Phenotypic and Genetic Characterization of *Botrytis cinerea* Population from Kiwifruit in Sichuan Province, China

Yan-gang Pei,¹ Qin-jun Tao,¹ Xiao-juan Zheng,¹ Ying Li,¹ Xiao-fang Sun,¹ Zhi-fei Li,^{1,2} Xiao-bo Qi,¹ Jing Xu,¹ Min Zhang,¹ Hua-bao Chen,¹ Xiao-li Chang,¹ Hui-min Tang,¹ Li-yun Sui,³ and Guo-shu Gong^{1,†}

¹College of Agronomy & Key Laboratory for Major Crop Diseases, Sichuan Agricultural University, Chengdu 611130, P. R. China; ²Sichuan HuaSheng Agricultural Co., Ltd, Deyang 618200, P. R. China; and ³Chengdu Academy of Agriculture and Forestry Sciences, Chengdu 611130, P. R. China

Abstract

Botrytis cinerea (anamorph of *Botryotinia fuckeliana*) causes gray mold on numerous plants, including kiwifruit. The primary aim of this study was to investigate the phenotypic and genetic characteristics of the *Botrytis cinerea* population from kiwifruit in Sichuan Province, China. In all, 176 isolates were collected from kiwifruit orchards from eight geographic regions in Sichuan. All isolates were identified as *B. cinerea* sensu stricto based on the combined datasets, including morphological criteria, determination of the *Bc-hch* allele, and phylogenetic analysis of the genes *RPB2*, *G3PDH*, and *HSP60*. Three colony types (i.e., sclerotial, mycelial, and conidial) were observed on potato dextrose agar after 2 weeks, with sclerotial isolates, the predominant category, accounting for 40.91%. No obvious differences in microscopic characteristics were observed among the three types. Three genotypes of transposable elements were identified in the *B. cinerea* population: *boty*, *flipper*, and *transposa* types. The most prevalent genotype from different geographic populations of *B. cinerea* was

transposa; in contrast, the *flipper* genotype accounted for only 3.98% of the total population, whereas the *vacuna* genotype was absent. According to MAT locus amplification, 87 and 89 isolates are *MAT1-1* and *MAT1-2* type, respectively, and the two mating types were found to be balanced overall in the population. Forty-eight representative isolates were all able to cause gray mold to some extent, and disease severities were significantly different between the cultivars Hongyang and Hort16A ($P < 0.01$). Disease severity was significantly greater on young leaves than on mature leaves ($P < 0.01$). No significant relationship was found between pathogenicity and geographical region, colony type, or transposon distribution. The results obtained in the present study suggest a relatively uniform species diversity of *Botrytis* but rich phenotypic and genetic differentiation within the *B. cinerea* population on kiwifruit in China. Utilizing resistant cultivars and rain-shelter cultivation instead of fungicides may be an effective approach to delaying pathogen variability.

Botrytis cinerea (teleomorph of *Botryotinia fuckeliana*), an air-borne necrotrophic fungus that attacks more than 200 plant species, causes gray mold disease of many economically important crops, including vegetables, ornamentals, bulbs, and fruit (Plesken et al. 2015b; Williamson et al. 2007). Indeed, *Botrytis cinerea* is considered one of the most destructive phytopathogenic fungi in the world (Sommer et al. 1992; Williamson et al. 2007). Gray mold disease is a serious problem for the kiwifruit industry in Sichuan Province, China (Liu and Li 2014; Qin and Gao 2013). The fungus causes leaf decay, flower rot, and fruit rot in kiwi plants during the growing season, with yield losses that can exceed 30% (Kulakiotu et al. 2004; Michailides and Elmer 2000; Shirzad et al. 2011).

B. cinerea is complex due to its marked instability while adapting to various environmental conditions and exhibits high phenotypic variability and genetic diversity (Giraud et al. 1997; Li et al. 2016; Mirzaei et al. 2009; Muñoz et al. 2002; Williamson et al. 2007). *B. cinerea* populations can generally be divided into three categories based on cultural and morphological characteristics: mycelial, sclerotial, and conidial types (Faretra et al. 1988; Huang et al. 2014).

[†]Corresponding author: G.-s. Gong; E-mail: guoshugong@126.com

Y. Pei and Q. Tao contributed equally to this work.

Funding: This study was funded by a project from Science & Technology Department of Sichuan Province (2014NZ0104 and 2017CC0035) and Cooperation projects of Yaan City and Sichuan Agricultural University.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure and two supplementary tables are published online.

The author(s) declare no conflict of interest.

Accepted for publication 24 October 2018.

Nevertheless, these categories are not sufficient for complete identification of the pathogen because environmental conditions are known to significantly influence colony morphology (Beever et al. 2004). The specific primer pair C729⁺/729⁻ was previously developed for specific identification of *B. cinerea* (Karakaya and Bayraktar 2009; Rigotti and Gindro 2002). Moreover, polygenic pedigree analysis has already revealed that the *B. cinerea* complex consists of two phylogenetic groups: group I and group II (Fournier et al. 2002; Martinez et al. 2005). Likewise, Fournier et al. (2003) developed polymerase chain reaction (PCR)-restriction fragment length polymorphism for the *Bc-hch* locus to divide *B. cinerea* into groups I and II. Two morphologically cryptic species had historically been treated as *B. pseudocinerea* (group I) and *B. cinerea* sensu stricto (group II) (Walker et al. 2011). *B. pseudocinerea* had sometimes previously been referred to as *B. cinerea* group I (Fournier et al. 2002; Martinez et al. 2005). Both *B. cinerea* and *B. pseudocinerea* have been reported on numerous hosts (Plesken et al. 2015a; Williamson et al. 2007) from different regions around the world (Adjebli et al. 2015; Johnston et al. 2014; Kumari et al. 2014; Li et al. 2015; Muñoz et al. 2016), although *B. cinerea* is reportedly more pathogenic (Muñoz et al. 2016; Walker et al. 2011). Currently, a combination of phylogenetic analyses of multiple genes, mainly including glyceraldehyde 3-phosphate dehydrogenase (*G3PDH*), heat shock protein 60 (*HSP60*), and DNA-dependent RNA polymerase subunit II (*RPB2*), has been widely used to corroborate the identification of *Botrytis* spp. (Ferrada et al. 2016; Staats et al. 2005; Zhang et al. 2010a,b; Zhang et al. 2018; Zhou et al. 2014); and, more recently, *B. sinoviticola* and *B. californica* were identified as two new species that once belonged to group I (Saito et al. 2016; Zhang et al. 2018; Zhou et al. 2014). In addition, a major subpopulation, provisionally called *B. cinerea* group S, was detected in all strawberry-growing regions in Germany by using multiple gene sequencing, and this group is closely related to *B. cinerea* and the host-specific species *B. fabae* (Leroch et al. 2012).

