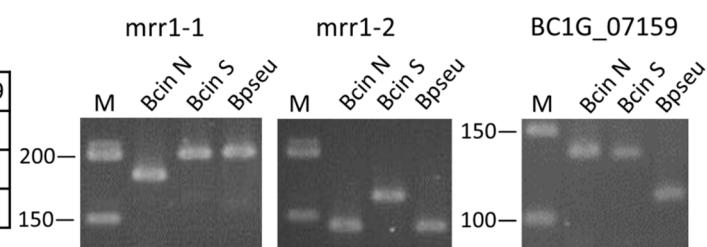


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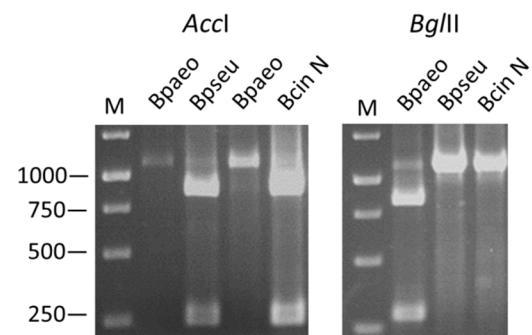
**A**

Primer pair	mrr1-1	mrr1-2	BC1G_07159
<i>B. pseudocinerea</i>	200 bp	144 bp	112 bp
<i>B. cinerea</i> N	182 bp	144 bp	136 bp
<i>B. cinerea</i> S	200 bp	165 bp	136 bp



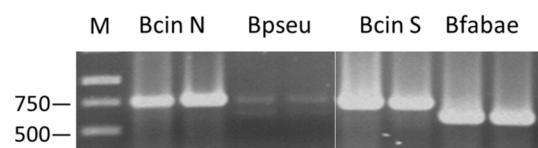
**B**

Primer pair	HSP60_fw/HSP60_rev	
Restriction digestion	<i>Bg</i> /II	
<i>B. pseudocinerea</i>	1111 bp	870 + 241 bp
<i>B. cinerea</i> N	1111 bp	870 + 241 bp
<i>B. cinerea</i> S	1111 bp	870 + 241 bp
<i>B. paeoniae</i>	830 + 281 bp	1111 bp



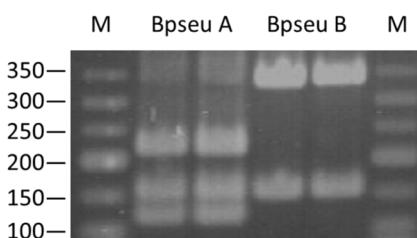
**C**

Primer pair	C729_for/ C729_rev
<i>B. pseudocinerea</i>	728 bp
<i>B. cinerea</i> N	728 bp
<i>B. cinerea</i> S	728 bp
<i>B. fabae</i>	606 bp



