

Rupp et al.: Supplemental data

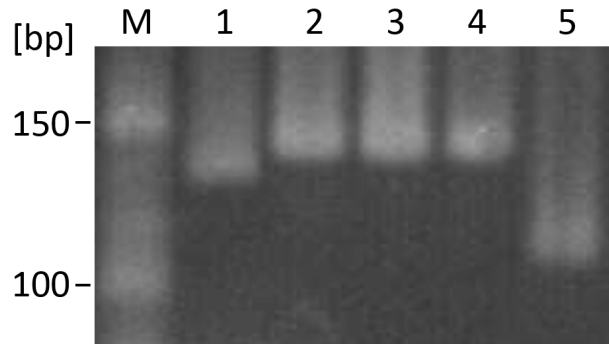
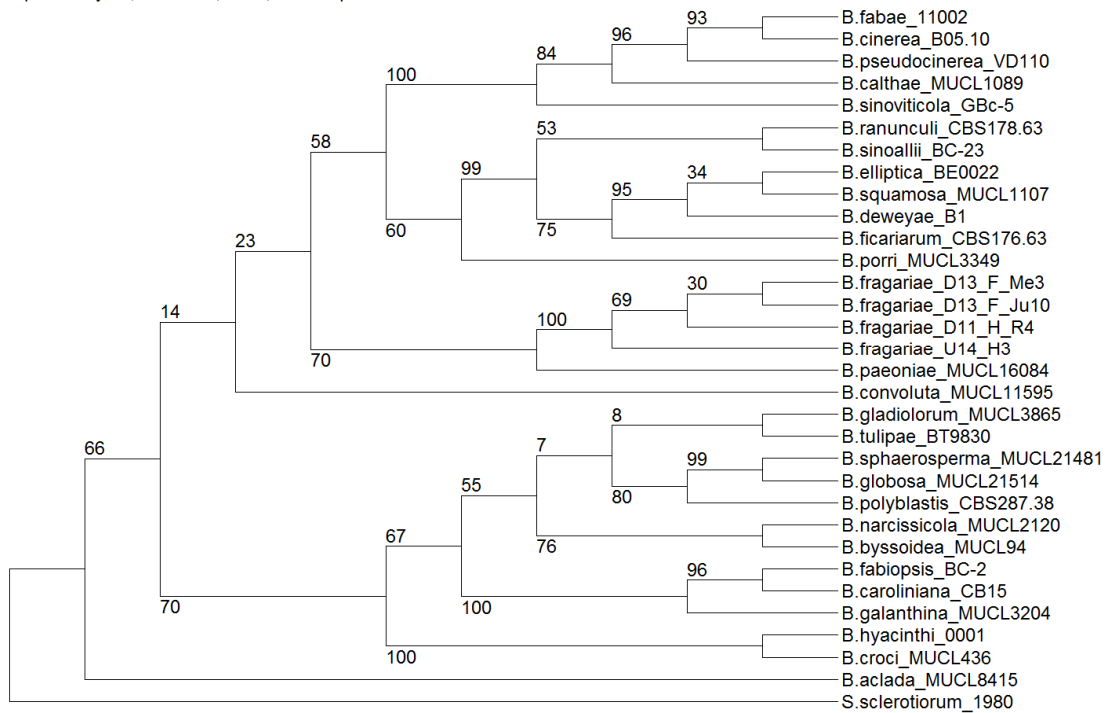


Fig. S1: PCR products of *B. cinerea* strain B05.10 (1), *B. fragariae* strains D13_F_Me3 (2), D11_H_R4 (3), D14_F_Ju20 (4), and *B. pseudocinerea* strain VD110 (5) with primer pair Mrr1-spez-F/-R .