Fungal transposable elements (TEs) are genes that can change position in the genome, leading to significant genetic changes. This

strategy allows natural populations to adapt to environmental constraints (Daboussi and Capy 2003). Two TEs, *boty* and *flipper*, are known to be associated with the ubiquitous fungus *B. cinerea* (Kecskeméti et al. 2014). The *B. cinerea* population is structured as four subgroups based on TEs: *transposa* (containing both *boty* and *flipper*), *boty* (only *boty*), *flipper* (only *flipper*), and *vacuma* (lacking both *boty* and *flipper*) (Giraud et al. 1997; Kumari et al. 2014). Most isolates of *B. cinerea* are heterothallic, and sexual compatibility is controlled by a single mating-type gene with two idiomorphs: *MAT1-1* and *MAT1-2*. A few isolates possessing both idiomorphs are self-fertile (Farettra et al. 1988; Martin et al. 2010). The two idiomorphs contain genes that determine mating identity and control sexual development, coding for proteins that regulate gene expression with typical functional domains (De Miccolis Angelini et al. 2016). Qiao and Li (2015) designed specific primers to detect mating types for PCR amplification based on the *MAT1-1-1* and *MAT1-2-1* gene sequences of *B. cinerea*. Mating-type distribution is the simplest way to determine the potential for sexual recombination and, thus, evolutionary potential: it is assumed that when a 1:1 ratio between mating types within a population is observed, the fungi are randomly mating due to frequency-dependent selection of each type (Kumari et al. 2014). Kumari et al. (2014) suggested the existence

of broad pathogenic variability in *B. cinerea*, with some degree of clustering with regard to location and pathogenicity group. In addition, the expression of pathogenicity varies among different TE types. Some previous studies suggested that *transposa* isolates are more virulent than *vacuma* isolates (Kumari et al. 2014; Muoz et al. 2010; Pande et al. 2010; Samuel et al. 2012). In addition, comparisons of disease severity have revealed that temperature affects both *transposa* and *vacuma* isolates (Samuel et al. 2012).

Recent research has illustrated the variability in the phenotypic instability (Corwin et al. 2016; Kuzmanovska et al. 2012), genetic diversity (Atwell et al. 2015; Decognet et al. 2009; Fournier et al. 2003; Leroch et al. 2012; Sniežana et al. 2004), population structure (Karchani-Balma et al. 2008; Konstantinou et al. 2015; Ma and Themis 2005; Martinez et al. 2003; Sniežana et al. 2004), fungicide resistance (Fernández-Ortuño et al. 2014; Konstantinou et al. 2015; Panebianco et al. 2015), and pathogenic variability (Kumari et al. 2014; Martinez et al. 2003; Samuel et al. 2012) of *B. cinerea* on various hosts. Kiwifruit is known as “the king of fruits” and is widely cultivated, with more than 40,000 ha in Sichuan Province. However, gray mold has become a serious issue for the kiwifruit industry. As a control measure, fungicides have been widely sprayed in most orchards, and this may influence the stability of the *B. cinerea*

Fig. 1. Disease symptoms of kiwi gray mold in the field. A, B, and C, Symptoms of leaves; D, symptoms of flowers.

population. Overall, the genetic features of the *B. cinerea* population from kiwifruit in China remain unclear. Therefore, the objectives of this study were to (i) characterize *B. cinerea* morphological traits in the kiwifruit population of Sichuan Province, (ii) investigate the *Botrytis* spp. diversity on kiwifruit in Sichuan Province, (iii) measure the frequency of four genotypes of TEs in Sichuan Province, (iv) determine the distributions of mating types within the collected population, and (v) compare pathogenic variability among different experimental groups. This study will provide useful information for managing gray mold in kiwifruit.

Materials and Methods

Sample collection and isolation. Kiwifruit samples ($n = 1,300$ leaves and 100 flowers) showing symptoms of gray mold (Fig. 1) were collected from major commercial kiwi orchards in eight geographic regions of Sichuan Province, China, during May and November of 2014 to 2017 (Supplementary Table S1). Each sample was composed of five leaves or flowers from five shoots per vine. Approximately 15 samples were taken in a hectare of orchard, and sampling was performed in approximately 80 orchards. The diseased leaves or flowers were placed inside an artificial climate box (VS-1300L-U; Suzhou) and kept in darkness with 90 to 95% relative humidity at 20°C for 3 days to facilitate sporulation or fruiting bodies of

Botrytis. Then, leaves or flowers with macroscopic symptoms of gray mold were examined under a microscope (SZX16; Olympus Corporation) for fruiting bodies of *Botrytis*. Diseased samples displaying the morphological characteristics of *B. cinerea* (Hawksworth 1994) were used to culture isolates. Single-spore cultures were obtained from each diseased sample according to the procedure described by Gong et al. (2010). Spores were removed directly from the diseased sample with sterilized acupuncture needles and placed in pieces (5 by 5 mm) of water agar. Then, a single spore was picked and removed to the next piece of water agar. Finally, a piece of water agar containing a single spore was transferred to a new potato dextrose agar (PDA) plate at 20°C, and pure cultures were obtained and stored at 4°C.

Cultural and morphological characteristics of *B. cinerea*. Cultural characteristics (i.e., colony morphology) were distinguished on 1% PDA after 7 days of incubation at 20°C in darkness. Three replicates were employed. Sclerotial production was also observed after 14 days. Conidiospores and conidiophores for microscopy were prepared using clear water or lactic acid and were observed and measured at $\times 100$ magnification using a Carl Zeiss Axio Imager Z2 microscope with differential interference contrast illumination. The length and width of 50 conidia and 20 conidiophores from each isolate were measured after 7 to 10 days of incubation at 20°C in darkness. The size of sclerotia ($n = 20$) on the plates was also examined.

Fig. 2. Characteristics of colonies, conidia, and conidiophores for three categories of colony type: **a**, sclerotial type (upper); **b**, conidial type (middle); and **c**, mycelial type (bottom).

Molecular characterization of *B. cinerea*. DNA extraction. DNA was extracted from aerial mycelia of *B. cinerea* isolates ($n = 176$ isolates) grown in 15 ml of potato dextrose agar (PDA) for 4 days at 20°C in the dark. Mycelia were harvested and washed in sterile water, snap-frozen in liquid nitrogen, and lyophilized. Fungal genomic DNA was extracted using a FastDNA Kit (Aidlab Biotechnologies Co., Ltd.). The integrity of the DNA was assessed on 1% agarose and observed with a UVP gel imager (Quantity One 1-D Analysis software; Bio-Rad). The concentration of genomic DNA was measured on a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific). DNA pellets were dissolved in 50 μ l of distilled water and stored at -20°C.

PCR conditions and primers used. DNA from all isolates ($n = 176$ isolates) was subjected to molecular identification using a *B. cinerea*-specific primer pair: C729⁺/729⁻ (Rigotti and Gindro 2002). To distinguish *B. cinerea* sensu stricto from other cryptic species (e.g., *B. pseudocinerea*, *B. sinoviticola*, and *B. californica*), the *Bc-hch* gene was also amplified from the 176 isolates by using the primer pair 262/520L (Fournier et al. 2003) and then digested with *Qui*lCut *Hha*I according to the manufacturer's instructions (Takara Biotech Co., Ltd.). Subsequently, 50 representative isolates (different morphological characteristics and different geographical regions) were selected for further identification by amplification of *RPB2*, encoding *G3PDH*, and encoding *HSP60* using the primers RPB2-F/RPB2-R, G3PDH-F/G3PDH-R, and HSP60-F/HSP60-R, respectively (Staats et al. 2005). The PCR products were resolved on 1.5% agarose in Tris-acetate EDTA buffer containing 0.01% Goldview I (Beijing Solarbio Science and Technology Co., Ltd.) and sequenced by Sangon Biotech Co., Ltd. The reactions were repeated three times for each PCR.

Phylogenetic analysis. A nucleotide BLAST search was performed for obtained *RPB2*, *G3PDH*, and *HSP60* sequences against recognized sequences of species of *Botrytis* in GenBank. All sequences were aligned with ClustalX 1.7, and characteristics were weighted equally. Then, a phylogenetic tree was constructed with the combined *RPB2*, *G3PDH*, and *HSP60* dataset. The evolutionary relationship was inferred by using the maximum-likelihood method based on the Kimura two-parameter model (Kimura 1980). Phylogenetic trees were constructed in MEGA7 (Kumar et al. 2016). Then, bootstrap consensus tree was inferred from 1,000 replicates. Branches corresponding to partitions reproduced in <50% bootstrap replicates were collapsed.

