Etiology of Moldy Core, Core Browning, and Core Rot of Fuji Apple in China

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Abstract

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'Fuji' apple fruit were collected in Shaanxi Province, China, and 186 fungal isolates were obtained from the fruit core region. Fungi were isolated from fruit with symptomless core regions, as well as from the core regions of fruit showing browning, typical moldy core, or core rot. Based on phylogenetic and morphological analysis, all fungi were identified to species. Pathogenicity was determined by cutting apple fruit into halves and daubing spore suspensions containing 1×10^4 up to 1×10^8 spores/ml on the carpel in the core region. Pathogenicity varied significantly among genera, with *Alternaria* and *Cladosporium* spp. causing core browning at lower spore concentrations and moldy

core at higher spore concentrations. Combinations of pathogens initiated more browning and moldy core than the pathogens applied alone. *Epicoccum* and *Phoma* spp. predominated in dry core rot, whereas primarily *Trichothecium* but also *Fusarium* and *Penicillium* spp. caused wet core rot. Core browning was introduced as a new type of core symptom, along with moldy core, dry core rot, and wet core rot. *Alternaria alternata*, *A. tenuissima*, *A. arborescens*, *Cladosporium cladosporioides*, and *C. tenuissimum* were the main pathogens causing core browning and moldy core.

Moldy core is an important disease of apple worldwide. It has been recorded in Australia, Canada, New Zealand, South Africa, The Netherlands, the United Kingdom, and the United States (28). It is characterized by the growth of mycelia within the locules, with or without penetration to the flesh immediately surrounding the core (7,28). Moldy core of apple is undetectable until the fruit is cut open or consumed, potentially affecting consumer confidence (22). In one test in Australia, 5% of stored 'Fuji' apple fruit exhibited moldy core (8). During late summer to early fall 1993, moldy core was very common in Fuji apple in the San Joaquin Valley of California (10). In 2003, the incidence of 'Red Delicious' apple fruit infected with moldy core in some orchards of northern Israel was up to 40% (15).

Many studies have shown that moldy core is caused by a variety of fungi. In Oregon, the most common fungi associated with moldy core were *Alternaria*, *Stemphylium*, *Cladosporium*, *Ulocladium*, *Epicoccum*, *Coniothyrium*, and *Pleospora* spp. (28). *Alternaria* spp. were the most commonly isolated fungi associated with moldy core of 'Delicious' apple fruit in Ohio (7). Fungi that were associated with core rot of 'Starking' apple fruit in South Africa included *Alternaria alternata*, *Pleospora herbarum*, *Coniothyrium* sp., *Penicillium funiculosum*, *P. expansum*, *P. ramulosum*, and *P. sp.* (aff. *dendriticum*) (3,35). During investigations in 2004 to 2006 in apple orchards in Slovenia, *Fusarium avenaceum* was identified as the causal agent of a wet apple core rot. Infections were associated with high amounts of mycotoxins, which may pose a risk for consumers of fresh or processed apple products (26,27).

Combrink et al. (4) indicated that core infection occurs progressively as fungal spores and bacteria become established on senescing blossoms after the full bloom stage of bud development. Little et al. (9) indicated that fungal spores may gain entry to the seed locules via the calycine sinus at any stage of fruit development. Snowdon (25) noted that the incidence of moldy core tended to increase following intensive spray programs to reduce other fungi.

Conditions of high relative humidity, mild temperatures, and tissue susceptibility are important factors that affect natural infection in orchards. Once inside the fruit, the pathogens are protected from contact fungicides and conditions for their continued growth are excellent (6,11,18,19,29,30).

The accepted common name "moldy core" includes the typical moldy core symptom, as well as the more advanced core rot symptom (American Phytopathological Society Common Names of Plant Diseases). The term "moldy core" as used herein refers to all the symptom types associated with core infections although, later in the article, we show that different pathogens are involved in the different symptom groups.

Although much is known about moldy core in other countries, very few studies have been conducted in China, the world's largest apple-producing country. Fuji is the main apple cultivar in China, comprising approximately 70% of current production. It is essential to know whether moldy core of Fuji apple in China has an etiology similar to that in other countries or whether other organisms are involved. The objective of this study was to identify the pathogens associated with moldy core of Fuji apple in China and investigate their various roles in production of moldy core symptoms.

