The Journal of
Eukaryotic Microbiology

Published by the International Society of Protistologists

J. Eukaryot. Microbiol., 57(2), 2010 pp. 206–212

DOI: 10.1111/j.1550-7408.2009.00467.x

Genetic Diversity of Populations of *Monilinia fructicola* (Fungi, Ascomycota, Helotiales) from China

JIN-YAN FAN,[a] LI-YUN GUO,[a] JIAN-PING XU,[b] YONG LUO[a,c] and THEMIS J. MICHAILIDES[c]

[a]*Department of Plant Pathology, China Agricultural University, Beijing 100193, China, and*
[b]*Department of Biology, McMaster University, Hamilton, ON, Canada L8S 4K1, and*
[c]*Department of Plant Pathology, University of California-Davis, Kearney Agricultural Center, Parlier, California 93648, USA*

ABSTRACT. The genetic variation among 128 isolates of *Monilinia fructicola* (Fungi, Ascomycota, Helotiales) from China was analyzed using Inter-Simple Sequence Repeat (ISSR) markers and compared with those of samples from California, USA and New Zealand. A total of 72 reproducible DNA fragments were scored, of which 87.5% (63/72) were polymorphic. The Nei's gene diversity and Shannon's diversity indices of three Chinese regional populations were very similar to that from California. However, several differences were observed among geographic populations of *M. fructicola* from both within China and between China and California. The analysis of molecular variance (AMOVA) of isolates from different geographic locations suggested that most of the observed genetic variation was found within populations. Results of this study are inconsistent with the hypothesis that the Chinese populations of *M. fructicola* were derived from a single or few recent migrants from other countries. Instead, our results suggest that *M. fructicola* has been in China long before its first official recording in 2003.

Key Words. Brown rot, genetic variation, geographic distribution, ISSR.

BROWN rot is an important disease of stone and pome fruits worldwide. *Monilinia fructicola* (G. Winter) Honey. 1928, is one of the major causal pathogens of brown rot of stone fruits. This fungus can infect various parts of stone fruit trees and cause blossom blight, twig blight, and brown rot of green and mature fruits (Ogawa et al. 1995), resulting in severe losses during the growth season and in post-harvest storage. The fungal pathogen *M. fructicola* was first discovered in 1883 in eastern United States (Batra 1991), and has since been found mainly in America and Australia (Byrde and Willetts 1977). Because the distribution of this fungus in Europe was limited to only a few local areas, it has been listed as an A2 quarantine pathogen by the European Union (EPPO 2006).

Peach and nectarine are considered important stone fruit crops and have been cultivated in China, where they originated, for >3,000 yr (Long 2000; Qu and Sun 2000). Since the late 1980s, both the production area and the total yield of these fruits have increased greatly in China. Unfortunately, brown rot caused by *Monilinia* spp. has been found also widely distributed in all the major stone fruit production areas in China (Zhu, Guo, and Chen 2008a). Xiang (1957) traced the earliest report of brown rot on stone fruits in China to the 1920s. By the 1950s, brown rot was found in many areas across China on a variety of stone fruits, including peach, plum, apricot, and mume (Dai, Xiang, and Zheng 1958; Xiang 1957). However, among the four pathogenic *Monilinia* species (i.e. *M. fructicola*, *Monilinia laxa*, *Monilinia fructigena*, and *Monilia polystroma*) that have been reported to cause brown rot on pome and stone fruit (Byrde and Willetts 1977; van Leeuwen et al. 2002), only *M. laxa* and *M. fructigena* were documented in China before 2003 (Wang et al. 1998; Xiang 1957; Zhu et al. 2005; Zhuang 1998).

Monilinia fructicola was recently reported as a new pathogen in China (Zhu et al. 2005) and is currently listed as a quarantine pest of China (Bulletin of China Agricultural Ministry 2007). However, during surveys carried out from 2003 to 2008, *M. fructicola* was found widely distributed in many areas in China, including Beijing, Shandong, Hebei, Zhejing, and Liaoning provinces. Indeed, it was the major pathogen causing brown rot on a diversity of stone fruits (Fan et al. 2007; L. Guo and X. Q. Zhu, unpubl. data; Zhong et al. 2008; Zhu, Guo, and Chen 2008b). In contrast, *M. laxa*, documented previously as the species widely distributed in China, was seldom found on the various hosts in these geographic areas.