Detection of TEs. The presence or absence of TEs *boty* and *flipper* were studied in 176 isolates of *Botrytis* using the primer pairs boty-SF/boty-SR and F300/F1550, respectively (Giraud et al. 1997; Kumari et al. 2014). PCR was performed as previously described by Kumari et al. (2014). Reaction mixtures contained 1 μ l of template (100 ng), 1 μ l (10 μ M) of each primer, and 23 μ l of 2 $\times$ Taq PCR Master Mix (Aidlab Biotechnologies Co., Ltd.). Amplified products were resolved on 1% agarose containing 0.01% Goldview I at 110 V for 30 min and photographed with a UVP gel imager (Quantity One 1-D Analysis software; Bio-Rad). Reactions were repeated three times for each PCR.

Determination of mating type. The presence of mating type *MAT1-1-1* or *MAT1-2* was determined in 176 isolates of *Botrytis* by using primer pairs BcMAT1-F/BcMAT1-R and BcMAT2-F/BcMAT2-R (Qiao and Li 2015). The reaction mixtures were prepared as described above. PCR was performed using the following conditions: initial denaturation of 5 min at 94°C; 32 cycles of denaturation for 1 min at 94°C, annealing for 45 s at 55°C, and extension for 1 min 30 s at 72°C; and a final extension at 72°C for 10 min. The amplified products were resolved on 1% agarose containing 0.01% Goldview I. Reactions were repeated three times for each PCR.

Pathogenicity tests. To simulate natural infections by *B. cinerea*, mycelial plugs and spore suspensions were used as inocula to conduct pathogenicity tests.

Mycelial plug as inoculum. Forty-eight representative isolates from eight geographical populations were selected to perform the experiment. Each *B. cinerea* isolate was cultured on PDA for 5 days at 20°C in the dark; then, 5-mm-diameter mycelial plugs cut from the

edge of the colony were adhered to each detached leaf, as described below. Healthy detached leaves (cultivars Hongyang and Hort16A) were collected as young leaves (25 to 30 days old) and mature leaves (60 to 70 days old) in early June from 8-year-old kiwifruit trees. Similarly, from Hongyang, healthy and near-ripe fruit (6% soluble solids) were collected in August. The leaves and fruit were surface disinfected with 75% ethanol for approximately 3 min and washed twice with sterilized distilled water. Three or four mycelial plugs were adhered on each wounded (slightly stabbed by anatomic needle) and nonwounded leaf and fruit, respectively. The inoculated leaves and fruit were placed on a moistened growth chamber at 20°C with a cycle of 12 h of light and 12 h of darkness. One day after inoculation, mycelial plugs were removed from the inoculated kiwifruit tissues. Three leaves and fruit were employed for each treatment and an equal number of controls (inoculated only with agar plugs) were included. Each experiment was repeated twice.

Spore suspension as inoculum. Twenty-four representative isolates were used to conduct another pathogenicity test by inoculation of spore suspensions. Healthy attached leaves were selected as young leaves (25 to 30 days old) and mature leaves (60 to 70 days old) in early June from kiwifruit vines on 8-year-old trees. Similarly, healthy fruit (6% soluble solids) from Hongyang (8-year-old kiwifruit trees) were collected in mid-August. A 20- μ l spore suspension (10^5 spores ml^{-1} , containing 0.05% Tween-20) taken from a 7-day-old culture grown on PDA at 20°C was inoculated on slightly wounded and nonwounded leaves and fruit, and an equal volume of sterilized water containing 0.05% Tween-20 was used as the control. Four inoculations were performed on each leaf and fruit. Three leaves and fruit were employed for each treatment and control. The leaves were covered with moistened plastic bags, and the fruit were placed on a moistened growth chamber at 20°C with a cycle of 12 h of light and 12 h of darkness. Each experiment was repeated twice.

In all pathogenicity tests, reisolation of pathogens from inoculated diseased sites was conducted using the same method as described previously (see "Sample collection and isolation"). After completing morphological identification, the reisolated fungus was reidentified by PCR amplification (see "PCR conditions and primers used") to ensure that Koch's rules were completely met.

Analysis of pathogenicity data. According to the lesion diameters on leaves for different isolates, we calculated significant differences for two leaf ages, wounded or nonwounded, and eight geographical populations and analyzed correlations between pathogenicity and transposon types or colony types. At the same time, two cultivars (Hongyang and Hort16A) were analyzed for their resistance to *B. cinerea*. Differences in sensitivity for leaf ages were compared using the *t* test. For genotypes and colony types, significant differences were identified using Pearson's χ^2 test. The average diameters of disease spots in each geographical population were analyzed by Duncan's least significant range test at $P = 0.05$, and the differences in

Fig. 3. Identification of a subset of *Botrytis cinerea* isolates based on molecular characteristics. **A**, Polymerase chain reaction (PCR) amplification of *B. cinerea* using the primer pair C729⁺/C729⁻; a band at 750 bp indicates *B. cinerea*. **B**, PCR amplification of *Bc-hch* using the primer pair 262/520L (top) and digestion of amplicons with *Hha*I (bottom); a band at 517 bp indicates *B. cinerea* sensu stricto. Positions of amplified fragments at specific sizes are marked with arrows.

virulence among different isolates in Sichuan Province were analyzed. All statistical tests were performed in SPSS v21.0.

Results

Identification of *B. cinerea*. In total, 176 isolates of *Botrytis* spp. were obtained from symptomatic leaves and flowers (Fig. 1) from kiwifruit orchards in eight regions in Sichuan Province. According to

morphological and molecular characteristics, all isolates were identified as *B. cinerea* sensu stricto.

The colonies formed fluffy, gray-white aerial mycelia on PDA, then turned gray and formed conidia on the mycelia after 7 days of incubation and, after 14 days, generated black sclerotia. The length and width of sclerotia varied from 1.1 to 4.5 and 1.0 to 3.5 mm, respectively (average = 3.4 by 2.3 mm) (Fig. 2). Conidiophores were

Fig. 4. Maximum-likelihood tree based on *RPB2*, *HSP60*, and *G3PDH* gene sequence data from 50 representative isolates. *Sclerotinia sclerotiorum* strain 484 was used as the outgroup. The bootstrap consensus tree was inferred from 1,000 replicates. Isolates obtained in this study are indicated in bold.

erect, solitary or clustered, brown-branched at the top, with the length and width ranging from 520 to 2,250 by 6 to 18 μm (average = 721 by 12 μm) (Fig. 2). The conidia were unicellular, hyaline to light brown, and ellipsoid to ovoid, with sizes ranging from 6.75 to 13.59 μm in length by 3.96 to 6.10 μm in width (average = 8.15 by 5.77 μm) (Fig. 2). All isolates were consistent with previous descriptions of *B. cinerea* (Mirzaei et al. 2008).