Materials and Methods

Isolation and identification of fungi. Samples were collected in 2008 and 2009 from trees of commercial orchards during the harvesting season; the trees were approximately 15 to 25 years old. Some fungicides were applied during the bloom period for management of moldy core, including carbendazim and polyoxin. During the 2 years, 440 Fuji apple fruit were collected from 13 different counties in Shaanxi Province of China (Table 1). Harvested fruit were stored at 4°C before the isolations were conducted. Apple fruit were rinsed under running tap water and then allowed to

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*The e-Xtra logo stands for "electronic extra" and indicates that Figures 1 and 2 appear in color in the online edition.

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air dry. Whole fruit were disinfested with 70% ethanol. The fruit were then dissected along the longitudinal axis to permit examination of the core region. Sampling was conducted by direct removal of fungal mycelium (1). If the core appeared uninfected, nine tissue pieces were removed (1 to 2 mm) using a sterile blade and consisted of three pieces each from flesh, carpel, and seed. Material sourced from apple cores was subsequently placed on potato dex-

trose agar (PDA) in 90-mm plastic petri dishes. After incubation at 25°C in the dark for 7 days, cultures were examined visually, and mixed cultures were separated to single cultures using sterile transfer. All the isolated fungi were preserved in hemolysis tubes (15 by 100 mm) with PDA and marked with a code number.

The fungal isolates were identified on the basis of morphology and molecular data. When morphology on PDA and internal tran-

Table 1. Identification, origin, and pathogenicity of some fungal isolates

Fungal species	Isolate code	Origin	Pathogenicity ^a	Symptom type
<i>Acremonium alternatum</i> Link	GL0801	Heyang	–	...
	GL09603	Yangling	–	...
<i>Alternaria alternata</i> (Fr.) Keissl.	GL09715	Fufeng	+	Moldy core
	GL0625	Luochuan	+	Moldy core
	GL4231	Yangling	+	Moldy core
<i>A. arborescens</i> E.G. Simmons	GL6273	Yangling	+	Browning
	GL4304	Yangling	+	Browning
	GL09614	Yangling	+	Browning
<i>A. infectoria</i> E.G. Simmons	GL6155	Fuxian	–	...
	GL5255	Yanan	–	...
<i>A. tenuissima</i> (Kunze) Wiltshire	GL4233	Baishui	+	Moldy core
	GL0451	Dali	+	Moldy core
	ZQ111521	Yangling	+	Moldy core
<i>Aphanocladium aranearum</i> (Petch) W. Gams	GL0836	Baishui	–	...
<i>Botryosphaeria dothidea</i> (Moug. ex Fr.) Ces. & De Not	GL0812	Baishui	+	Wet core rot
<i>Chaetomium globosum</i> Kunze ex Fr.	GL0411	Yangling	–	...
	GL3301	Yangling	–	...
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	GL0839	Baishui	+	Browning
	ZQ241422	Baishui	+	Browning
	GL7315	Liquan	+	Browning
<i>C. tenuissimum</i> Cooke	GL0850	Baishui	+	Browning
	GL0849	Heyang	+	Browning
	GL0848	Huangling	+	Browning
	ZQ150821	Yangling	+	Browning
<i>Colletotrichum gloeosporioides</i> complex	GL0852	Baishui	+	Wet core rot
	GL0831	Hancheng	+	Wet core rot
<i>Coniothyrium diplodiella</i> (Speg.) Sacc.	GL4301	Baishui	–	...
<i>Epicoccum nigrum</i> Link	GL5254	Luochuan	+	Dry core rot
	GL3303	Yangling	+	Dry core rot
<i>Eutypella caricae</i> (De Not.) Berl.	GL08362	Baishui	–	...
<i>E. vitis</i> (Schwein.) Ellis & Everh.	GL4151	Baishui	–	...
	GL5161	Baishui	–	...
<i>Fusarium equiseti</i> (Corda) Sacc.	GL09304	Yangling	+	Wet core rot
	GL3311	Yangling	+	Wet core rot
<i>F. proliferatum</i> (Matsush.) Nirenberg ex Gerlach & Nirenberg	GL1222	Baishui	+	Wet core rot
	GL8122	Yangling	+	Wet core rot
<i>F. solani</i> (Mart.) Sacc.	GL09415	Yangling	+	Wet core rot
	GL4104	Yangling	+	Wet core rot
<i>F. tricinctum</i> (Corda) Sacc.	GL0844	Luochuan	+	Wet core rot
	GL8442	Luochuan	+	Wet core rot
<i>Mucor circinelloides</i> Tiegh.	GL4120	Yangling	–	...
<i>Nigrospora oryzae</i> (Berk. & Broome) Petch	GL0481	Baishui	–	...
	GL9142	Yangling	–	...
<i>Penicillium chrysogenum</i> Thom	GL09809	Jiangzhang	+	Wet core rot
<i>P. expansum</i> Link	GL0822	Baishui	+	Wet core rot
	GL0823	Yanan	+	Wet core rot
<i>P. paneum</i> Frisvad	GL4121	Baishui	+	Wet core rot
	GL09420	Yangling	+	Wet core rot
<i>P. viridicatum</i> Westling	GL0832	Baishui	+	Wet core rot
	GL0827	Yangling	+	Wet core rot
<i>Phoma exigua</i> Sacc.	GL0826	Tongchuan	+	Dry core rot
<i>Ph. glomerata</i> (Corda) Wollenw. & Hochapfel	GL09806	Jiangzhang	+	Dry core rot
	GL0833	Luochuan	+	Dry core rot
	GL0461	Yangling	+	Dry core rot
<i>Ph. samarorum</i> Desm.	GL71510	Baishui	+	Dry core rot
<i>Phomopsis</i> sp.	GL9725	Liquan	–	...
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	GL8912	Baishui	–	...
	GL8921	Yangling	–	...
<i>Spencermartinsia viticola</i> (A.J.L. Phillips & J. Luque)				
A.J.L. Phillips, A. Alves & Crous	GL3314	Yangling	+	Wet core rot
	GL09306	Yangling	+	Wet core rot
<i>Trichoderma inhamatum</i> Veerkamp & W. Gams	GL0834	Baishui	–	...
<i>Trichothecium roseum</i> (Pers.) Link	GL0800	Yangling	+	Wet core rot
	GL0824	Yangling	+	Wet core rot