Recently, up to 100% incidence of fruit rot has been reported in fruit packinghouses in Beijing (Chen et al. 2003). In addition, isolates of *M. fructicola* were collected from Shandong province in 2004 and from various locations in Shandong and Beijing in 2005. Some of these isolates were found highly resistant to the thiophanate-methyl fungicide (Fan, Fang, and Guo 2009). This fungicide is routinely sprayed in stone fruit orchards. The wide occurrence of *M. fructicola* determined right after this species was first reported in China led to our suspicion that this pathogen might not be a newly introduced fungus, but might have existed in China for a long time. If a population was established from one or a few newly introduced strains of a species, the genetic diversity in the population should be much lower than that of its source population or a population of comparable size but which has had a much longer colonization history. Thus, the objectives of this study were to determine the genetic diversity of *M. fructicola* isolates from areas in China, using the PCR-based Inter-Simple Sequence Repeat (ISSR) technique (Zietkiewicz, Rafalski, and Labuda 1994), to compare them with isolates from California, USA, and New Zealand, and test the hypothesis that *M. fructicola* might have been in China for a long time before it was first reported in 2003.

MATERIALS AND METHODS

Isolates. A total of 164 isolates of *M. fructicola* were analyzed in this study. Among them, 128 isolates were from peach ($n = 96$), nectarine ($n = 5$), plum ($n = 19$), pear ($n = 7$), and ornamental peach ($n = 1$). These isolates were collected from three provinces and the City of Beijing in China including Beijing ($n = 77$), Hebei ($n = 5$), Shandong ($n = 43$), and Zhejiang ($n = 3$). In addition, 28 isolates from the United States (California, $n = 27$; North Carolina, $n = 1$), 7 from New Zealand, and 1 from France were included for comparison (Table 1). All isolates were identified as *M. fructicola* based on both morphological characteristics (Lane 2002) and molecular features developed by Ioos and Frey (2000). In addition, one isolate of *M. laxa* was included as a reference. Mono-conidial cultures of each isolate were maintained on potato dextrose agar slants and stored at 4 °C.

DNA extraction and quantification. The mycelia of each isolate were obtained by culturing the fungus in pea broth for 4 d in the dark at 21 °C. The mycelia were vacuum-filtered through Whatman No. 1 filter paper. About 1 g of fresh mycelia was

Corresponding Author: L. Y. Guo, Department of Plant Pathology, China Agricultural University, Beijing 100193, China—Telephone number: +86 10 6273 3027; FAX number: +86 10 6273 3027; e-mail: ppguo@cau.edu.cn