PCR amplification using the specific primer pair C729⁺/C729⁻ showed that all isolates ($n = 176$ isolates) produced a specific 750-bp fragment (Fig. 3A). The *Bc-hch* gene amplified using the specific primer pair 262/520L from the 176 *B. cinerea* isolates revealed a fragment of 1,149 bp (Fig. 3B). All PCR products were digested using the enzyme *Hha*I and produced the same 294- and 517-bp fragments (Fig. 3B), of which the 517-bp fragment is a *B. cinerea* group

Table 1. Relative percent distribution of colony types, genotypes of transposable elements (TEs) and mating types with respect to location

Location	N ^z	Colony types (%) ^y			Genotypes of TEs (%)				Mating types (%)	
		Scl	Con	Myc	<i>transposa</i>	<i>boty</i>	<i>flipper</i>	<i>vacuma</i>	MAT1-1	MAT1-2
Dujiangyan	33	27.27	15.15	57.58	69.70	24.24	6.06	0.00	42.42	57.58
Shuangliu	16	62.50	6.25	31.25	87.50	12.50	0.00	0.00	43.75	56.25
Mianzhu	22	63.64	27.27	9.09	59.09	40.91	0.00	0.00	59.09	40.91
Pujiang	38	50.00	26.32	23.68	60.53	39.47	0.00	0.00	47.37	52.63
Yucheng	18	38.89	11.11	50.00	83.33	16.67	0.00	0.00	61.11	38.89
Anzhou	13	7.69	38.46	53.85	69.23	7.69	23.08	0.00	69.23	30.77
Pengzhou	14	50.00	14.29	35.71	92.86	0.00	7.14	0.00	42.86	57.14
Cangxi	22	22.73	18.18	59.09	77.27	18.18	4.55	0.00	40.91	59.09
Total	176	40.91	19.89	39.20	72.16	23.86	3.98	0.00	49.43	50.57

^y Scl = sclerotial, Con = conidial, and Myc = mycelial.

^z Number of isolates.

Fig. 5. Disease symptoms on kiwifruit leaves at 3 days after inoculation with mycelial plugs of *Botrytis cinerea*. CK denotes the control, isolates YA7 and CX10 belong to the *boty* type, and D2 and M13 belong to the *transposa* type.

II-specific fragment (only *B. cinerea* sensu stricto), matching previous reports (Fournier et al. 2005; Muñoz et al. 2016). These results suggest that all 176 isolates belonged to *B. cinerea* sensu stricto.

The sequences of *RPB2*, *G3PDH*, and *HSP60* from 50 representative isolates were 100% similar to those of *B. cinerea* isolate CLMR451-1 (accession number KR076780), *B. cinerea* isolate CHM137423 (accession number KR055048), and *B. cinerea* isolate MHT4 (accession number KY364368), respectively, as reported in GenBank. The sequences for the 50 isolates were deposited in the National Center for Biotechnology Information database (Supplementary Table S2). A phylogenetic tree was constructed using the combined sequences of *RPB2*, *G3PDH*, and *HSP60* on 72 isolates (50 isolates from this study and 22 reference isolates from GenBank), with *Sclerotinia sclerotiorum* strain 484 used as outgroups (Staats et al. 2005). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site, and showed that all of the 50 kiwifruit gray mold isolates clustered with *B. cinerea* and

distinctly away from other species of *Botrytis* (e.g., *B. pseudocinerea* and *B. sinoviticola*) (Fig. 4).

Partition of three morphological categories of *B. cinerea* population. The colony characteristics of all isolates showed obvious differences on PDA medium after 14 days. The isolates were divided into three colony types: sclerotial, conidial, and mycelial (Fig. 2). The sclerotial-type isolates produced a number of sclerotia, a small number of hyphae, and few or no conidiospores, whereas the conidial-type isolates produced a large number of conidiospores and few or no sclerotia. The mycelial-type isolates appeared as thick colonies with abundant aerial hyphae that did not produce or only produced small amounts of conidiospores (or sclerotia); the sclerotial type was the richest, with a separation frequency of 40.91%. The separation frequency of the mycelial type was 39.20%, and the separation frequency of the conidial type was only 19.89% (Table 1). All three colony types were found in all eight geographical regions.

Table 2. Analysis of variance for lesion diameter of kiwifruit^a

Parameters	Source of variance	SS	df	MS	F value	P value
Different isolates (mycelial plug as inoculum)	Between groups	3,677.131	47	78.237	5.807	0.000
	Within groups	1,940.167	144	13.473	...	...
Different isolates (spore suspension as inoculum)	Between groups	7,953.935	23	345.823	13.272	0.000
	Within groups	1,876.063	72	26.056	...	...
Different leaf ages (mature leaves and young leaves)	Between groups	3,252.749	1	3252.749	94.690	0.000
	Within groups	9824.595	286	34.352	...	...
Different cultivars (Hongyang and Hort16A)	Between groups	2,664.538	1	2664.538	85.337	0.000
	Within groups	4,433.767	142	31.224	...	...
Wounded and nonwounded (mycelial plug as inoculum)	Between groups	4.117	1	4.117	0.258	0.612
	Within groups	1,497.212	94	15.928	...	...
Wounded and nonwounded (spore suspension as inoculum)	Between groups	1,647.363	1	1647.363	33.564	0.000
	Within groups	2,257.731	47	49.081	...	...

^a Values obtained by mean of three replicates.

Fig. 6. Disease symptoms on fruit of cultivar Hongyang with *Botrytis cinerea*. **A**, Symptoms 3 days after inoculation mycelial plugs; **B**, symptoms 5 days after inoculation with conidial suspension. CK indicates inoculation with agar discs or water; strains T5, T1, T2, and T3 are from Pujiang.

The microscopic characteristics of the three colony types, including conidiophores and conidia, are shown in Figure 2. However, no obvious differences were observed among the three types.

Detection of TEs. The analysis of the detection of TEs *boty* and *flipper* revealed that three genotypes are present: *transposa* (*boty* and *flipper*), *boty* (only *boty*), and *flipper* (only *flipper*). The distribution of TEs presented a similar tendency when investigated with respect to location (Table 1). The most frequent transposon genotype was *transposa* (72.16%), followed by *boty* (23.86%) and *flipper* (3.98%), while *vacuma* was absent. Both *boty* and *transposa* isolates were found in all geographical populations. In contrast, *flipper* isolates were found in only four regions (Dujiangyan, Cangxi, Pengzhou, and Anzhou) (Table 1).

Distribution of mating types in the *B. cinerea* population. Amplification of the *MAT1-1* locus yielded a product of approximately 1,000 bp, and amplification of the *MAT1-2* locus yielded a product of approximately 800 bp. In total, 87 isolates were the *MAT1-1* type and the remaining 89 isolates were the *MAT1-2* type. The mating-type distribution of the sampled population is presented in Table 1. The overall sampled population showed a distribution of mating loci in a ratio of 1:1. However, ratio deviations were observed in several subpopulations. Analysis according to location revealed *MAT1-1* and *MAT1-2* to be equally distributed among isolates collected from Dujiangyan, Shuangliu, Mianzhu, Pujiang, and Pengzhou. In Anzhou, the frequency of the *MAT1-1*-containing population (69.23%) exceeded that of *MAT1-2* (30.77%). A similar case was observed in Yucheng: a population dominated by the *MAT1-1* allele (61.11%) followed by *MAT1-2* (38.89%). However, the opposite trend was observed for isolates from Cangxi, with a higher number of populations containing the *MAT1-2* locus (59.09%) than the *MAT1-1* locus (40.91%).

Pathogenicity differentiation. All representative isolates were found pathogenic on wounded and nonwounded leaves of the kiwifruit cultivar Hongyang, inoculated with either mycelia or conidia (Fig. 5; Supplementary Fig. S1). The pathogens were successfully reisolated from symptomatic tissues but not from healthy tissues (controls). White mycelial growth was observed along the inoculated sites after 24 h. Disease symptoms then developed on inoculated leaves after 48 h, changing in color from taupe to dark brown by 4 days. It was clear that pathogenicity variability existed among the isolates even from the same geographic population (Table 2). In the pathogenicity test with mycelial plugs as inocula, the diameter of disease lesions ranged from 1.0 to 22.7 mm after 3 days on young leaves of cultivar Hongyang, whereas it ranged from 1.0 to 33.7 mm after 5 days in the field pathogenicity test using spore suspensions as inocula. In addition, all representative isolates were also found pathogenic on the fruit when pathogenicity was conducted on Hongyang kiwifruit. The development of lesions inoculated with mycelial plugs expand faster than inoculation with conidia on either wounded or nonwounded fruit (Fig. 6).