^a Symbols: +, fungus can infect apple and apple shows symptoms; –, fungus can not infect apple, pathogenicity tested with spore suspensions of 1×10^6 spores/ml.

scribed spacer (ITS) region parameters were insufficient to identify some species, more gene sequences and culture characteristics were used based on fungal groups. For *Alternaria* spp., isolates were identified according to sporulation patterns on potato carrot agar (PCA) under an alternating light-and-dark cycle consisting of 8 h of cool-white daylight followed by 16 h of darkness at 25°C for 4 to 7 days (23,24). For species of *Cladosporium*, microscopic observations of the isolates were made from colonies cultivated on synthetic nutrient-poor agar (SNA) and characteristic colonies were cultivated on malt extract agar (MEA) and oatmeal agar (OA) for 14 days at 25°C in the dark (21). Partial gene sequences were determined as described by Crous et al. (5) and Schubert et al. (21) for actin (ACT) and translation elongation factor 1- α (TEF). Isolates of *Fusarium* were identified based on cultural and macroconidial morphology on carnation leaf agar (CLA) at 25°C in the dark (12) in combination with DNA sequences of the β -tubulin gene (13). The identification of *Penicillium* isolates was achieved using morphological characteristics on Czapek yeast autolysate agar (CYA) at 25°C in the dark (14), in combination with DNA sequences of the β -tubulin gene (20). All sequences used in molecular identifications were submitted to GenBank (Table 2).

Genomic DNA was isolated from fungal mycelium grown on MEA using the UltraClean Microbial DNA Isolation Kit (Mo Bio

Laboratories, Inc.), according to the manufacturer's protocols. The gene sequences used for the phylogenetic analysis were aligned using the Clustal X program (34). Phylogenetic analysis was performed using PAUP 4.0 (32).

Type of symptoms. Harvested apple fruit showed varying symptoms during the isolation of fungi. To facilitate characterization, symptoms were separated into three types: browning, moldy core, and core rot (Fig. 1). Browning was characterized by brown carpal walls and no mycelial growth in the core. Moldy core was characterized by black to gray fungus growth over the seed and carpal walls, and the entire central portion of the core was often filled with mycelium. Core rot was characterized by white mycelia or pink conidia groups growing in the core region and flesh appearing necrotic (dry core rot or wet core rot). The occurrence of each type was noted and frequencies were determined.