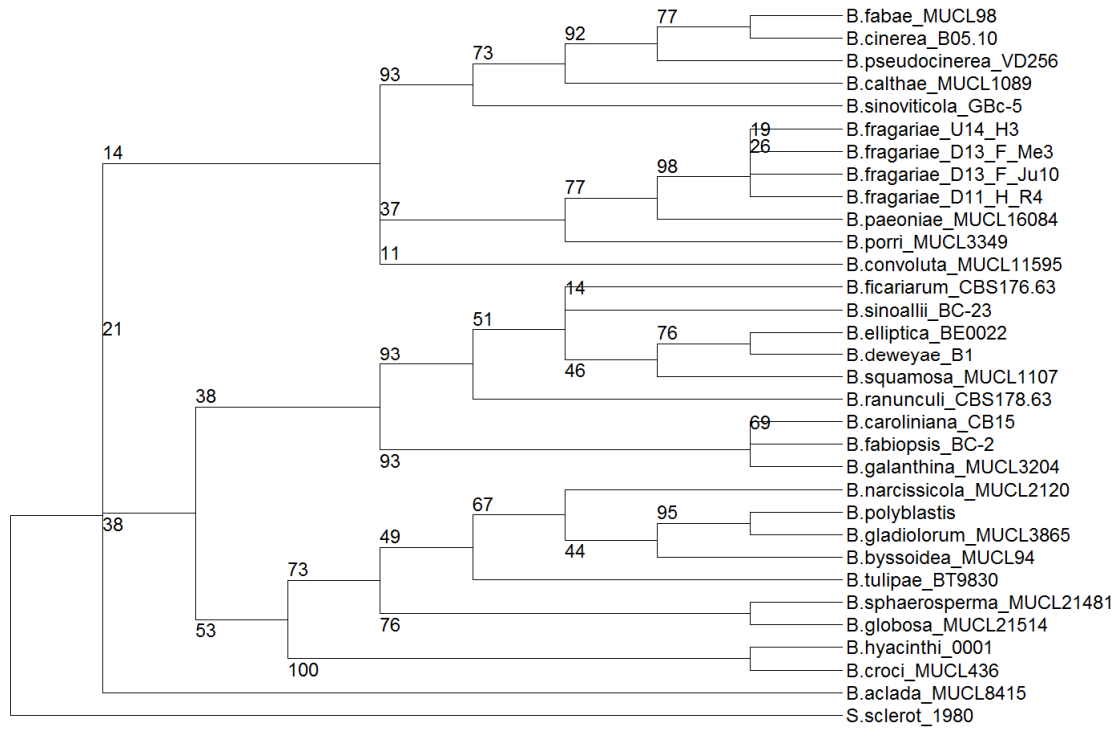
A

hsp60: PhyML, 826 sites, GTR, 1000 replicates



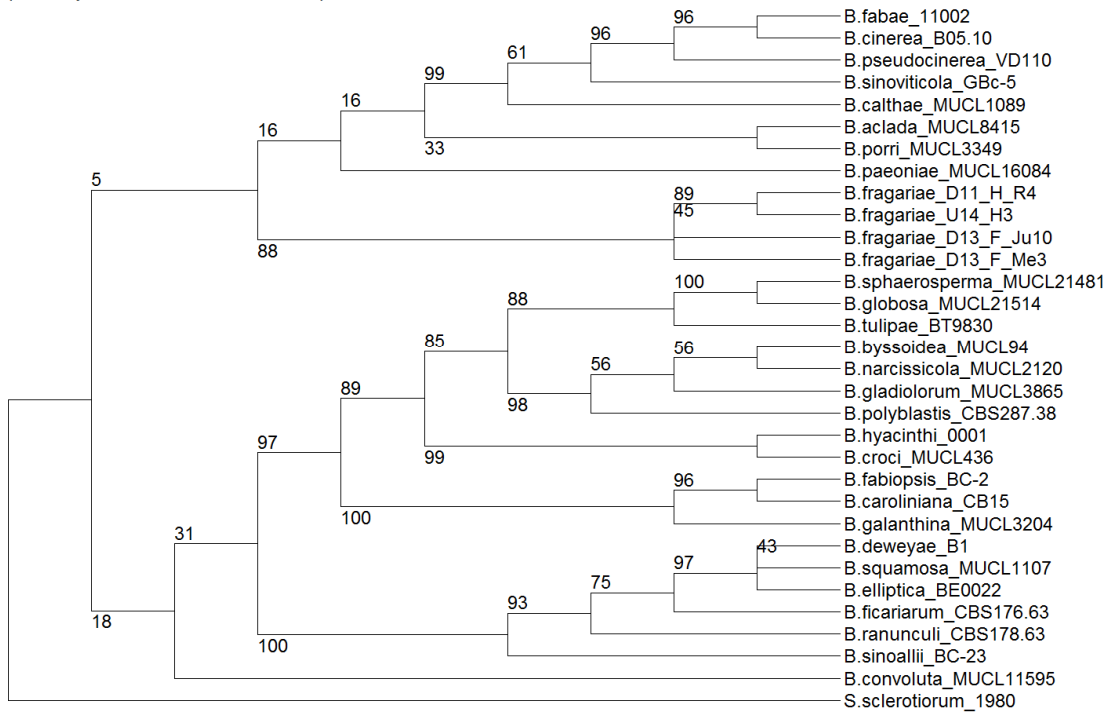
B

g3pdh: PhyML, 804 sites, GTR, 1000 replicates



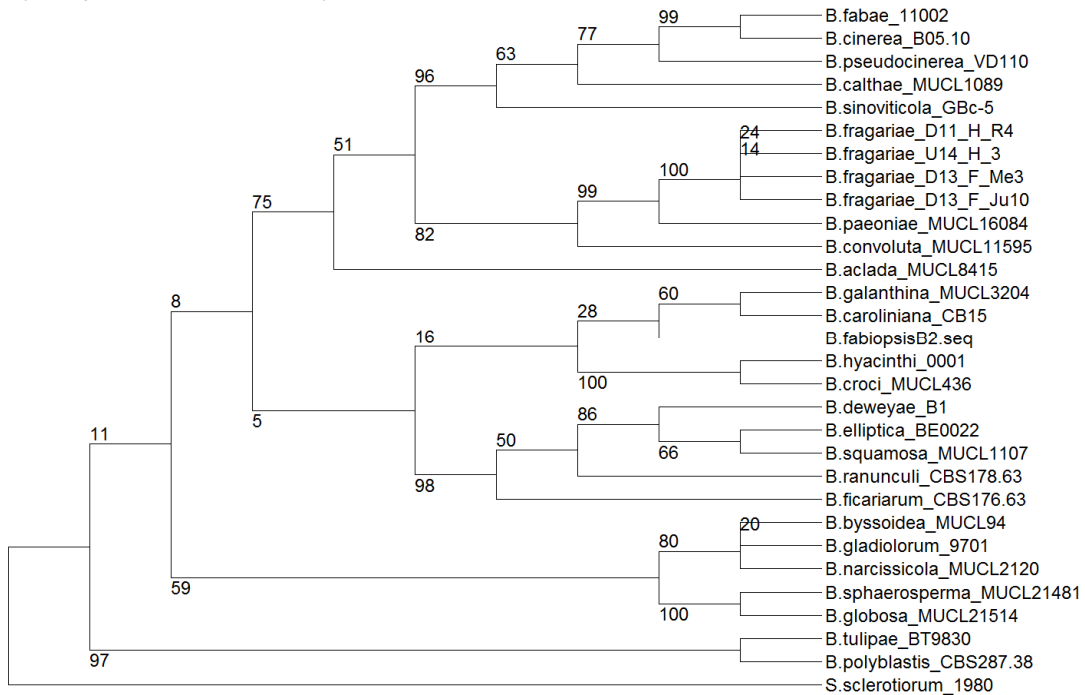
C

rpb2: PhyML, 794 sites, GTR, 1000 replicates



D

nep1: PhyML, 581 sites, GTR, 1000 replicates



E

nep2: PhyML, 799 sites, GTR, 1000 replicates