Table **1.** Isolates of *Monilinia* spp. used in this study

Location	Host	Number of isolates	Isolate name	Year of isolation
M. fructicola				
Hanzhuang, Pinggu, Beijing, China	Peach	3	BH1A3[a], BH2A3, BH3A3	2003
	Peach	5	BH1A4, BH2A4, BH3A4, BH4A4, BH5A4	2004
	Peach	4	BH1A5, BH2A5, BH3A5, BH4A5	2005
Dayuzi, Pinggu, Beijing, China	Peach	4	BD1A3, BD2A3, BD3A3, BD4A3	2003
	Nectarine	5	BD1B4, BD2B4, BD3B4, BD4B4, BD5B4	2004
	Plum	5	BD1C5, BD2C5, BD3C5, BD4C5, BD5C5	2005
	Peach	6	BD1A5, BD2A5, BD3A5, BD4A5, BD5A5, BD6A5	2005
Jinhaihu, Pinggu, Beijing, China	Peach	2	BJ1A3, BJ2A3	2003
Xiaoyuzi, Pinggu, Beijing, China	Peach	1	BS1A3	2003
Machangying, Pinggu, Beijing, China	Peach	3	BM1A5, BM2A5, BM3A5	2005
Dahuashan, Pinggu, Beijing, China	Peach	1	Bd2A5	2005
Liangzhongchang, Pinggu, Beijing, China	Peach	3	BL1A5, BL2A5, BL3A5	2005
Xiongerzhai, Pinggu, Beijing, China	Peach	2	BN1A5, BN2A5	2005
Liudian, Pinggu, Beijing, China	Peach	1	Bl1A5	2005
Xiagezhuang, Pinggu, Beijing, China	Peach	1	Bx1A5	2005
Zhenluoying, Pinggu, Beijing, China	Peach	5	BZ1A5, BZ2A5, BZ3A5, BZ4A5, BZ5A5	2005
Fangshan, Beijing, China	Plum	4	BF1C5, BF2C5, BF3C5, BF4C5	2005
	Peach	4	BF1A5, BF2A5, BF3A5, BF4A5	2005
Xiedao, Chaoyang, Beijing, China	Peach	3	BX1A5, BX2A5, BX3A5	2005
Guolinsuo, Haidian, Beijing, China	Peach	1	BG1A5	2005
Zhiwuyuan, Haidian, Beijing, China	Flowering peach	1	Bh1E5	2005
Tailing, Changping, Beijing, China	Peach	3	BC1A5, BC2A5, BC3A5	2005
Huzhuang, Changping, Beijing, China	Peach	5	BC4A5, BC5A5, BC6A5, BC7A5, BC8A5	2005
	Plum	1	BC1C5	2005
Heishanzhai, Changping, Beijing,	Plum	4	BCH1C7, BCH2C7, BCH3C7, BCH4C7	2007
Handan, Hebei, China	Peach	2	HH1A5, HH2A5	2005
Hebei, China	Peach	3	HS1A4, HS2A4, HS3A4	2004
Pingyi, Linyi, Shandong, China	Peach	4	SL1A7, SL2A7, SL3A7, SL4A7	2007
	Pear	5	SL1D7, SL2D7, SL3D7, SL4D7, SL5D7	2007
Xujiazhuang, Yiyuan, Zibo, Shandong, China	Peach	1	SZ1A5	2005
Zhanglong,Yiyuan,Zibo, Shandong,	Peach	4	SZ1A7, SZ2A7, SZ3A7, SZ4A7,	2007
Zhongzhuang,Yiyuan, Zibo, Shandong, China	Peach	2	SZ5A7, SZ6A7	2007
Zhifu, Yantai, Shandong, China	Peach	3	SYZ1A7, SYZ2A7, SYZ3A7	2007
Fushan, Yantai, Shandong, China	Peach	5	SYF1A5, SYF2A5, SYF3A5, SYF4A5, SYF5A5	2005
Jinan, Shandong, China	Peach	9	SJ1A4, SJ2A4, SJ3A4, SJ4A4, SJ5A4, SJ6A4, SJ7A4, SJ8A4, SJ9A4	2004
Muyudian, Laiyang, Shandong, China	Peach	1	SLM1A6	2006
Chengxiang, Laiyang, Shandong, China	Plum	5	SLC1C6, SLC2C6, SLC3C6, SLC4C6, SLC5C6	2006
	Peach	2	SLC1A6, SLC2A6	2006
	Pear	2	SLC1D6, SLC2D6	2006
Ningbo, Zhejiang, China	Peach	1	ZN1A6	2006
Zhejiang, China	Peach	2	Z1A6, Z2A6	2006
New Zealand	Peach	7	NZ1A0, NZ2A0, NZ3A0, NZ4A0, NZ5A0, NZ6A0, NZ7A0	Unknown
France	Unknown	1	Ft	Unknown
California, US	Peach	1	C1A2	2002
California, US	Unknown	2	C1F0, C2F0	Unknown
KAC, Fresno, CA, US	Peach	1	CFK1A2	2002
KAC, Fresno, CA, US	Unknown	3	CFK1F0, CFK2F0, CFK3F0	Unknown
Sanger, Fresno, CA, US	Plum	1	CFS1C2	2002
Sanger, Fresno, CA, US	Nectarine	3	CFS1B2, CFS2B2, CFS3B2	2002
Parlier, Fresno, CA, US	Nectarine	2	CFP1B2, CFP2B2	2002
Reedley, Fresno, CA, US	Nectarine	3	CFR1B2, CFR2B2, CFR3B2	2002
Dinuba, Fresno, CA, US	Plum	1	CFD1C8	1998
Merced, CA, US	Peach	7	CMe1A2, CMe2A2, CMe3A2,CMe4A2, CMe5A2,CMe6A2, CMe7A2	2002
Merced, CA, US	Nectarine	1	CMe1B2	2002
Madera, CA, US	Nectarine	1	CMa1B2	2002
Madera, CA, US	Peach	1	CMa1A2	2002
North Carolina, US	Peach	1	NC1A6	2006
M. laxa				
United States	Unknown	1	ML1F0	Unknown

[a]The letters ahead of first number stand for location, the last second letter stands for host, A, peach; B, nectarine; C, plum; D, pear; E, flowering peach; F, unknown; the last number stands for year, 2, 2002; 3, 2003; 4, 2004; 5, 2005; 6, 2006; 8, 1998; 0, unknown.

placed into a sterile 2-ml centrifuge tube with 1 ml of DNA extracting buffer, prepared based on the CTAB/NaCl method (Murray and Thompson 1980; Wilson 1987). The macerate was homogenized at speed setting 5.0 for 40 s in a FastPrep Breaker (MP Biomedicals Inc., Irvine, CA). The total DNA was then extracted following the CTAB/NaCl procedure (Murray and Thompson 1980; Wilson 1987).