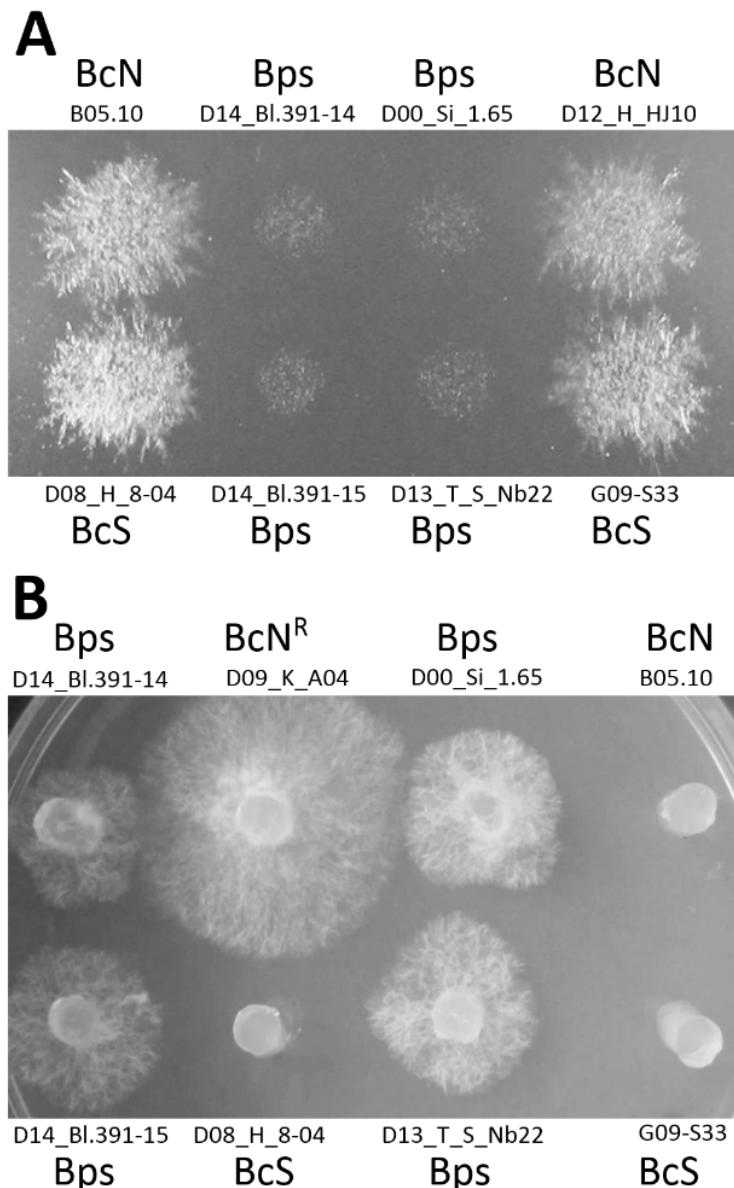
**D**

Primer pair	MS547-685_R/ MS547-201_F	
Restriction digestion	undigested	
<i>B. pseudocin.</i> group A	484 bp	205+121+158 bp
<i>B. pseudocin.</i> group B	484 bp	326 +158 bp

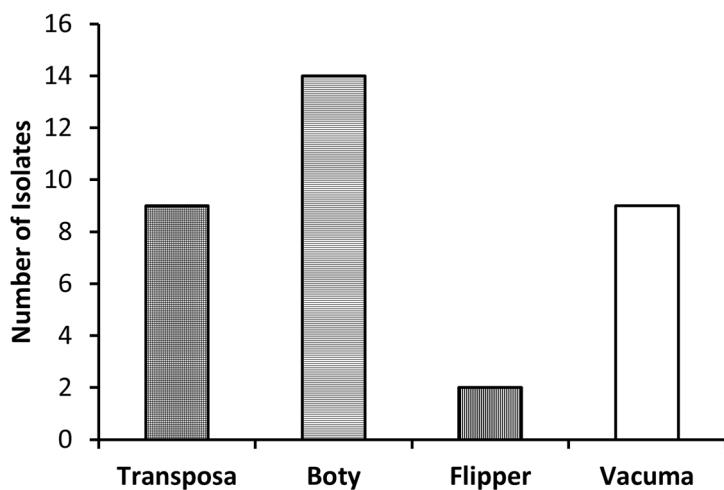


**Fig. S1.** PCR-based differentiation of *B. pseudocinerea* and *B. cinerea* genotypes. On the left side, primer pairs, restriction enzymes (if used), and predicted fragment sizes are indicated. On the right side, the resulting DNA fragments after gel electrophoresis are shown A: Indel PCR for differentiation of *B. cinerea* N, *B. cinerea* S and *B. pseudocinerea*, using primer pairs mrr1-1 (BcinN-in-F/ BcinN-in-R, flanking an 18 bp indel) and mrr1-2 (Mrr1-spez-F/ Mrr1-spez-R, flanking the 21 bp indel) in *mrr1* (Leroch et al., 2013). Identification of *B. pseudocinerea* is preliminary, because the same band sizes are obtained by a rare genotype of *B. cinerea*, referred to as *B. cinerea* X. Final identification of *B. pseudocinerea* is achieved by detection of a 24 bp deletion with primer pair BC1G\_07159 (g2944\_137\_F/ g2944\_273\_R). B: Identification of *B. paeoniae* by PCR-RFLP. *B. paeoniae* *hsp60* contains a *Bgl*III site that is lacking in *B. pseudocinerea* and *B. cinerea*, and lacks an *Acc*I site that is present in *B.*