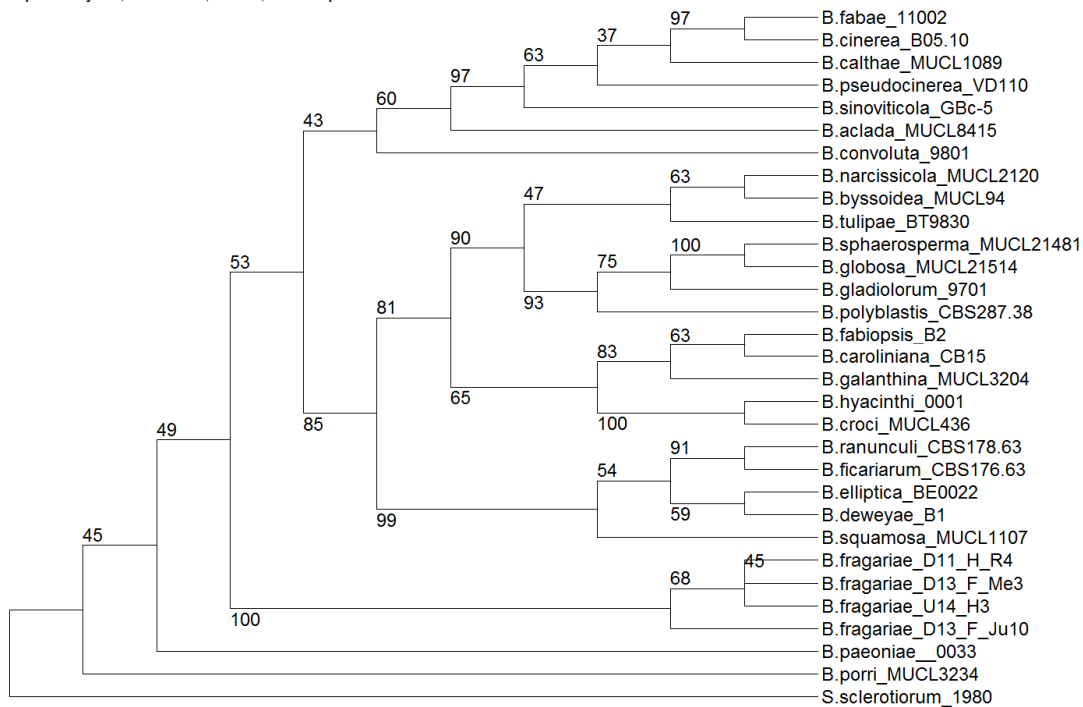


Fig. S2: Phylogenetic trees of *Botrytis* spp., including four strains of *B. fragariae* from Germany (strains D11_H_R4, D13_F_Me3, D13_D_F_Ju10) and South Carolina (strain U14_H3), based on combined sequences of *hsp60* (A), *g3pdh* (B), *rpb2* (C), *nep1* (D) and *nep2* (E). *Sclerotinia sclerotiorum* was used as outgroup.