Primer selection and PCR amplification. Eight isolates (Ft, CMe1B2, NZ1A0, BS1A3, HH2A5, SJ6A4, SYF5A5, and ZN1A6) obtained from different hosts and geographic locations were first used in a pilot experiment to screen for primers that might generate polymorphic fingerprinting patterns. Thirty-five primers previously reported to be polymorphic for *M. fructicola* and *Beauveria bassiana* (Balsamo) Vuillemin, 1912 were screened (Li et al. 2006; Ma and Michailides 2005). Primers were synthesized by Beijing SBS Genetech Co. Ltd. (Beijing, China). Each PCR reaction was carried out in 25 µl containing 20 ng of fungal DNA template, 0.2 mmol/L each of dNTP, $MgCl_2$ 0.16 mmol/L, 1 × PCR buffer (Mg^{2+} free), 0.8 µmol/L ISSR primer, and 1 U *Taq* DNA polymerase [Reagents were from Po Bio-engineering (Dalian) Co. Ltd., Dalian, Liaoning Province, China].

The amplification program consisted of an initial preheating for 5 min at 95 °C, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 49 °C for 1 min, and extension at 72 °C for 1.5 min, with a final extension at 72 °C for 10 min. The amplification reactions were performed in a Gene Amp 9700 thermal cycler (Applied Biosystems Inc., San Mateo, CA). The PCR was performed at least twice for each isolate and the PCR products were separated by agarose (1.5%, w/v) gels in Tris-borate-EDTA (TBE) buffer, stained with 10 mg/ L ethidium bromide solution, and photographed using the MultiImage™ Light Cabinet (Alpha Innotech Corporation, San Leandro, CA). Primers that generated reproducible and easily scorable polymorphic banding patterns were selected and used in subsequent amplification of the full set of natural samples.

Data analysis. For analysis of the ISSR data set, the presence or absence of amplified fragments ranged in size from 0.2–2 kb in the same position for each isolate was recorded as 1 or 0, respectively. For population genetic analyses, isolates were grouped hierarchically based on their geographic locations. Only populations with more than 20 isolates were included in the population analysis. Isolates from China were divided into three regional populations, isolates from Shandong (with sampling areas of 25,000 km^2), isolates from Pinggu, Beijing (with sampling areas of 1,075 km^2), and isolates from other places within Beijing (with sampling areas of 15,345 km^2). Although, Pinggu is a relatively small area, it is a major production area of peach and nectarine in Beijing and many varieties of peach are grown there. Furthermore, it is the place where *M. fructicola* was first discovered in China. Isolates from three counties in southern San Joaquin Valley of California came from an area of 26,262 km^2. This region is a major production area of stone fruits in the United States and is used here for comparison.

The genetic diversity of each population was estimated by calculating the Nei's gene diversity (Nei 1973) and Shannon's diversity index (Shannon and Weaver 1949) using the GenAlEx6 software (Peakall and Smouse 2006), and genotypic diversity (*D*) was estimated by using MultiLocus software (version 1.3, Agapow and Burt 2003). The similarity among different populations was measured by calculating Nei's genetic identity as described by Nei (1978) using the GenAlEx6 software (Peakall and Smouse 2006). An Analysis of molecular variance (AMOVA) was carried out using GenAlEx6 Software to determine the main source of variation observed in the dataset (Excoffier, Smouse, and Quattro 1992; Huff, Peakall, and Smouse 1993; Michalakis and Excoffier 1996; Peakall, Smouse, and Huff 1995). The genetic differentiation among geographic populations was measured by calculating Wright's F_{st} (fixation index) as described by Wright (1946, 1951, 1965) and N_m (effective migration rate) as described by McDermott and McDonald (1993) using the GenAlEx6 software (Peakall and Smouse 2006). All the data analyzed by this program were treated as haploid with the nature of the markers being dominant/recessive (McDonald 1997).

The genetic similarities (*S*) between all pairs of isolates were calculated using Dice's similarity coefficient (Dice 1945). A phenogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) by the program Sequential, Agglomerative, Hierarchical, and Nested clustering methods (SAHN) of the software package NTSYS-pc 2.1 (Department of Ecology and Evolution, State University of New York, NY).