Pathogenicity. For inoculations, freshly harvested Fuji apple fruit were sterilized with 70% ethanol and cut into halves along the longitudinal axis with a flame-sterilized knife. Fruit halves were inoculated without additional wounding, using sterile swabs to gently daub the spore suspension into the core region, away from the area injured by the original cut. Spores were harvested from cultures by adding a small amount of sterile distilled water to each dish and gently rubbing the sporulating mycelial mats with a bent

Table 2. Information on molecular identification of some fungal isolates

Isolate code	Gene region ^a	GenBank accession number	GenBank number of reference isolate	Sequence length (bp)	Identity (%)	Fungal species
GL0801	ITS	JX241639	AY566992	518	100	<i>Acremonium alternatum</i>
GL4231	ITS	JX241640	AF397222	525	99	<i>Alternaria alternata</i>
GL6273	ITS	JX241641	AF397237	538	100	<i>A. arborescens</i>
GL5255	ITS	JX241642	AF397246	553	99	<i>A. infectoria</i>
GL0451	ITS	JX241643	AF397221	515	99	<i>A. tenuissima</i>
GL0836	ITS	JX241644	AJ292431	539	100	<i>Aphanocladium araneum</i>
GL0812	ITS	JX241645	JF501225	433	99	<i>Botryosphaeria dothidea</i>
GL0411	ITS	JX241646	GU563374	532	99	<i>Chaetomium globosum</i>
GL0839	ITS	JX241647	HM148003	473	100	<i>Cladosporium cladosporioides</i>
	TEF	JX241672	HM148280	205	99	<i>C. cladosporioides</i>
	ACT	JX241674	HM148490	198	97	<i>C. cladosporioides</i>
GL0848	ITS	JX241648	HM148197	473	100	<i>C. tenuissimum</i>
	TEF	JX241673	HM148442	222	97	<i>C. tenuissimum</i>
	ACT	JX241675	HM148687	229	99	<i>C. tenuissimum</i>
GL0852	ITS	JX241649	AJ301988	510	99	<i>Colletotrichum gloeosporioides</i> complex
GL4301	ITS	JX241650	EU520203	517	100	<i>Coniothyrium diplodiella</i>
GL3303	ITS	JX241651	FJ426997	479	99	<i>Epicoccum nigrum</i>
GL08362	ITS	JX241652	AJ302460	520	99	<i>Eutypella caricae</i>
GL5161	ITS	JX241653	FJ790855	539	99	<i>E. vitis</i>
GL09304	ITS	JX241654	GQ505752	513	99	<i>Fusarium equiseti</i>
	β -tubulin	JX241676	GQ915441	806	98	<i>F. equiseti</i>
GL1222	ITS	JX241655	AF291061	557	99	<i>F. proliferatum</i>
	β -tubulin	JX241677	AF291055	844	99	<i>F. proliferatum</i>
GL09415	ITS	JX241656	AB551705	522	99	<i>F. solani</i>
	β -tubulin	JX241678	AB587061	742	98	<i>F. solani</i>
GL0844	ITS	JX241657	HM068317	509	99	<i>F. tricinatum</i>
	β -tubulin	JX241679	AB587078	839	99	<i>F. tricinatum</i>
GL4120	ITS	JX241658	DQ118991	615	99	<i>Mucor circinelloides</i>
GL9142	ITS	JX241659	DQ219433	494	99	<i>Nigrospora oryzae</i>
GL09809	ITS	JX241660	EF200101	525	99	<i>Penicillium chrysogenum</i>
	β -tubulin	JX241680	EF198568	409	100	<i>P. chrysogenum</i>
GL0823	ITS	JX241661	DQ339547	562	99	<i>P. expansum</i>
	β -tubulin	JX241681	FJ457084	526	99	<i>P. expansum</i>
GL09420	ITS	JX241662	HQ442346	550	99	<i>P. paneum</i>
	β -tubulin	JX241682	AY674387	428	99	<i>P. paneum</i>
GL0832	ITS	JX241663	AF033477	561	99	<i>P. viridicatum</i>
	β -tubulin	JX241683	FJ004441	459	99	<i>P. viridicatum</i>
GL0826	ITS	JX241664	AY787726	467	99	<i>Phoma exigua</i>
GL0833	ITS	JX241665	FJ427005	486	99	<i>Ph. glomerata</i>
GL71510	ITS	JX241666	FJ427061	534	99	<i>Ph. samarorum</i>
GL9725	ITS	JX241667	EF432292	562	97	<i>Phomopsis</i> sp.
GL8921	ITS	JX241668	AY625075	600	99	<i>Rhizopus stolonifer</i>
GL3314	ITS	JX241669	AY905555	474	99	<i>Spenceriartinsia viticola</i>
GL0834	ITS	JX241670	DQ093708	546	99	<i>Trichoderma inhamatum</i>
GL0800	ITS	JX241671	EU552162	590	100	<i>Trichothecium roseum</i>