Disease severity showed no significant differences among most of the geographic populations (Table 3). For the pathogenicity test on detached leaves with mycelial plug inoculation, the isolates from Dujiangyan showed the strongest virulence (18.04 ± 1.03); the second most virulent was from Cangxi (16.4 ± 1.3) (Table 3). Notably, χ^2 analysis of isolate pathogenicity revealed similar capacities between the *transposa* and *boty* types ($\chi^2 = 15.19$, $df = 19$, $P > 0.05$) and no significant correlation between pathogenicity and colony type ($\chi^2 = 38.6$, $df = 38$, $P > 0.05$).

Significant differences were found for disease severity between the two age groups. Disease development was much more severe on young leaves than on mature leaves ($P < 0.01$) (Tables 2 and 3; Fig. 5). The cultivar Hort16A showed strong resistance to *B. cinerea*, whereas the cultivar Hongyang was very sensitive to the pathogen ($P < 0.01$) (Tables 2 and 3; Figs. 5 and 7). There were no significant differences in disease severity between the wounded leaves and the nonwounded leaves at 3 days after inoculation with mycelial plugs of *B. cinerea* ($P > 0.05$). However, the disease severity of wounded leaves was significantly higher than that of nonwounded leaves in the pathogenicity test with spores ($P < 0.01$) (Table 2).

Discussion

In this study, 176 isolates of *B. cinerea* were obtained and consistently isolated from gray mold disease in leaves or flowers of kiwifruit in Sichuan Province, China. All isolates displayed a morphological phenotype corresponding to *B. cinerea* (Mirzaei et al. 2008). Phenotypic and genotypic diversity in *B. cinerea* isolates has been studied in various locations around the world (Baraldi et al. 2002; Giraud et al. 1999; Grindle 1979; Kuzmanovska et al. 2012; Mirzaei et al. 2009; Muñoz et al. 2002; Sniežana et al. 2004). However, no detailed population analysis of *B. cinerea* isolates from kiwifruit has been published to date. Eight geographical populations from Sichuan Province were examined in this study using molecular methods, and all were found to belong to *B. cinerea* sensu stricto. Thus, *B. cinerea* sensu stricto constitutes the *B. cinerea* population from kiwifruit in Sichuan Province, whereas *B. pseudocinerea* was not found in this study. Similar results have been obtained previously for the *Botrytis* populations obtained from other hosts by Muñoz et al. (2002), Esterio et al. (2011), Huang et al. (2014), Váczy et al. (2008), and Samuel et al. (2012). Overall, *B. pseudocinerea* isolates have been reported at low frequency levels of 2.5 to 15% (Kretschmer and Hahn 2008; Ma and Themis 2005; Martínez et al. 2005; Walker et al. 2011). However, recently, *B. pseudocinerea* was found on tomato in central China (Li et al. 2015), and Plesken et al. (2015a) found that *B. pseudocinerea* is common and sometimes dominant over *B. cinerea* on several crop plants. Thus, the absence of *B. pseudocinerea* isolates should be investigated further in Sichuan populations. In addition, a new subpopulation, *Botrytis* group S, was detected in Germany in 2011 (Walker et al. 2011) and was repeatedly found on different hosts in other countries (Giraud et al. 1999;

Table 3. Pathogenicity of isolates from different geographical areas toward leaves at two ages of Hongyang and Hort16A kiwifruit at 3 days after inoculation with mycelial plugs

Location	Number of isolate	Average lesion diameter (mm) ^a			
		Hongyang		Hort16A	
		Young leaf	Mature leaf	Young leaf	Mature leaf
Dujiangyan	6	18.0 ± 1.0 a	6.0 ± 0.3 abc	8.9 ± 0.5 a	2.5 ± 0.3 a
Cangxi	6	16.4 ± 1.3 ab	6.7 ± 0.3 ab	7.8 ± 0.5 b	2.2 ± 0.2 a
Mianzhu	6	15.0 ± 0.9 abc	4.6 ± 0.2 d	7.2 ± 0.3 bc	1.8 ± 0.1 b
Anzhou	6	15.0 ± 0.8 abc	5.3 ± 0.5 cd	6.8 ± 0.6 c	1.7 ± 0.2 b
Shuangliu	6	13.5 ± 0.6 bc	6.4 ± 0.3 abc	5.4 ± 0.4 d	1.3 ± 0.1 c
Yucheng	6	13.0 ± 1.2 bc	6.2 ± 0.5 abc	7.3 ± 0.3 bc	2.3 ± 0.1 a
Pujiang	6	12.8 ± 1.3 c	5.4 ± 0.2 bcd	5.4 ± 0.5 d	1.6 ± 0.1 bc
Pengzhou	6	11.9 ± 1.0 c	6.8 ± 0.3 a	6.7 ± 0.6 c	1.5 ± 0.2 bc

^a Data followed by the same lowercase letters in the same column are not significantly different at $\alpha = 0.05$ by Duncan's least significant range test.

Johnston et al. 2014). Recently, several new species have been reported in China and the United States: *B. californica* reported in California (Saito et al. 2014); *B. sinoallii* identified on green onion, garlic, and garlic chives in China (Zhang et al. 2010b); *B. fabiopsis* found on broad bean in Hubei Province, China (Zhang et al. 2010a); and *B. sinoviticola* identified in China (Zhou et al. 2014). The discovery of these new species suggests that the *B. cinerea* is rapidly evolving into other new, independent species. Therefore, it is vital to successively monitor the dynamics of the pathogen in the future and to provide useful information for controlling kiwifruit gray mold caused by *B. cinerea*.

Previous studies have reported that *B. cinerea* contains a wealth of phenotypic diversity and can be classified into at least three colony types (sclerotial, conidial, and mycelial) (Grindle 1979; Martinez et al. 2005; Mirzaei et al. 2009). This study confirmed the three categories, with the sclerotial type as the most prevalent type (40.91% of all isolates), which was lower than in previous reports (Huang et al. 2014; Martinez et al. 2005). In fact, no obvious differences (including in conidia and conidiophores) were observed among the three types. Because colony characteristics are unstable and controlled by the environment as well as genetics, they are not sufficient for complete identification of the pathogen. *B. cinerea* has a high degree of variation as a pathogenic fungus, and its genetic diversity may be

caused by nucleation, sexual recombination, sexual reproduction, parasexual reproduction, and mobile TEs (Beever et al. 2004).

TE genotype identification constitutes a research area for *B. cinerea* and is an important means of detecting genetic diversity (Beever et al. 2004; Daboussi and Capy 2003; Giraud et al. 1999). In our study, the *transposa* isolates of *B. cinerea* were predominant in the Sichuan population. The *transposa* isolates accounted for 72.16%, *boty* for 23.86%, and *flipper* for 3.98% of the population, whereas the *vacuma* isolates were not detected. The distributions of *transposa* and *boty* isolates obtained were similar to those in previous works on *B. cinerea* populations in other hosts in China (Huang et al. 2014). In populations of *B. cinerea* in kiwifruit from Greece, a high frequency of *flipper* and *vacuma* isolates was detected (Samuel et al. 2012). However, our findings regarding the distribution frequencies of the four transposon types were completely different from their results, possibly due to differences in populations from different geographic areas (Kumari et al. 2014). The results suggested that the genetic diversity of *B. cinerea* populations is very rich, which may explain why the fungus is highly variable. Martinez et al. (2005) reported that *vacuma* isolates are more predominant at the full-bloom stage and the *transposa* type at harvest. *Vacuma* isolates were mainly distributed in northern China, although in only small amounts (Zhang et al. 2018). In our study, *vacuma* was absent in the isolate samples,

Fig. 7. Symptoms of attached leaves in the orchard at 5 days after inoculation with spore suspensions of *Botrytis cinerea*.

no matter whether isolated from flowers or leaves, which may be due to the warm and humid climate in Sichuan, which is located in the southwest of China.