RESULTS

Inter-Simple Sequence Repeat (ISSR) analysis. Among the 35 primers tested, 12 primers [$(GACA)_4$, $(GTG)_5$, $(AAG)_8$, $(AC)_8T$, $(AG)_8S$, $(AG)_8YT$, $(AG)_8YC$, $(AC)_8YC$, $VHV(GT)_7$, $(AC)_8YT$, $(GTC)_6$, and $(AAG)_6$] produced reproducible polymorphic bands. These primers were then used to genotype all 165 isolates. Each primer amplified two to seven bands. From the 165 isolates, a total of 72 bands were amplified and 63 of them were polymorphic (see Fig. 1, e.g. using ISSR primer $(AG)_8S$).

Population diversity and similarity. The Nei's gene diversity and Shannon's diversity index of the three Chinese regional populations were 0.337 and 0.527, respectively, for that from Pinggu District, Beijing; 0.337 and 0.496, respectively, for samples from the other districts combined in Beijing; and 0.348 and 0.532, respectively, for the population from Shandong (Table 2). These values were very similar to those of the Californian *M. fructicola* population (Nei's gene diversity $h = 0.355$, and Shannon's diversity index $I = 0.543$). Furthermore, the genotypic diversities of the above four populations were all equal to 1 (Table 2), the maximum value.

When the genetic similarity among populations was calculated using Nei's identity, the indices between populations in China varied from 0.959 to 0.976, and those between the Californian population and each of the three regional populations in China varied from 0.923 to 0.940 (Table 3). This result again suggested that the three populations in China were very similar to each other and to the California population. Consistent with the above results, the results based on AMOVA showed that 93% of the genetic variance was found within regional populations. About 7% of the total genetic variation was due to geographic separations among the regional populations (Table 4).

Analysis of genetic differentiation between pairs of populations. When three populations from China were compared, the values of F_{st} ranged from 0.014 ($P = 0.049$) to 0.051 ($P = 0.001$) (Table 5). Slightly higher genetic differentiations were found between the California population and each of the three population in China (F_{st} varied from 0.067 ($P = 0.001$) to 0.098 ($P = 0.001$) (Table 5).

The effective migration rates (N_m) between the three populations from China and California were > 2.295 (Table 5). Within China, a low-level genetic differentiation and high-level of gene flow ($F_{st} = 0.014$, $P = 0.049$; $N_m = 17.532$) were found between the populations from Beijing (excluding Pingguo) and Shandong (Table 5).

Cluster analysis. Each of the 164 *M. fructicola* isolates had a distinct multilocus genotype and they were all distinctively different from the isolate of a sister species *M. laxa* (Fig. 2). The similarity coefficients between pairs of the 164 *M. fructicola* isolates