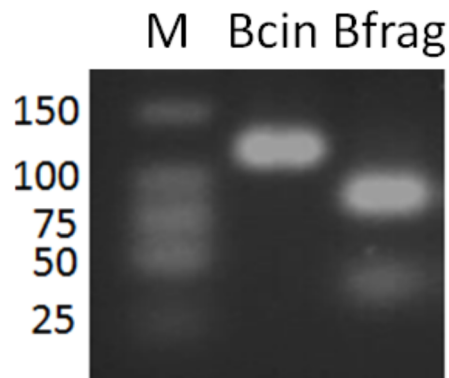


Fig. S3: Identification of *B. fragariae* by PCR-RFLP. Amplification with primers G3PDH_785_R/ G3PDH_665_F results in a 121 bp product that is cleaved by BsaJI, resulting in 86 bp and 35 bp fragments only with DNA of *B. fragariae* but not with *B. cinerea* or any other *Botrytis* species (data not shown).

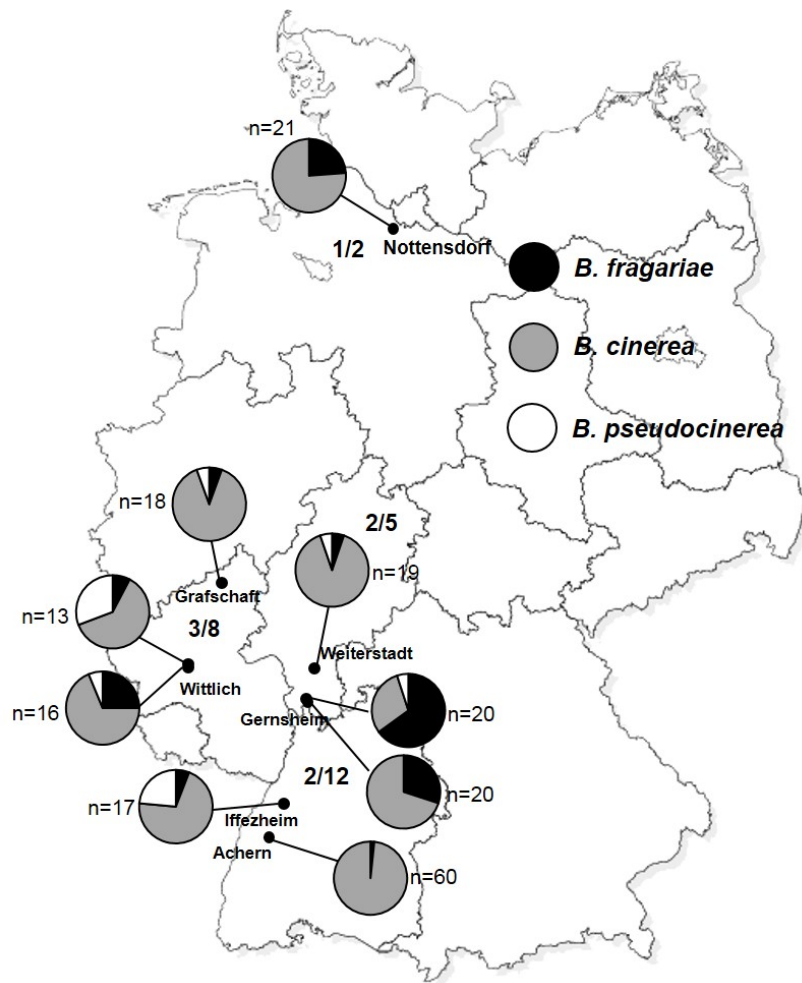


Fig. S4: Frequency of occurrence of *B. fragariae*, *B. cinerea* and *B. pseudocinerea* in Germany. Fields are shown in which *B. fragariae* was found and all isolates (n) were identified to the species level. The proportion of fields in which *B. fragariae* was observed is also indicated.