The fungal species *B. cinerea* is an ascomycete, and its teleomorph is *Botryotinia fuckeliana*. The first studies of *Botrytis cinerea* sexual behavior and mating system were reported by Faretra et al. (1988), and indoor sexual hybridization experiments demonstrated that the mating-type gene follows the 1:1 ratio of Mendel's laws. Isolates are considered to be heterothallic if either the *MAT1-1* or *MAT1-2* locus is amplified (Wessels et al. 2013). Sexual recombination may be a significant factor in determining population dynamics, because it results in the generation of new genotypes and, thus, contributes to genetic diversity and evolutionary potential (Esterio et al. 2011). According to previous studies, most isolates possess only one mating type; in most cases, the population distribution of mating loci exists at a ratio of 1:1. Insight into the *MAT* locus and the fundamental principles of the mating process can prompt new strategies for controlling gray mold caused by *B. cinerea* (Faretra et al. 1988; Martin et al. 2010). This study revealed the *MAT1-1-1* and *MAT1-1-2* loci at frequencies of 49.43 and 50.57%, respectively, among 176 isolates collected from kiwifruit in Sichuan Province, though isolates carrying both loci were not found. However, ratio deviations were observed in Anzhou, Yucheng, and Cangxi; these results may be related to the presence of recessive homozygous lethal alleles (Byrne et al. 1995) or may also be due to structural rearrangements, deletions, insertions, and mutations in the chromosome during hybridization (Raper 1985). The different sampling methods and the number of samples also have an effect on the mating-type ratio (Qiao and Li 2015), and the specific reasons require further study. Overall, the two loci maintained an equilibrium in kiwifruit populations, and each locus appeared in each of the populations. The results suggest that sexual reproduction likely occurs under natural conditions.

All representative isolates inoculated in kiwifruit leaves and fruit caused gray mold disease. This study revealed obvious pathogenic differences among *B. cinerea* isolates from the eight geographical regions. Although most geographical populations showed no obvious differences, the Dujiangyan populations exhibited greater virulence than the other geographic populations. The isolates from the same geographic population showed different virulence levels. The resistance of cultivar Hort16A was overwhelmingly superior to that of Hongyang. The resistant Hort16A, one of the major kiwifruit cultivars in New Zealand, may be one of the reasons why kiwi gray mold is not severe in New Zealand. However, the susceptible Hongyang, as the main cultivar of kiwifruit planted in Sichuan Province, leads to a serious gray mold disease that threatens the kiwifruit industry. Therefore, expanding the use of resistant cultivars should be an extremely efficient method of controlling gray mold disease. Mature leaves showed distinct pathogen resistance, whereas young leaves were extremely sensitive to *B. cinerea*. In general, there are two peaks of gray mold epidemic every year in Sichuan, in the spring and autumn; they are attributed to the young leaf stage coinciding with a suitable climate (cool and damp weather) for the development of *B. cinerea*. The humid climate in Sichuan Province is very suitable for the growth of *B. cinerea*. Thus, rain-shelter cultivation with a hostile environment for *B. cinerea* growth may be an effective measure to control gray mold disease. In China, this approach has been effectively applied to control plant disease on grape, kiwifruit, and other crops (Yu et al. 2017). In Sichuan, kiwifruit are often bagged from young to ripe, which can reduce the impact of pests and diseases on the fruit; thus, gray mold is mainly reflected in leaves and flowers during the growing season. According to our observations, fragments of diseased flowers or leaves can fall onto healthy tissues (leaves, fruit, and so on) and cause gray mold, which can be seen as a mycelial inoculation. Through our multiple pathogenicity tests comparison, the infection with wounded inoculation was faster than that of non-wounded inoculation. Our result is a good explanation of why gray mold is often prevalent after a storm or cold spell in later spring. In the autumn, many young leaves from autumn shoots are often induced due to inappropriate agricultural practices or *Corynespora* leaf spot disease (Cui et al. 2015), resulting in defoliation and, thus, an

epidemic of gray mold. Therefore, delaying the growth of mature leaves of spring and summer shoots from September to November can decrease gray mold disease caused by *B. cinerea*. In New Zealand, gray mold disease can be controlled by proper field management instead of fungicide use (Manning et al. 2010). Disease risk can be decreased by vine canopy management and by timely cleanup of the diseased plant residues from the orchard.

In conclusion, the above results indicated that *B. cinerea* sensu stricto is the causal agent of kiwifruit gray mold. The *B. cinerea* population from kiwifruit in Sichuan Province shows rich phenotypic and genetic diversity, including three colony types and three TE types, and the two mating types are balanced overall in the population. The virulence of isolates differed between regions of Sichuan. In addition, the resistances of different cultivars and leaf ages to *B. cinerea* differed significantly. This work will provide a valuable theoretical basis for the future prevention and control of kiwifruit gray mold.

Acknowledgments

We thank X. Huang, H. Chen, C. Zhang, C. Zhang, and Y. Zhou (Plant Protection Department, Sichuan Agriculture University, Sichuan Province) for collecting *B. cinerea* isolates and analyzing the data; M. I. Khaskheli for improving the writing of manuscript; and the anonymous reviewers for their constructive suggestions.

Literature Cited

- Adjebli, A., Leyronas, C., Aissat, K., and Nicot, P. C. 2015. Comparison of *Botrytis cinerea* populations collected from tomato greenhouses in Northern Algeria. *J. Phytopathol.* 163:124-132.
- Atwell, S., Corwin, J. A., Soltis, N. E., Subedy, A., Denby, K. J., and Kliebenstein, D. J. 2015. Whole genome resequencing of *Botrytis cinerea* isolates identifies high levels of standing diversity. *Front. Microbiol.* 6:996.
- Baraldi, E., Bertolini, P., Chierici, E., and Luiselli, D. 2002. Genetic diversity between *Botrytis cinerea* isolates from un-stored and cold stored kiwi fruit. *J. Phytopathol.* 150:629-635.
- Beever, R. E., Weeds, P. L., Elad, Y., Williamson, B., Tudzynski, P., and Delen, N. 2004. Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. Pages 29-52 in: *Botrytis: Biology, Pathology and Control*. Y. Elad, B. Williamson, P. Tudzynski, and N. Delen, eds. Springer, Dordrecht, The Netherlands.
- Byrne, M., Murrell, J. C., Allen, B., and Moran, G. F. 1995. An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. *Theor. Appl. Genet.* 91:869-875.
- Corwin, J. A., Subedy, A., Eshbaugh, R., and Kliebenstein, D. J. 2016. Expansive phenotypic landscape of *Botrytis cinerea* shows differential contribution of genetic diversity and plasticity. *Mol. Plant-Microbe Interact.* 29:287-298.
- Cui, Y. L., Gong, G. S., Yu, X. M., Xu, J., Wen, X., Zhang, M., Chen, H. B., Zheng, X. J., Zhou, Y., and Chang, X. L. 2015. First report of brown leaf spot on kiwifruit caused by *Corynespora cassiicola* in Sichuan, China. *Plant Dis.* 99:725.
- Daboussi, M. J., and Capy, P. 2003. Transposable elements in filamentous fungi. *Annu. Rev. Microbiol.* 57:275-299.
- Decognet, V., Bardin, M., Trottin, Y., and Nicot, P. C. 2009. Rapid change in the genetic diversity of *Botrytis cinerea* populations after the introduction of strains in a tomato glasshouse. *Phytopathology* 99:185-193.
- De Miccolis Angelini, R. M., Rotolo, C., Pollastro, S., and Faretra, F. 2016. Molecular analysis of the mating type (*MAT1*) locus in strains of the heterothallic ascomycete *Botrytis cinerea*. *Plant Pathol.* 65:1321-1332.
- Esterio, M., Muñoz, G., Ramos, C., Cofré, G., Estévez, R., Salinas, R., and Auger, J. 2011. Characterization of *Botrytis cinerea* isolates present in Thompson seedless table grapes in the central valley of Chile. *Plant Dis.* 95:683-690.
- Faretra, F., Antonacci, E., and Pollastro, S. 1988. Sexual behaviour and mating system of *Botryotinia fuckeliana*, teleomorph of *Botrytis cinerea*. *J. Gen. Microbiol.* 134:2543-2550.
- Fernández-Ortuño, D., Grabke, A., Bryson, P. K., Amiri, A., Peres, N. A., and Schnabel, G. 2014. Fungicide resistance profiles in *Botrytis cinerea* from strawberry fields of seven southern U.S. States. *Plant Dis.* 98:825-833.
- Ferrada, E. E., Latorre, B. A., Zoffoli, J. P., and Castillo, A. 2016. Identification and characterization of *Botrytis* blossom blight of Japanese plums caused by *Botrytis cinerea* and *B. prunorum* sp. nov. in Chile. *Phytopathology* 106:155-165.
- Fournier, E., Eournie, V., Giraud, I., Tatiana, A., and Catherine, B. 2005. Partition of the *Botrytis cinerea* complex in France using multiple gene genealogies. *Mycologia* 97:1251-1267.
- Fournier, E., Giraud, T., Loiseau, A., Vautrin, D., Estoup, A., Solignac, M., Comuet, J., and Brygoo, Y. 2002. Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (Ascomycota). *Mol. Ecol. Notes* 2:253-255.
- Fournier, E., Levis, C., Fortini, D., Leroux, P., and Brygoo, Y. 2003. Characterization of *Bc-hch*, the *Botrytis cinerea* homolog of the *Neurospora crassa* vegetative incompatibility locus, and its use as a population marker. *Mycologia* 95:251-261.