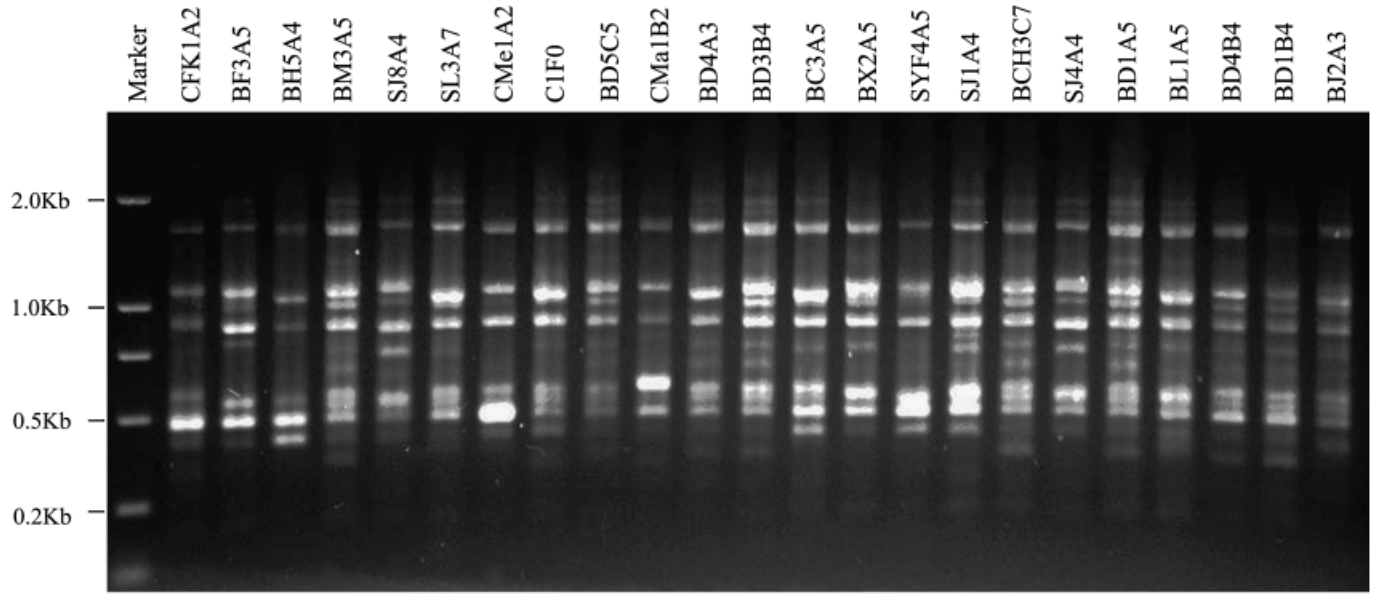

Fig. **1.** Electrophoretic patterns of amplified fragments generated from isolates of *Monilinia fructicola* with primer $(AG)_8S$ on a 1.5% agarose gel. M, marker DNA (DL2000).

were all >0.46. The 164 isolates of *M. fructicola* can be arbitrarily divided into seven groups at the similarity coefficient of 0.61: each of the seven groups consisted of isolates from different locations or different hosts, consistent with the lack of geographic or host tree-based clustering between isolates. For example, among the 7 isolates from New Zealand, 5 were clustered together with the isolate from North Carolina, and the remaining 2 were clustered together with isolates from China or California (Fig. 2). Among the 27 isolates from California, 7 isolates were clustered together with the isolate from France in group C; the rest were clustered with isolates from China in groups A, D–F, and most of them shared a similarity coefficient larger than 0.85 with Chinese isolates. These results suggested that the populations from China were not a subset of that from California or New Zealand.

DISCUSSION

The results of this study showed that the level of genetic diversity, especially genotypic diversity, among Chinese population of *M. fructicola* isolates is high and very similar to that of the California population. The analysis of genetic differentiation among the populations from China and California also indicated limited genetic differentiation but significant gene flow between the Chinese and California populations of *M. fructicola*. Because *M. fructicola* was found in North America over a 100 yr ago, the comparably high genetic diversity within the Chinese population of *M. fructicola* is inconsistent with the hypothesis that the Chinese population of this species existed only since its first official report in 2003 (Zhu et al. 2005). Instead, our results support the hypothesis that *M. fructicola* had existed in China for a long time, likely comparable to the populations in California.

Monilinia fructicola has been found in both eastern and western Unites States where stone fruits are grown. In California, this fungus was first found in 1936 (Hewitt and Leach 1939). The 27 isolates examined in this study from California were from orchards of three counties in the San Joaquin Valley, where sexual reproduction of this fungus was frequently found (Hong, Michailides, and Holtz 1996). Therefore, we believe the genetic diversity

Table **2.** Genetic diversity within populations of *Monilinia fructicola* from China and United States.

Population[a]	Number of isolates	Mean N_a[b]	Mean N_e[c]	Number of private alleles	Mean h[d]	Mean I[e]	Genotypic diversity (D)[f]
Pinggu, Beijing	51	2.317	1.612	0.175	0.337	0.527	1.000
Other areas in Beijing	26	1.889	1.595	0.000	0.337	0.496	1.000
Shandong	43	2.190	1.620	0.032	0.348	0.532	1.000
South San Joaquin Valley, California	27	2.175	1.651	0.190	0.355	0.543	1.000

[a]One hundred and twenty of the 128 isolates from China were from three regional populations: Pinggu, Beijing; other areas within Beijing, and Shandong. The California population in the United States contained isolates from South San Joaquin Valley of California.
[b]Observed number of alleles.
[c]Effective number of alleles.
[d]Nei's gene diversity.
[e]Shannon's diversity index.
[f]Genotypic diversity within populations was calculated as $(n/n-1)(1-\sum p_i^2)$, where p_i is the frequency of the ith genotype and n is the number of individuals sampled, which is the probability that two individuals taken at random have unique genotypes.

Table **3.** Nei's genetic identity between pairs of geographic populations of *Monilinia fructicola*.

Geographic population[a]	Pinggu, Beijing	Other areas in Beijing	Shandong	South San Joaquin Valley, California
Pinggu, Beijing	1.000[b]			
Other areas in Beijing	0.959	1.000		
Shandong	0.960	0.976	1.000	
South San Joaquin Valley, CA	0.928	0.940	0.923	1.000

[a]For divisions of populations, see footnote to Table 2.
[b]Nei's genetic identity.