PhyML ln(L)=-14202.6 4163 sites GTR 1000 replic. 4 rate classes

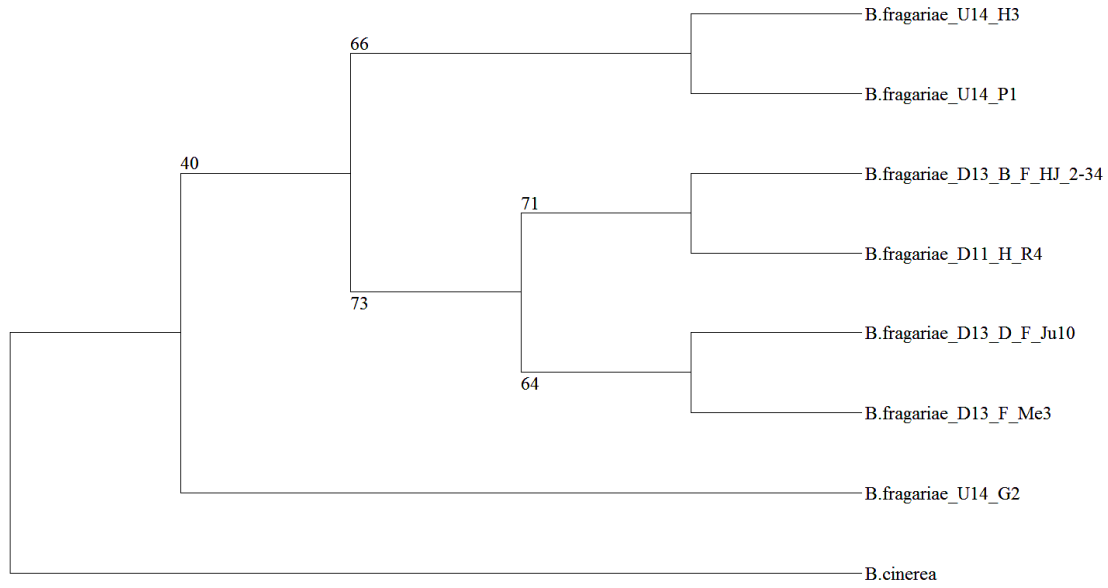


Fig. S5: Phylogenetic tree of four German and three US-American *B. fragariae* strains, based on *hsp60*, *g3pdh*, *rpb2*, *nep1* and *nep2*, using *B. cinerea* as outgroup.

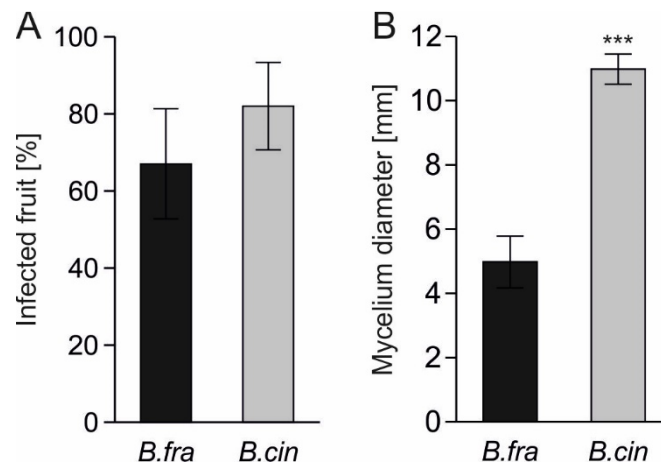


Fig. S6: Infection of strawberry tissues by *B. fragariae* and *B. cinerea* (4 d p.i.). A: Infection rate on fruit. B: Lesion formation on leaves. Significant differences between the species are indicated (***: p<0.001, two-sided t-test).

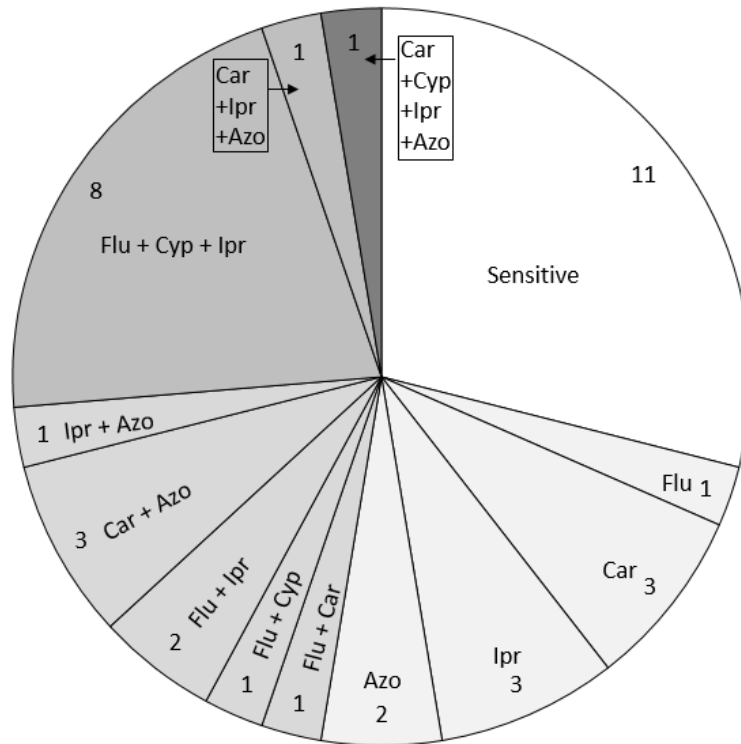


Fig. S7: Distribution of fungicide resistance in 38 *B. fragariae* isolates (cf. Table 2). Fen: Fenhexamid; Bos: Boscalid; Cyp: Cyprodinil; Azo: Azoxystrobin. Flu: Fludioxonil; Ipr: Iprodione; Car: Carbendazim.

Table S1: Primers used in this study.