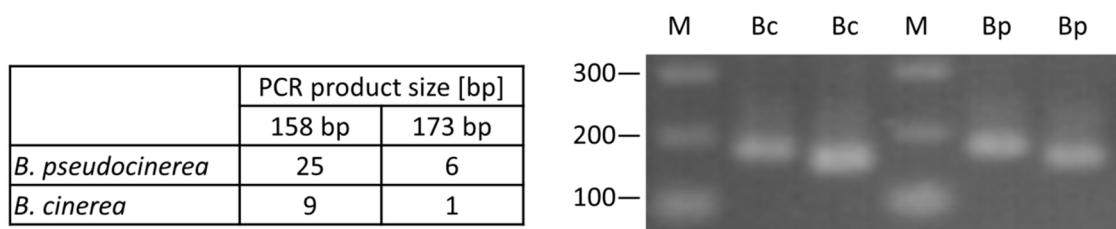
*pseudocinerea* and *B. cinerea*. C: Indel PCR for identification of *B. fabae*. *B. fabae* contains a unique 122 bp deletion in a gene corresponding to *B. cinerea* BC1G\_06014 (Rigotti et al., 2002), amplified with primer pair C729\_for/ C719\_rev. With *B. pseudocinerea* strains, only weak duplex bands are obtained. D: PCR-RFLP based differentiation of *B. pseudocinerea* groups A and B. The *ms547* sequence of *B. pseudocinerea* group A contains two *EarI* site one of which is missing in group B.



**Fig. S2.** Differentiation of *B. cinerea* and *B. pseudocinerea* by growth tests on 0.5% sucrose agar containing discriminatory fungicide concentrations. A: Agar with 1 mg fenpropidin l<sup>-1</sup>, inoculated with conidia. B: Agar with 5 mg fenhexamid l<sup>-1</sup>, inoculated with agar discs with 24 h old germlings. Pictures were taken 3 days after inoculation. Bps: *B. pseudocinerea* strains; BcN: *B. cinerea* N strains; BcS: *B. cinerea* S strains. BcN<sup>R</sup>: Fenhexamid resistant *B. cinerea* N strain. Strains B05.10, D08\_H\_8-04 and G09-S33 are described in Leroch et al. (2013).



**Fig. S3.** Frequency of occurrence of the transposable elements Boty and Flipper in *B. pseudocinerea*, determined by PCR with Boty- and Flipper-specific primers. Transposa strains contain both elements, Vacuma strains none of these elements.



**Fig. S4.** PCR detection of a 15 bp indel in the mitochondrial *rpsS3* coding region in strains of *B. pseudocinerea* (Bp) and *B. cinerea* (Bc). Left: Numbers of strains yielding the larger and the smaller PCR product. Right: Ethidium-bromide stained gel with PCR products from strains of both species. Fungal DNA was amplified with primers rps3\_55\_fw/ rps3\_213\_rev.

Table S2: Growth inhibition of *B. pseudocinerea* (Bps) and *B. cinerea* (Bcin) strains by different concentrations of fenpropidin in liquid and on solid medium. SA: Agar containing 0.5% sucrose. The tests were performed twice, with two replicates each. (+) Weak growth.

Species/ strain	Growth in liquid medium with fenpropidin (ppm)								Growth on SA with 1 mg fenpropidin l <sup>-1</sup>
	0	0.01	0.03	0.1	0.3	1	2	4	
Bps VD110	+	+	(+)	-	-	-	-	-	-
Bps D11_T_B42	+	+	-	-	-	-	-	-	-
Bps D00-Si-1.65	+	+	(+)	-	-	-	-	-	-
Bps D00_R0_1.41	+	+	+	(+)	-	-	-	-	-
Bps D97_Ve_1.13	+	+	+	(+)	-	-	-	-	-
Bps D12_Gö_1361	+	+	-	-	-	-	-	-	-
Bps D13_K_Mai_Mu14	+	+	+	(+)	-	-	-	-	-
Bps D11_T_B41 (group B)	+	+	+	+	-	-	-	-	(+)
Bcin B05.10	+	+	+	+	+	+	(+)	-	+
Bcin G09-S33	+	+	+	+	+	+	+	-	+
Bcin D09_K_A04	+	+	+	+	+	+	(+)	-	+
Bcin D08_H_8-07a	+	+	+	+	+	+	+	-	+
Bcin D13_K_S_Ge1	+	+	+	+	+	+	(+)	-	+