^a ACT = actin; ITS = internal transcribed spacer, and TEF = translation elongation factor 1- α .

glass rod (18). The spore concentration was determined and adjusted with the aid of a hemacytometer to obtain a series of five suspensions, containing 1×10^4 to 1×10^8 spores/ml.

Each apple was inoculated with several isolates from every species and each isolate inoculation was repeated three times in each of the 2 years of study. The control fruit were treated similarly to the inoculated fruit but with sterile water instead of spore suspensions. After inoculation, fruit were placed on trays, covered with plastic wrap to maintain high humidity, and held at 25°C in the dark. Fruit were examined daily and types of symptom expression were recorded.

To understand the effects of combined species from the genera *Alternaria* and *Cladosporium* on symptom formation, fruit were inoculated with combined concentrations of two different species in pathogenicity tests, with each pathogen present at 1×10^4 to $1 \times$

10^6 spores/ml (essentially 2×10^4 to 2×10^6 spores/ml). The inoculation method and assessment were the same as described above.

Results

Isolation and identification of fungi. The fungal genera *Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Phoma*, and *Trichothecium* were isolated more frequently than other fungi (fungi with isolation frequency above 1% are shown in Table 3). Some fungi appeared one or two times, including *Botryosphaeria dothidea*, *Coniothyrium diplodiella*, and *Mucor circinelloides* (data not shown).

Based on the combined phylogenetic and morphological analyses, all fungal isolates belonged to 33 species in 20 genera: *Acremonium alternatum*; *Alternaria alternata*, *A. arborescens*, *A. infectoria*, and *A. tenuissima*; *Aphanocladium araneum*; *B. dothidea*; *Chaetomium globosum*; *Cladosporium cladosporioides* and *C. tenuissimum*; *Colletotrichum gloeosporioides* complex; *Coniothyrium diplodiella*; *Epicoccum nigrum*; *Eutypella caricae* and *E. vitis*; *Fusarium equiseti*, *F. proliferatum*, *F. solani*, and *F. tricinctum*; *Mucor circinelloides*; *Nigrospora oryzae*; *Penicillium chrysogenum*, *P. expansum*, *P. paneum*, and *P. viridicatum*; *Phoma exigua*, *Ph. glomerata*, and *Ph. samarorum*; *Phomopsis* sp.; *Rhizopus stolonifer*; *Spencermartinsia viticola*; *Trichoderma inhamatum*; and *Trichothecium roseum* (Table 2).

Type of symptoms. Three types of symptoms were observed on infected samples (Fig. 1). Browning occurred most frequently (43.2%), with the occurrence rates of core rot and moldy core at 30.7 and 26.1%, respectively.

Pathogen composition for the three symptom types was different (Fig. 2). In browning (Fig. 2A), the genera *Alternaria* and *Cladosporium* individually occurred in 24.3 and 8.8% of the apple fruit, respectively. These two genera appeared simultaneously in 43.7% of isolations, and also appeared with other fungi, including species of *Epicoccum*, *Phoma*, *Penicillium*, and *Fusarium* in 13.4% of isolations. In total, *Alternaria* and *Cladosporium* spp. could be isolated from 81.4 and 65.9% of diseased fruit, respectively, indicating that both *Alternaria* and *Cladosporium* spp. are the main pathogens associated with core browning.

For moldy core (Fig. 2B), the genera *Alternaria* and *Cladosporium* individually occurred in 34.7 and 14.4% of affected apple fruit, respectively. These two fungi appeared with other fungi, such as species of *Fusarium*, *Penicillium*, and *Phoma* in 41.0% of isolations. In total, *Alternaria* and *Cladosporium* spp. could be isolated from 75.7 and 55.5% of diseased fruit, respectively, indicating that both *Alternaria* and *Cladosporium* spp. are also the main pathogens associated with moldy core.