Name	Sequence (5' – 3')	Gene	Reference
BpsID_137F	GCAGATGAGGCGGATGATAG	BC1G_07159/ Bcin09g02270	Plesken et al. 2015
BpsID_273R	TCCACCCAAGCATCATCTTC		
BcinN-in-F	GCGACCTCATCGTTCTTTAC	<i>mrr1</i>	Plesken et al. 2015
BcinN-in-R	GGCTCTCGATGAGCTGTTTC		
Mrr1-spez-F	TATCGGTCTTGACAGTCCGC	<i>mrr1</i>	Leroch et al., 2013
Mrr1-spez-R	TTCCGTACCCGATCTTCGGAA		
BC-hch262	AAGCCCTTCGATGTCTTGGA	<i>hch</i>	Fournier et al., 2005
BC-hch520L	ACGGATTCCGAACTAAGTAA		
Bc_Tub_for	AAGATCCGCGAGGAGTTCCC	<i>tubA</i>	This work
Bc_Tub_rev	GGCGTGGGACGTTGTTAG		
BF1_56_opt	TTAGCAGCCTGGCTGCATATAC	<i>bos1</i>	This work
BR1_1084_opt	TTGTGCCATGACGTTGAC		
BF2	CAACGTTATGGCACAAAATCTCA	<i>bos1</i>	Ma et al., 2007
BR2	AAGTTTCTGGCCATGGTGTTC		
BF3_opt	TCGAGGTTCCAGGGAGAAATC	<i>bos1</i>	This work
BR3_opt	CTATCCACGCGGTAAGTGAG		
BF4	GCAAACCGTATGATCATGGA	<i>bos1</i>	Ma et al., 2007
BR4_opt	TCTTCGGCCAAGAGAATGTC		This work
BR5	AAGTACTCGCAGTCGGTGGT	<i>bos1</i>	Ma et al., 2007
BF5_opt	TTCTCGCAATGGAAGAACC		This work
Qo13ext	GGTATAACCCGACGGGTTATAGAATAG	<i>cytB</i>	Leroux et al., 2010
Qo14ext	AACCATCTCCATCCACCATACTACAAA		
HSP60_fw	CAACAATTGAGATTTGCCACAAG	<i>hsp60</i>	Staats et al., 2005
HSP60_rev	GATGGATCCAGTGGTACCGAGCAT		
G3PDH_fw	ATTGACATCGTCGCTGTCAACGA	<i>g3pdh</i>	Staats et al., 2005
G3PDH_rev	ACCCACTCGTTGTCGTACCA		
G3PDH_785_R	GTCACCGTTCATGTCAGTAG	<i>g3pdh</i>	This work
G3PDH_665_F	ATGGTCTCTCAAGGGTAAG		
RPB2_fw	GATGATCGTGATCATTTCGG	<i>rpb2</i>	Staats et al., 2005
RPB2_rev	CCCATAGCTTGCTTACCCAT		
FG1020_fw	GGAGGATGATATGGCAAAGTC	<i>fg1020</i>	Plesken et al. 2015
FG1020_rev	GGATTAAGAGCTTCACTACCA		
Nep1for	CCAACGCAAATTCCTTTCTATCC	<i>nep1</i>	Grant-Downton et al., 2014
Nep1rev	GTTGGCGAAGTTGTGGTCATTGAA		
NEP2forD	TTGCCTTCTCAAATCATTACAGC	<i>nep2</i>	Staats et al., 2007
NEP2revD	TCTAGAAAGTAGCCTTCGCAAGAT		
IGS1a	TCCCGGTGAGCCTTTTA	(IGS)	Kretschmer and Hahn, 2008
IGS1b	CCATCGGCCAGTAATCCAC		
atrB_RT_for	CACCGGAGCAGGATTGAGTG	<i>atrB</i>	This work
atrB_RT_rev	GCGGAAGGTTGATGGATAG		
BcAct_RTfor	TCTGTCTTGGGTCTTGAGAG	<i>actin</i>	Leroch et al., 2013
BcAct_RTrev	GGTGCAAGAGCAGTGATTTTC		
Tub_RT_fw	TTCCGTGCTGTCAACGTTTC	<i>tubA</i>	Leroch et al., 2013
Tub_RT_rev	ACGTTGCGCATTGGTCCTC		
MATalpha5	ATGACGGCTCCCTCAAAC	Mat1-1	This work
MATalpha3	GGTGGTGAAGGGACATCTTC		
HMG5	ATGTCTCTCTCTCTCCG	Mat1-2	This work
HMG3	GGAAAAGAATGTGTAGAGATCCTG		
Flipper-fw	AGCCCTACCCATCGTCAAATAC	(Flipper)	This work
Flipper-rev	GCTCGGGATCATCATCTGAAAC		

Table S2: Accession numbers of sequences used for assembling the phylogenetic trees.*

