was used as the excitation source at 405 nm, and fluorescence was recorded between 425 nm and 475 nm.

Detection of luciferase activity of *B. cinerea* P_*Bccpdh*:*Luc* transformant

B. cinerea P_*Bccpdh*:*Luc* transformant was grown on PDA at 23°C. Three mycelia blocks (approx. 2 mm^3) were excised from the growing edge of the colony and submerged in 50 µl of water or indicated phytoalexin containing 50 µM D-luciferin in a sealed 96-well microplate (Nunc 96F microwell white polystyrene plate, Thermo Fisher Scientific, Waltham, MA, USA). Changes in luminescence intensity were measured over time with Mithras LB 940 (Berthold Technologies, Bad Wildbad, Germany).

DNA sequencing and Bioinformatics

DNA fragments were sequenced by the dideoxynucleotide chain termination method using Big-Dye ver. 3 chemistry (Applied Biosystems, USA). Products were separated on an ABI 3130

analyzer (Applied Biosystems). Sequence data was analyzed and annotated using MacVector (version 18.2 or earlier; MacVector Inc., Apec, NC, USA). Draft genome sequences of fungal species used for the analysis shown in Fig. 6, S20, S22-25 were obtained from Ensembl Genomes project (Ensembl Fungi, http://fungi.ensembl.org/index.html).

For phylogenetic analysis (Fig. S23), the deduced amino acid sequences were aligned by ClustalW (Thompson et al., 1994), and the phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987), and drawn using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

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