Table **4.** Analysis of molecular variance among populations of *Monilinia fructicola* from different geographic locations.

Source of variation[a]	Degree of freedom	Sum of squares	Variance component	Percentage of variation	P[b]
Among regions	2	82.772	0.236	2	0.001
Among populations of region	1	29.836	0.543	5	0.001
Within populations[c]	143	1590.188	11.120	93	0.001
Total sample	146	1702.796	11.899		

[a]Variances are from different countries or from different regions of China or from different populations.
[b]P values are based on 1,000 permutations.
[c]Different populations are from California in United States, Beijing, Shandong in China.

found here for the California isolates is representative of the North American population that has had a relatively long population history.

If *M. fructicola* has existed in China for a long time, then why was it not found earlier? The most likely explanation was the lack of detailed surveys and analyses. In an extensive survey, Wang et al. (1998) investigated *Monilinia* spp. from peach, plum, apple, pear, and other plants in the Rosaceae family in >20 provinces (cities) in China, including geographically distant regions of Beijing, Yunnan (southwest China), and Jilin (northeast China). They attributed the brown rot pathogens of stone fruits in these areas as *M. cinerea* based on colony morphology and reported the sexual stage of the fungus as *M. laxa*. However, there was no reference or type strain included in their analyses and comparisons. Moreover, the Chinese name used for *M. fructicola* in the most popular Chinese identification handbook at that time (Wei 1979) was the ''America–Australia type brown rot'' pathogen, and this species was described as limited to America, Australia, and New Zealand. As a result, investigators at that time presumed what they found was not *M. fructicola*. It would be extremely informative to examine the cultures collected by Wang et al. (1998) to determine the extent of *M. fructicola* in their survey but these cultures and specimens were not retained.

Population genetic variations of *M. fructicola* have been studied previously. Fulton, van Leeuwen, and Brown (1999) compared 4 isolates from Japan with isolates from United States, New Zealand, and Australia using RAPD markers and concluded that the Japanese isolates had similar genotypes as those from New Zealand. Ma, Yoshimura, and Michailides (2003) studied the genetic relationships among isolates of *M. fructicola* sensitive to, low resistant to, and high resistant to benzimidazole fungicides from California using microsatellite primers. They found that low resistant and high-resistant isolates did not cluster independently from the sensitive isolates. However, as far as we know, the present study is the first on populations of *M. fructicola* from stone and pome fruits in China.

Previous studies have revealed the differential distributions of the three species of *Monilinia* that caused brown rot of stone and pome fruits: *M. fructigena* was mainly found in Europe and Asia, *M. fructicola* in North and South Americas, New Zealand, and Australia, and *M. laxa* was widely distributed in Europe, Asia, the Americas, and Australia (Byrde and Willetts 1977). However, all

Table **5.** Pairwise genetic differentiation between populations of *Monilina fructicola*.

Population[a]	Pinggu, Beijing	Other areas in Beijing	Shandong	South San Joaquin Valley, CA
Pinggu, Beijing		4.953[b]	4.616[b]	2.377[b]
Other areas in Beijing	0.048[c] (0.001)[d]		17.532[b]	3.508[b]
Shandong	0.051[c] (0.001)	0.014[c] (0.049)		2.295[b]
South San Joaquin Valley, California	0.095[c] (0.001)	0.067[c] (0.001)	0.098[c] (0.001)	

[a]For divisions of populations, see footnote to Table 2.
[b]Effective migration rate (N_m).
[c]F statistics (F_{st}) values.
[d]Numbers in parentheses are P values based on 1,000 permutations.