	<i>hsp60</i>	<i>g3pdh</i>	<i>rpb2</i>	<i>nep1</i>	<i>nep2</i>
<i>B. fabae</i> CBS109.57	AJ716074	AJ705013	AJ745685	AM087025	---*1
<i>B. pseudocinerea</i> 10091	JN692400	JN692414	JN692428	---*1	---*1
<i>B. calthae</i> MUCL1089	AJ716061	AJ705000	AJ745672	AM087031	AM087088
<i>B. sinoviticola</i> GBc-5	JN692399	JN692413	JN692427	---*2	---*2
<i>B. ranunculi</i> CBS178.63	AJ716095	AJ705034	AJ745706	AM087054	AM087086
<i>B. sinoalii</i> BC-23	EU514488	EU519217	EU514479	---*2	---*2
<i>B. elliptica</i> BE0022	AJ716071	AJ705010	AJ745682	AM087050	AM087081
<i>B. squamosa</i> MUCL1107	AJ716098	AJ705039	AJ745710	AM087052	AM087084
<i>B. deweyae</i> B1	HG799519	HG799521	HG799518	HG799527	HG799520
<i>B. ficariarum</i> CBS176.63	AJ716076	AJ705015	AJ745687	AM087055	AM087085
<i>B. parri</i> MUCL3349-3234	AJ716093	AJ705032	AJ745704	AM087060	AM087063
<i>B. paeoniae</i> MUCL16084	AJ716089	AJ705028	AJ745700	AM087033	AM087064
<i>B. convoluta</i> MUCL11595	AJ716069	AJ705008	AJ745680	AM087035	AM087062
<i>B. gladiolorum</i> MUCL3865	AJ716081	AJ705020	AJ745692	AM087041	AM087072
<i>B. tulipae</i> BT9830	AJ716102	AJ705041	AJ745713	AM087037	AM087077
<i>B. sphaerosperma</i> MUCL21481	AJ716096	AJ705035	AJ745708	AM087042	AM087068
<i>B. globosa</i> MUCL21514	AJ716082	AJ705021	AJ745694	AM087044	AM087070
<i>B. polyblastis</i> CBS287.38	AJ716091	AJ705030	AJ745702	AM087039	AM087074
<i>B. narcissicola</i> MUCL2120	AJ716087	AJ705026	AJ745697	AM087046	AM087078
<i>B. byssoidea</i> MUCL94	AJ716059	AJ704998	AJ745670	AM087045	AM087079
<i>B. fabiopsis</i> BC-2	EU514482	EU519211	EU514473	---*2	---*2
<i>B. caroliniana</i> _CB17	JN672675	JN672671	JN672677	JF811593	---*1
<i>B. galanthina</i> MUCL3204	AJ716078	AJ705017	AJ745690	AM087058	AM087067
<i>B. hyacinthi</i> 0001	AJ716084	AJ705023	AJ745695	AM087048	AM087066
<i>B. croci</i> MUCL436	AJ716070	AJ705009	AJ745681	AM087047	AM087065
<i>B. aclada</i> MUCL8415	AJ716050	AJ704992	AJ745664	AM087059	AM087087

* Sequences of *B. cinerea* strain B05.10 and *Sclerotinia sclerotiorum* strain 1980 were obtained from genome assemblies provided by the EnsemblFungi website (<http://fungi.ensembl.org/index.html>).

*1: Own unpublished sequence data. *2: Unpublished sequence data (provided by Dr. Jing Zhang, Huazhong Agricultural University, Wuhan, China).

Table S3: Occurrence of *Botrytis species* on wild strawberries (*Fragaria vesca*) in Western Germany.

Site*	No of isolates	<i>B. cinerea</i>		<i>B. pseudocinerea</i>	<i>B. fragariae</i>
		Group N	Group S		
A	5	0	2	3	0
B	17	4	11	2	0
C	11	4	1	6	0
D	10	2	5	3	0

*Four sites located in the Palatine forest, Germany, Kaiserslautern.

Table S4: Fungicide treatments in fields from which *B. fragariae* isolates were recovered. *For GE-Nottensdorf (the only site where isolates were obtained after the treatments), treatments of the 2011 season are shown.

Field code	Year	Treatments in the previous year*
GE-Nottensdorf	2011	Fenhexamid* ¹ (1x), Fludioxonil + Cyprodinil* ² (2x), Boscalid + Pyraclostrobin* ³ (1x), Trifloxystrobin* ⁴ (1x)
GE-Iffezheim	2012	None
GE-Weiterstadt	2012	Fludioxonil + Cyprodinil (2x), Azoxystrobin, Fenhexamid
GE-Grafschaft	2011	Unknown
GE-Wittlich-A	2012	Fludioxonil + Cyprodinil (1x)
GE-Wittlich-B	2013	None
GE- Gernsheim-1	2012	Unknown
GE- Gernsheim-1	2013	Boscalid + Pyraclostrobin, Fludioxonil + Cyprodinil (2x) , Fenhexamid
GE-Wagshurst	2014	None
US-Pelion-SC	2015	None
US-Gilbert-SC	2015	Captan, Fenhexamid, Fludioxonil + Cyprodinil
US-Holly Hill-SC	2015	Unknown

*Trade names: ¹ Teldor®, ² Switch®, ³ Signum®, ⁴ Flint®.