Table S3: Primers used in this study

Name	Sequence	Gene	Reference
BcinN-in-F	GCGACCTCATCGTTCTTCAC		
BcinN-in-R	GGCTCTCGATGAGCTGTTTC	mrr1	This work
Mrr1-spez-F	TATCGGTCTGCAGTCCGC		
Mrr1-spez-R	TTCCGTACCCGATCTCGGAA	mrr1	Leroch et al., 2013
g2944_137_F	GCAGATGAGCGGATGATAG		
g2944_273_R	TCCACCCAAGCATCATCTTC	BC1G_07159	This work
BC-hch262	AAGCCCTTCGATGCTTGGAA		
BC-hch520L	ACGGATTCCGAACTAAGTAA	hch	Fournier et al., 2005
C729_for	AGCTCGAGAGAGATCTCTGA		
C729_rev	CTGCAATGTTCTGCGTGGAA	BC1G_06014	(20) (20)
PIRA-fw-P225L	CGAATGTATTCTCTGCGCATGCTGCTGACATCTTGT	sdhB	This work
PIRA-rev-P225L	GCAAGCCATCTGTAACTCTG		
Bc_Tub_for	AAGATCCCGAGGAGTTCCC	tubA	This work
Bc_Tub_rev	GGCGGTTGGACGTTGTTAG		
155_betaTub_F	CAACCTCAAAATGCGTGAG	tubA	Fournier et al., 2005
1174_betaTub_R	AGATGGGTTGCTGAGCTTC		
PIRA-Tub1	GCCTCGTTATCGATAACAGAACATC	tubA	This work
PIRA-Tub2	TATGATGGCTACCTCTCCGTC		
Bc-Boty1	CTTACCGAACACAAGCCAT		
Bc-Boty2	GGTCTTCCATTCTCGCCCTTC	Boty pol	Kretschmer and Hahn, 2008
F300	GCACAAAACCTACAGAAGA		
F1550	ATTCGTTCTGGACTGTA	Flipper	Levis et al., 1997
Qo13ext	GGTATAACCCGACGGGGTTATAGAATAG		
Qo14ext	AACCATCTCCATCCACATACCTACAAA	cytB	Leroux et al., 2010
mito_55_fw	TTGCTCAGGGCTCCGTAAC		
mito_213_rev	GCTGGTCCAGAGTGTAAGG	rps3 (mitoch.)	This work
HSP60_fw	CAACAATTGAGATTGCCAACAG		
HSP60_rev	GATGGATCCAGTGGTACCGAGCAT	hsp60	Staats et al., 2005
G3PDH_fw	ATTGACATCGTCGCTGTCAACGA		
G3PDH_rev	ACCCCACACTGTTGTCGTACCA	g3pdh	Staats et al., 2005
MS547-F	AAGGAGGACGTTGAGGAT		
MS547-R	AAAGTCCAGAAATCTCGATGTATTGT	ms547	Walker et al., 2011
ms547_685_R	GTCGATAGATGGGCTGCAAG		
ms547_201_F	GTTGGTGGAGGTAGGAAGAC	ms547	This work
Mat1-F	GTGACCAGGAAACAGCTATGACCGGAGTGTGTTGATCGTGGAGCCGAG	(MAT1-1)	
Mat1-R	GTGACTGTAAAACGACGGCCAGTCCACACATCATGACGGCTCCC	(MAT1-2)	Amselem et al., 2011

## References

- Amselem J, Cuomo CA, van Kann JAL, Viaud M, Benito EP, Couloux A, et al.** 2011. Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLoS Genet 7: e1002230.
- 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.
- Kretschmer M., Hahn M.** 2008. Fungicide resistance and genetic diversity of *Botrytis cinerea* isolates from a vineyard in Germany. J Plant Dis Prot 115:214–219.
- 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.
- Levis C, Fortini D, Brygoo Y.** 1997. Flipper, a mobile Fot1-like transposable element in *Botrytis cinerea*. Mol Gen Genet 254:674-680
- Muñoz G, Hinrichsen P, Brygoo Y, Giraud T.** 2002. Genetic characterization of *Botrytis cinerea* populations in Chile. Mycol Res 106:594-601.
- Rigotti R, Gindro L, Richter H, Viret O.** 2002. Characterization of molecular markers for specific and sensitive detection of *Botrytis cinerea* Pers.: Fr. in strawberry (*Fragaria×ananassa* Duch.) using PCR. FEMS Microbiol Lett 209:169-174.
- Staats M, van Baarlen P, van Kan JAL.** 2005. Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of host specificity. Mol Biol Evol 22:333-346.
- Walker AS, Gautier AL, Confais J, Martinho D, Viaud M, Le Pêcheur P, Dupont J, Fournier E.** 2011. *Botrytis pseudocinerea*, a new cryptic species causing grey mould in French vineyards in sympatry with *Botrytis cinerea*. Phytopathology 101:1433-1445.