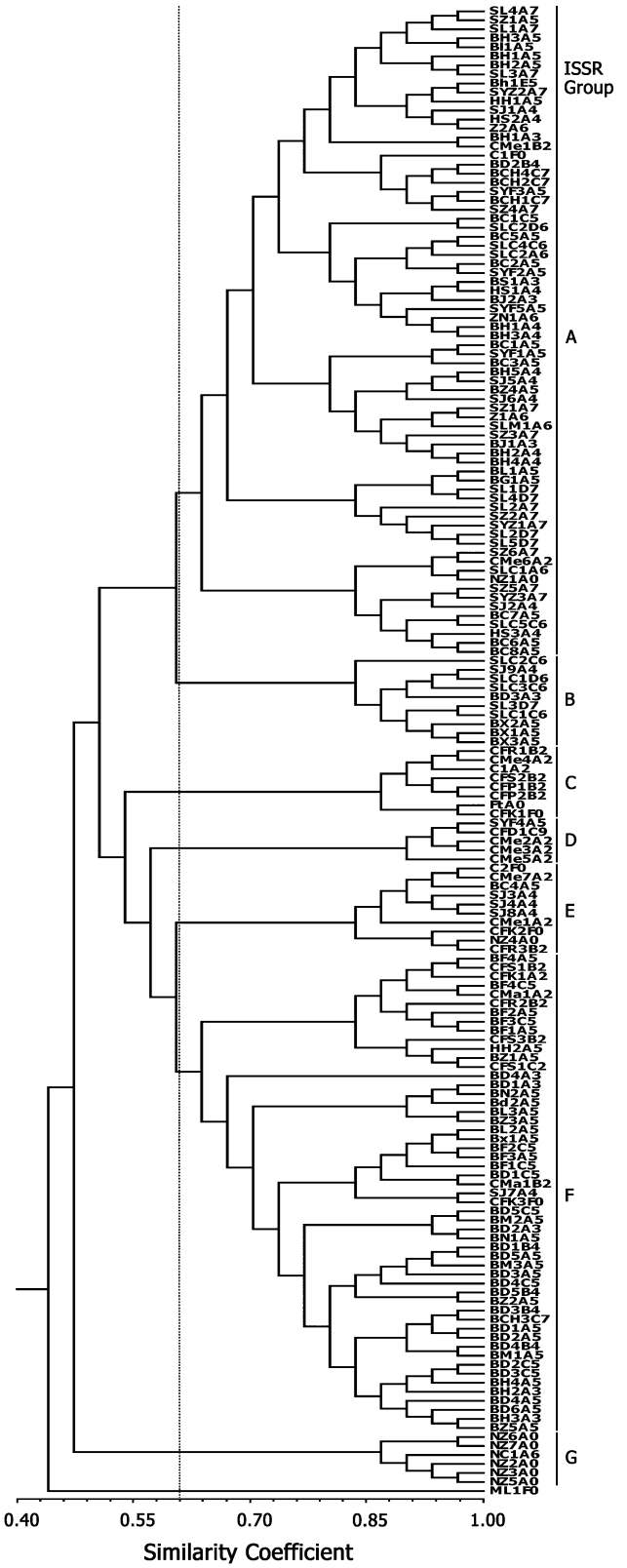

Fig. **2.** A dendrogram showing the relationships among 165 isolates of *Monilinia* spp. using data from the Inter-Simple Sequence Repeat genotyping analysis and clustered using the Unweighted Pair-Group Method with Arithmetic Average. Scale bar represents Dice's coefficient of similarity. The letters A–G stand for different ISSR groups of *Monilinia fructicola*. *Monilinia laxa* (isolate ML1F0) is used as the outgroup.

three species have been found in Japan (Terui and Harada 1966). Unlike *M. laxa* and *M. fructigena*, which are believed to have originated close to the center of their hosts' origins, *M. fructicola* was thought to originate in North America (Byrde and Willetts 1977). If this was the case, *M. fructicola* could have been introduced into China with the introduction of stone fruit cultivars from the Unites States or Japan. Based on historical records, foreign cultivars were first brought to China by missionaries and/or visitors from Europe and North America in the early twentieth century and later by the Japanese through agricultural organizations associated with the Japanese occupying army (Wang and Zhuang 2001). Previous studies suggested that the Japanese isolates of *M. fructicola* likely had the same origin as those from New Zealand (Fulton et al. 1999). More detailed DNA sequence analyses, including populations of these fungi from China and Japan as well as from other parts of the world, might reveal the likely routes of migration and the patterns of evolution in this important group of plant pathogens.

ACKNOWLEDGMENTS

This work was funded by the International Science Foundation (D3938-1) and Chinese Ministry of Agriculture (Grant number 3-22). We thank Xiaoyu Chen of Beijing Plant Protection Station, Yingzi Wang and Peisong Wang of Yantai Academy of Agricultural Sciences, Shandong, and Xiaojun Li of the Fruit Institute of Shandong Academy of Agricultural Science for help on collecting fruit disease samples. We also thank Dr. R. Ioos of the French Ministry of Agriculture and Forestry, for providing the reference isolate of *M. fructicola*.

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Received: 07/24/09, 10/04/09, 11/17/09; accepted: 11/20/09