Table S5: *Botrytis* strains used for quantitative fungicide sensitivity assays (cf. Fig. 6) and *atrB* expression studies (cf. Fig. 7).

Species	Strain	Fungicide sensitivity or resistance			Reference
		Fludioxonil	Cyprodinil	Iprodione	
<i>B. cinerea</i>	B05.10*	S	S	S	Leroch et al., 2013
<i>B. cinerea</i>	D06_5-16*	MDR1	MDR1	n.a.	Kretschmer et al., 2009
<i>B. cinerea</i>	D08-H-8-07a*	MDR1h	MDR1h	n.a.	Leroch et al., 2013
<i>B. cinerea</i>	D09_K_A04	n.a.	n.a.	HR	This work
<i>B. cinerea</i>	D09_K_4_01	n.a.	HR	n.a.	This work
<i>B. cinerea</i>	D09_K_F04	n.a.	n.a.	MR	This work
<i>B. fragariae</i>	D11_H_R4*	S	S	MR	This work
<i>B. fragariae</i>	D13_F_HJ_2-34	S	S	S	This work
<i>B. fragariae</i>	D13_F_Me3	S	S	S	This work
<i>B. fragariae</i>	D13_F-Ju21	MDR1	MDR1	MR	This work
<i>B. fragariae</i>	D13_F-Ju26	MDR1	MDR1	n.a.	This work
<i>B. fragariae</i>	D14_F-Ju20*	MDR1	HR	HR	This work
<i>B. fragariae</i>	D13_F_Nba13	n.a.	MDR1	MR	This work
<i>B. fragariae</i>	D13_F_Ju10	S	S	n.a.	This work
<i>B. fragariae</i>	D13_F_Ju1	S	S	n.a.	This work
<i>B. fragariae</i>	D13_F_Ju29	n.a.	HR	n.a.	This work
<i>B. fragariae</i>	D14_F_Ju12	n.a.	HR	n.a.	This work
<i>B. fragariae</i>	D13_F_Nba10	n.a.	n.a.	S	This work
<i>B. fragariae</i>	D15_W_R3	n.a.	n.a.	HR	This work
<i>B. fragariae</i>	D14_Bl.427-22	n.a.	n.a.	HR	This work

*: Used for *atrB* expression studies. Abbreviations: S: Sensitive; MR: Medium resistant; HR: Highly resistant. n.a.: Not analysed in Fig. 6.

References

- Fournier E, Giraud T, Albertini C, Brygoo Y.** 2005. Partition of the *Botrytis cinerea* complex in France using multiple gene genealogies. *Mycologia* **97**:1251–1267.
- Grant-Downton RT, Terhem RB, Kapralov MV, Mehdi S, Rodriguez-Enriquez MJ, Gurr SJ, van Kan JA, Dewey FM.** 2014. A novel *Botrytis* species is associated with a newly emergent foliar disease in cultivated *Hemerocallis*. *PLoS One* **9(6)**:e89272.
- Kretschmer M, Hahn M.** 2008. Fungicide resistance and genetic diversity of *Botrytis cinerea* isolates from a vineyard in Germany. *J Plant Dis Protect* **115**:214–219.
- Kretschmer M, Leroch M, Mosbach A, Walker AS, Fillinger S, Mernke D, Schoonbeek HJ, Pradier JM, Leroux P, de Waard MA** 2009. Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathog* **5**:e1000696.
- Leroch M, Plesken C, Weber RWS, Kauff F, Scalliet G, Hahn M.** 2013. Gray mold populations in German strawberry fields are resistant to multiple fungicides and dominated by a novel clade closely related to *Botrytis cinerea*. *Appl Environ Microbiol* **79**:159–167.
- Leroux P, Gredt M, Leroch M, Walker AS.** 2010. Exploring mechanisms of resistance to respiratory inhibitors in field strains of *Botrytis cinerea*, the causal agent of gray mold. *Appl Environ Microbiol* **76**:6615-6630.
- Ma Z, Yan L, Luo Y, Michailides TJ.** 2007. Sequence variation in the two-component histidine kinase gene of *Botrytis cinerea* associated with resistance to dicarboximide fungicides. *Pestic Biochem Physiol* **88**:300–306.
- Plesken C, Weber RWS, Rupp S, Leroch M, Hahn M.** 2015. *Botrytis pseudocinerea* is a significant pathogen of several crop plants but susceptible to displacement by fungicide-resistant *B. cinerea* strains. *Appl Environ Microbiol* **81**:7048-7056.
- Staats M, van Baarlen P, Schouten A, van Kan JAL, Bakker FT.** 2007. Positive selection in phytotoxic protein-encoding genes of *Botrytis* species. *Fungal Genet. Biol.* **44**:52–63.