- Giraud, T., Fortini, D., Levis, C., Lamarque, C., Leroux, P., Lobuglio, K., and Brygoo, Y. 1999. Two sibling species of the *Botrytis cinerea* complex, *transposa* and *vacuina*, are found in sympatry on numerous host plants. *Phytopathology* 89:967-973.
- Giraud, T., Fortini, D., Levis, C., Leroux, P., and Brygoo, Y. 1997. RFLP markers show genetic recombination in *Botryotinia fuckeliana* (*Botrytis cinerea*) and transposable elements reveal two sympatric species. *Mol. Biol. Evol.* 14: 1177-1185.
- Gong, G., Xu, Q., Zhang, M., Yang, J., and Chen, H. 2010. A simple method for single fungal spore isolation. *J. Maize Sci.* 18:126-127.
- Grindle, M. 1979. Phenotypic differences between natural and induced variants of *Botrytis cinerea*. *J. Gen. Microbiol.* 111:109-120.
- Hawksworth, D. L., ed. 1994. *Ascomycete Systematics: Problems and Perspectives in the Nineties*. Springer, New York.
- Huang, Y., Zhu, Z. D., Duan, C., Wu, X. F., and Dong, Y. 2014. Genetic diversity of *Botrytis cinerea* isolates from Broad Bean. *Sci. Agric. Sin.* 47:2335-2347.
- Johnston, P. R., Hoksbergen, K., Park, D., and Beever, R. E. 2014. Genetic diversity of *Botrytis* in New Zealand vineyards and the significance of its seasonal and regional variation. *Plant Pathol.* 63:888-898.
- Karakaya, A., and Bayraktar, H. 2009. *Botrytis* disease of kiwifruit in Turkey. *Australas. Plant Dis. Notes* 4:87-88.
- Karchani-Balma, S., Gautier, A., Raies, A., and Fournier, E. 2008. Geography, plants, and growing systems shape the genetic structure of Tunisian *Botrytis cinerea* populations. *Phytopathology* 98:1271-1279.
- Keckskeméti, E., Brathuhn, A., Kogel, K.-H., Berkemann-Löhnertz, B., and Reineke, A. 2014. Presence of transposons and mycoviruses in *Botrytis cinerea* isolates collected from a German grapevine growing region. *J. Phytopathol.* 162:582-595.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111-120.
- Konstantinou, S., Veloukas, T., Leroch, M., Meneses, G., Hahn, M., and Karaoglanidis, G. 2015. Population structure, fungicide resistance profile, and *sdhB* mutation frequency of *Botrytis cinerea* from strawberry and greenhouse-grown tomato in Greece. *Plant Dis.* 99:240-248.
- Kretschmer, M., and Hahn, M. 2008. Fungicide resistance and genetic diversity of *Botrytis cinerea* isolates from a vineyard in Germany. *J. Plant Dis. Prot.* 115: 214-219.
- Kulakiotu, E. K., Thanassouloupoulos, C. C., and Sfakiotakis, E. M. 2004. Postharvest biological control of *Botrytis cinerea* on kiwifruit by volatiles of "Isabella" grapes. *Phytopathology* 94:1280-1285.
- Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33: 1870-1874.
- Kumari, S., Tayal, P., Sharma, E., and Kapoor, R. 2014. Analyses of genetic and pathogenic variability among *Botrytis cinerea* isolates. *Microbiol. Res.* 169:862-872.
- Kuzmanovska, B., Rusevski, R., Jankulovski, L., Jankulovska, M., Ivic, D., and Bandzo, K. 2012. Phenotypic and genetic characterization of *Botrytis cinerea* isolates from tomato. *Genetika* 44:633-647.
- Leroch, M., Plesken, C., Weber, R. W. S., Kauff, F., Scalliet, G., and Hahn, M. 2012. 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.
- Li, N., Zhang, J., Yang, L., Wu, M. D., and Li, G. Q. 2015. First report of *Botrytis pseudocinerea* causing gray mold on tomato (*Lycopersicon esculentum*) in central China. *Plant Dis.* 99:283.
- Li, Y., Sun, S., Du, C., Xu, C., Zhang, J., Duan, C., and Zhu, Z. 2016. A new disease of mung bean caused by *Botrytis cinerea*. *Crop Prot.* 85:52-56.
- Liu, Q., and Li, X. 2014. SWOT analysis on development of kiwifruit industry and countermeasures in Sichuan Province. *Guizhou Agric. Sci.* 42:224-228.
- Ma, Z. H., and Themis, J. M. 2005. Genetic structure of *Botrytis cinerea* populations from different host plants in California. *Plant Dis.* 89:1083-1089.
- Manning, M. A., Pak, H. A., and Beresford, R. M. 2010. Non-fungicidal control of *Botrytis* storage rot in New Zealand kiwifruit through pre- and postharvest crop management. Pages 183-196 in *Postharvest Pathology. Plant Pathology in the 21st Century* (Contrib. 9th Int. Congress), Vol 2. D. Prusky and M. Gullino, eds. Springer, Dordrecht, The Netherlands.
- Martin, T., Lu, S., van Tilbeurgh, H., Ripoll, D., Dixelius, C., Turgeon, B., and Debuchy, R. 2010. Tracing the origin of the fungal α 1 domain places its ancestor in the HMG-Box super family: Implication for fungal mating-type evolution. *PLoS One* 5:e15199.
- Martinez, F., Blancard, D., Lecomte, P., Levis, C., Dubos, B., and Fermaud, F. 2003. Phenotypic differences between *vacuina* and *transposa* subpopulations of *Botrytis cinerea*. *Eur. J. Plant Pathol.* 109:479-488.
- Martinez, F., Martinez Bordeaux, I., Dubos, B., and Fermaud, M. 2005. The role of saprotrophy and virulence in the population dynamics of *Botrytis cinerea* in vineyards. *Phytopathology* 95:692-700.
- Michailides, T. M., and Elmer, P. A. G. 2000. *Botrytis* gray mold of kiwifruit caused by *Botrytis cinerea* in the United States and New Zealand. *Plant Dis.* 84: 208-223.
- Mirzaei, S., Goltapeh, E. M., Shams-Bakhsh, M., Safaie, N., and Chaichi, M. 2009. Genetic and phenotypic diversity among *Botrytis cinerea* isolates in Iran. *J. Phytopathol.* 157:474-482.
- Mirzaei, S., Mohammadi Goltapeh, E., Shams-Bakhsh, M., and Safaie, N. 2008. Identification of *Botrytis* spp. on plants grown in Iran. *J. Phytopathol.* 156:21-28.
- Muñoz, C., Talquena, S. G., Oriolani, E., and Combina, M. 2010. Genetic characterization of grapevine-infecting *Botrytis cinerea* isolates from Argentina. *Rev. Iberoam. Micol.* 27:66-70.