For core rot (Fig. 2C), the genus *Trichothecium* occurred in 24.7% of isolations and was mixed with species of *Alternaria*, *Epicoccum*, *Fusarium*, *Penicillium*, and *Phoma* in 28.1% of isolations. Thus, *Trichothecium* was the main pathogen associated with core rot and was found in 52.8% of isolations. Although the genera *Penicillium*, *Epicoccum*, *Phoma*, and *Fusarium* occurred alone (6.7, 3.9, 3.4, and 2.2%, respectively) or in various combinations (18.4%) in low frequency, altogether they amounted to about one-

Fig. 1. Symptoms of three types: A, browning; B, moldy; and C, core rot.

Table 3. Isolation frequency of pathogenic fungi from different symptom types

Fungi	Isolation frequency (%) ^a		
	Browning	Moldy core	Core rot
<i>Alternaria</i> spp.	78.32	72.39	8.72
<i>Cladosporium</i> spp.	53.11	16.68	2.30
<i>Epicoccum nigrum</i>	4.76	2.20	13.59
<i>Fusarium</i> spp.	3.21	5.86	8.84
<i>Penicillium</i> spp.	1.59	4.11	16.25
<i>Phoma</i> spp.	3.72	7.73	15.13
<i>Trichothecium roseum</i>	2.82	1.63	52.77

^a Isolation frequency = (number of apple fruit infected with fungi/total number of fruit of each type) × 100.

third of isolations (34.6%) and, when combined with co-occurrences with *Trichothecium* (28.1%), they amounted to 62.7% of the isolations. Therefore, even though the genus *Trichothecium* is a predominant pathogen in core rot isolations, other species of *Penicillium*, *Epicoccum*, *Phoma*, and *Fusarium* also contribute to the occurrence of core rot.

Species of *Phoma*, *Epicoccum*, and *Penicillium* could cause core rot (Table 1) but they also occurred in samples with browning, alone (9.8%) or simultaneously with *Alternaria* and or *Cladosporium* spp. (13.4%) (Fig. 2A). Although *Alternaria* and or *Cladosporium* spp. were the predominant organisms associated with both browning and moldy core, they also could be isolated,

Fig. 2. Pathogen composition of the three symptom types: A, browning; B, moldy; and C, core rot.

although at much lower frequencies, from samples with core rot and co-occurring with *Colletotrichum*, *Botryosphaeria*, and *Spencer-martinsia* spp. (12.6%) (Fig. 2C).

When identified to species level, three pathogenic *Alternaria* spp. (*Alternaria alternata*, 48.8%; *A. tenuissima*, 32.6%; and *A. arborescens*, 18.6%) and two *Cladosporium* spp. (*Cladosporium cladosporioides*, 48.8%; and *C. tenuissimum*, 51.2%) were present in a high ratio of isolations from diseased fruit, regardless of the symptom type.

Pathogenicity. On nonwounded apple halves, variation in symptom appearances of core browning, moldy core, and core rot were related to the fungal species utilized. At a spore concentration of 1×10^6 spores/ml, *A. alternata* and *A. tenuissima* caused the moldy core symptom, whereas *A. arborescens*, *C. cladosporioides* and *C. tenuissimum* caused the browning symptom. Fungi causing wet core rot included *B. dothidea*, *Colletotrichum gloeosporioides* complex, *F. equiseti*, *F. proliferatum*, *F. tricinctum*, *Penicillium chrysogenum*, *P. expansum*, *P. paneum*, *P. viridicatum*, *Spencer-martinsia viticola*, and *Trichothecium roseum*. Dry core rot was caused by *Epicoccum nigrum*, *Phoma exigua*, *Ph. glomerata*, and *Ph. samarorum* (Table 1).

Inoculum concentration affected the symptom appearances with some of the *Alternaria* and *Cladosporium* spp. When a series of suspension concentrations was used in pathogenicity tests, it was found that the species *A. alternata*, *A. arborescens*, *A. tenuissima*, *Cladosporium cladosporioides*, and *C. tenuissimum* caused browning in relatively lower concentrations and moldy core under relatively higher spore concentrations (Table 4). For example, for *A. alternata* and *A. tenuissima*, browning occurred following inoculation with 1×10^5 spores/ml, whereas moldy core occurred following inoculation with 1×10^6 spores/ml. Furthermore, the lowest inoculum concentrations required for symptom formation varied among species (i.e., for *Alternaria* and *Cladosporium* spp., 1×10^5 spores/ml and 1×10^6 spores/ml, respectively, were required to initiate symptoms). For *C. tenuissimum*, the higher inoculation concentration, 1×10^8 spores/ml, was needed to initiate moldy core symptoms.