- Muñoz, G., Campos, F., Salgado, D., Galdames, R., Gilchrist, L., Chahin, G., and Andrade, O. 2016. Molecular identification of *Botrytis cinerea*, *Botrytis paeoniae* and *Botrytis pseudocinerea* associated with gray mould disease in peonies (*Paeonia lactiflora* Pall.) in southern Chile. *Rev. Iberoam. Micol.* 33:43-47.
- Muñoz, G., Hinrichsen, P., Brygoo, Y., and Giraud, T. 2002. Genetic characterisation of *Botrytis cinerea* populations in Chile. *Mycol. Res.* 106:594-601.
- Pande, S., Sharma, M., Kishore, G. K., Shivram, L., and Mangala, U. N. 2010. Characterization of *Botrytis cinerea* isolates from chickpea: DNA polymorphisms, cultural, morphological and virulence characteristics. *Afr. J. Biotechnol.* 9:7961-7967.
- Panebianco, A., Castello, I., Cirvilleri, G., Perrone, G., Epifani, F., Ferrara, M., Polizzi, G., Walters, D. R., and Vitale, A. 2015. Detection of *Botrytis cinerea* field isolates with multiple fungicide resistance from table grape in Sicily. *Crop Prot.* 77:65-73.
- Plesken, C., Weber, R. W., Rupp, S., Leroch, M., and Hahn, M. 2015a. *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.
- Plesken, C., Westrich, L. D., and Hahn, M. 2015b. Genetic and phenotypic characterization of *Botrytis calthae*. *Plant Pathol.* 64:128-136.
- Qiao, G. H., and Li, X. H. 2015. Analysis and molecular detection of *Botrytis cinerea* mating type genes. *Mycosystema* 34:10-16.
- Qin, X. B., and Gao, J. H. 2013. Studies on genetic diversity for specialty kiwifruits from southwestern regions of China using AFLP system. *J. Trop. Subtrop. Bot.* 21:315-322.
- Raper, C. A. 1985. B-mating-type genes influence survival of nuclei separated from heterokaryons of schizophyllum. *Exp. Mycol.* 9:149-160.
- Rigotti, S., and Gindro, K. 2002. Characterization of molecular markers for species and sensitive detection of *Botrytis cinerea* Pers.: Fr. in strawberry (*Fragaria × ananassa* Duch.) using PCR. *FEMS Microbiol. Lett.* 209:169-174.
- Saito, S., Michailides, T. J., and Xiao, C. L. 2014. First report of *Botrytis pseudocinerea* causing gray mold on blueberry in North America. *Plant Dis.* 98:1743.
- Saito, S., Margosan, D., Michailides, T. J., and Xiao, C. L. 2016. *Botrytis californica*, a new cryptic species in the *B. cinerea* species complex causing gray mold in blueberries and table grapes. *Mycologia* 108:330-343.
- Samuel, S., Veloukas, T., Papavasileiou, A., and George, S. K. 2012. Differences in frequency of transposable elements presence in *Botrytis cinerea* populations from several hosts in Greece. *Plant Dis.* 96:1286-1290.
- Shirzad, H., Hassani, A., Ghosta, Y., Abdollahi, A., Finidokht, R., and Meshkatsadat, M. H. 2011. Assessment of the antifungal activity of natural compounds to reduce postharvest gray mould (*Botrytis cinerea* Pers.: FR.) of kiwifruits (*Actinidia deliciosa*) during storage. *J. Plant Prot. Res.* 51:1-6.
- Snježana, T., Miličević, T., and Cvjetković, B. 2004. Genetic diversity and dynamic of pyrimethanil resistant phenotype in population of *Botrytis cinerea* Pers. Fr. in one wine-growing area in Croatia. *J. Plant Dis. Prot.* 111:451-460.
- Sommer, N., Fortlage, R. J., and Edwards, D. C. 1992. Postharvest diseases of selected commodities. *J. Plant Dis. Prot.* 3:3-11.
- Staats, M., van Baaren, P., and van Kan, J. A. L. 2005. Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of the specificity. *Mol. Biol. Evol.* 22:333-346.
- Váczy, K. Z., Sándor, E., Karaffa, L., Fekete, E., Fekete, E., Ármay, M., Czeglédi, L., Kövics, G. J., Druzhinina, I. S., and Kubicek, C. P. 2008. Sexual recombination in the *Botrytis cinerea* populations in Hungarian vineyards. *Phytopathology* 98:1312-1319.
- Walker, A., Gautier, A., Confais, J., Martinho, D., Viaud, M., Le Pêcheur, P., Dupont, J., and Fournier, E. 2011. *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. *Phytopathology* 101:1433-1445.
- Wessels, B. A., Lamprecht, S. C., Linde, C. C., Fourie, P. H., and Mostert, L. 2013. Characterization of the genetic variation and fungicide resistance in *Botrytis cinerea* populations on rooibos seedlings in the western cape of South Africa. *J. Plant Pathol.* 136:407-417.
- Williamson, B., Tudzynski, B., Paul, T., and Van Kan, J. 2007. *Botrytis cinerea*: The cause of grey mould disease. *Mol. Plant Pathol.* 8:561-580.
- Yu, S., Liu, C., Liang, C., Zang, C., Liu, L., Wang, H., et al. 2017. Effects of rain-shelter cultivation on the temporal dynamics of grape downy mildew epidemics. *J. Phytopathol.* 165:331-341.
- Zhang, J., Wu, M. D., Li, G. Q., Yang, L., Yu, L., Jiang, D. H., Huang, H. C., and Zhuang, W. Y. 2010a. *Botrytis fabiopsis*, a new species causing chocolate spot of broad bean in central China. *Mycologia* 102:1114-1126.
- Zhang, J., Zhang, L., Li, G. Q., Yang, L., Jiang, D. H., Zhuang, W. Y., and Huang, H. C. 2010b. *Botrytis sinoallii*, a new species of the gray mold pathogen on *Allium* crops in China. *Mycoscience* 51:421-431.
- Zhang, Y., Li, X., Shen, F., Xu, H., Li, Y., and Liu, D. 2018. Characterization of *Botrytis cinerea* isolates from grape vineyards in China. *J. Plant Dis.* 102:40-48.
- Zhou, Y. J., Zhang, J., Wang, X. D., Yang, L., Jiang, D. H., Li, G. Q., Hsiang, T., and Zhuang, W. Y. 2014. Morphological and phylogenetic identification of *Botrytis sinoviticola*, a novel cryptic species causing gray mold disease of table grapes (*Vitis vinifera*) in China. *Mycologia* 106:43-56.