Because an inoculum concentration of up to 1×10^8 spores/ml seemed unreasonably high for an individual pathogen under natural conditions, we tested the effects of combined inoculum concentrations on symptom formation (Table 5). It was found that lower combined inoculum concentrations of individual pathogens com-

Table 4. Symptom types of *Alternaria* and *Cladosporium* spp. under different spore concentrations after inoculation

Fungal species	Isolate code	Spore concentrations (spores/ml) ^a				
		1×10^4	1×10^5	1×10^6	1×10^7	1×10^8
<i>Alternaria alternata</i>	GL09715	–	B	M	M	M
<i>A. tenuissima</i>	ZQ111521	–	B	M	M	M
<i>A. arborescens</i>	GL09614	–	B	B	M	M
<i>Cladosporium cladosporioides</i>	ZQ241422	–	–	B	M	M
<i>C. tenuissimum</i>	ZQ150821	–	–	B	B	M

^a Symbols: – = no infection observed, B = browning, and M = moldy core.

Table 5. Symptom types of *Alternaria* and *Cladosporium* spp. under different spore concentrations after combined inoculation

Combination	Isolate code	Spore concentrations (spores/ml) ^a		
		1×10^4	1×10^5	1×10^6
<i>Alternaria alternata</i> + <i>A. arborescens</i>	GL09715 + GL09614	–	B	M
<i>A. alternata</i> + <i>A. tenuissima</i>	GL09715 + ZQ111521	–	B	M
<i>A. arborescens</i> + <i>A. tenuissima</i>	GL09614 + ZQ111521	–	B	M
<i>A. alternata</i> + <i>Cladosporium cladosporioides</i>	GL09715 + ZQ241422	–	B	M
<i>A. alternata</i> + <i>C. tenuissimum</i>	GL09715 + ZQ150821	–	B	M
<i>A. arborescens</i> + <i>C. cladosporioides</i>	GL09614 + ZQ241422	–	B	M
<i>A. arborescens</i> + <i>C. tenuissimum</i>	GL09614 + ZQ150821	–	B	M
<i>A. tenuissima</i> + <i>C. cladosporioides</i>	ZQ111521 + ZQ241422	–	B	M
<i>A. tenuissima</i> + <i>C. tenuissimum</i>	ZQ111521 + ZQ150821	–	B	M
<i>C. cladosporioides</i> + <i>C. tenuissimum</i>	ZQ241422 + ZQ150821	–	B	M

^a Symbols: – = no infection observed, B = browning, and M = moldy core.

bined with a second organism also initiated browning and moldy core symptom formation. For *A. arborescens*, *C. cladosporioides*, and *C. tenuissimum*, moldy core occurred when combined by inoculation with 1×10^6 spores/ml (Table 5), but only browning appeared when inoculated separately under the same concentrations (Table 4). For *C. cladosporioides* and *C. tenuissimum*, browning occurred when combined by inoculation with 1×10^5 spores/ml (Table 5) but no symptoms formed when inoculated separately under the same concentrations (Table 4). The control fruit did not show any symptoms in all of the experiments described above.

Discussion

The symptoms of moldy core mentioned in various studies are generally inconsistent in the literature. Ellis and Barrat (7) indicated that moldy core was characterized by the growth of mycelia within the locules, with or without penetration into the mesoderm, and may become invasive and lead to a slow, dry rot confined to the flesh immediately surrounding the core. However, Archer pointed out that, if the mycelia penetrated into flesh and caused rot of flesh, these symptoms belonged to core rot rather than moldy core (1). In this article, moldy core/core rot included all these symptoms and was subdivided into three types. The browning type was not the early symptom of moldy core; rather, it could be found from harvest through 3 months of refrigerated storage. Therefore, we have described core browning as a new individual symptom type, along with moldy core and wet and dry core rot. Sometimes, moldy core and core rot had some similar symptoms (i.e., flesh was necrotic); therefore, if the core region was filled by mycelia and the flesh was rotted but the core was not rotted, we divided this type into moldy core rather than core rot. In nature, we could find the moldy core symptom commonly but this symptom occurred just under a higher spore concentration in our inoculation experiments. It is proposed that the moldy core symptom can only be caused by sufficiently high inocula of one pathogen or combined pathogens at lower inoculum levels. The core rot symptom could separate into two types in the orchard: dry core rot and wet core rot. Wet core rot, mainly caused by *Trichothecium roseum*, *Fusarium equiseti*, *F. proliferatum*, *F. tricinatum*, *Penicillium chrysogenum*, *P. expansum*, *P. paneum*, and *P. viridicatum*, appeared more frequently than dry core rot, mainly caused by *Epicoccum nigrum*, *Phoma exigua*, *Ph. glomerata*, and *Ph. samarorum*; and this phenomenon was also demonstrated in the pathogenicity experiment. Because we didn't specifically study the pathogenic composition of these two types of core rot, additional research on the conditions that favor their development is needed.

The method of inoculation used in previous research involved injecting a spore suspension into the core region of apple fruit (10). The problem with this method is that before inoculation, it is difficult to know whether fruit were healthy or not. The advantages of the method we used could ensure that fruit were healthy before inoculation and the pathogenic progress of fungi could be observed daily without destructive sampling. Because inoculum concentration affects symptom development and the type of symptom, different inoculation concentrations are needed for successful assessments of moldy core pathogenesis. Results from this study suggest that inoculation concentrations from 1×10^4 to approximately 1×10^8 spores/ml should be used.

Management of moldy core/core rot relies on the use of fungicides applied during the bloom period, although their effectiveness varies due to weather conditions and other unknown factors. Worldwide, fungicides used in recent years include boscalid; captan; polyoxin B; potassium phosphate; and steroidal saponine; the demethylation inhibitor fungicides difenoconazole and bromuconazole; and the strobilurins pyraclostrobin, kresoxim methyl, azoxystrobin, and trifloxystrobin. Most of these fungicides have been reported to be effective against *A. alternata* (2,15,16,17,18). In this study, although *Alternaria* spp. could be isolated from samples with core browning (81.4%) and moldy core (75.5%), *Cladosporium* spp. could be isolated in high proportions as well (65.9 and 55.5%, respectively); therefore, *Cladosporium* spp.

should be included as primary causal agents of core browning and moldy core. For core rot, *T. roseum* was the main pathogen recovered (about 52.8% of isolations), and species of *Penicillium*, *Epicoccum*, *Phoma*, and *Fusarium* occurred alone or co-occurred with *T. roseum* (62.7% of the isolations). These results showed that the core browning, moldy core, and core rot pathogens were a highly diverse fungal complex and the role of any particular species could not be overemphasized. We suggest that most of the pathogens should be examined when conducting a preliminary screening of fungicides.

Many genera, including *Alternaria*, *Botryosphaeria*, *Botrytis*, *Candida*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, *Fusarium*, *Gloeosporium*, *Mucor*, *Penicillium*, *Pestalotia*, *Phoma*, *Pleospora*, *Sporathrix*, *Stemphylium*, and *Ulocladium*, were reported as pathogens associated with moldy core/core rot (3,7,28,31,33,35). We found that fungi in 10 genera (*Alternaria*, *Botryosphaeria*, *Cladosporium*, *Colletotrichum*, *Epicoccum*, *Fusarium*, *Penicillium*, *Phoma*, *Spenceriartinsia*, and *Trichothecium*) could cause browning, moldy core, or core rot symptoms in China, and identified them to species level. In our study, *C. cladosporioides* and *C. tenuissimum* are reported for the first time to cause moldy core. The species *B. dothidea*, *F. equiseti*, *F. proliferatum*, *F. solani*, *F. tricinatum*, *P. chrysogenum*, *P. paneum*, *P. viridicatum*, and *S. viticola* were found for the first time to cause wet core rot. The species *E. nigrum*, *Ph. exigua*, *Ph. glomerata*, and *Ph. samarorum* were found for the first time to cause dry core rot. In China, *T. roseum* was a main pathogen of core rot but has not been a reported in other regions.

We identified the fungi to species level, because this may prove useful for choosing an effective fungicide. Future work will examine regional disease incidence, annual variation in the pathogen complex, and the management of moldy core/core rot.

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