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Black Spot of Peach Caused by *Alternaria alternata* (Fr.) Keissler

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ABSTRACT

A new disease of peach (*Prunus persica* Batsch var. *vulgaris* Maxim.), causing brown, sunken lesions and brownish to blackish brown spots with cracks on peach fruits, was found in Okayama prefecture, Japan, in 1995. The disease was observed not only on peach fruits but also on twigs and leaves. An *Alternaria* sp. was consistently isolated from these diseased fruits, twigs and leaves. The isolates were pathogenic to peach fruits and leaves. Based on the morphological characteristics, the causal fungus was identified as *Alternaria alternata* (Fr.) Keissler. After cross-inoculation with isolates from peach, Japanese pear and apple, the isolates were found to be pathogenic only to their original host. This is the first report on a peach disease caused by a host-specific *A. alternata* ; therefore, the common name of black spot ('Kokuhanbyo' in Japanese) was proposed.

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Key words : peach, black spot, *Alternaria alternata*.

INTRODUCTION

Peach fruits produced from mid-June to mid-August in Okayama prefecture are famous in Japan for their high quality. However, since 1989, a new disease of peach (*Prunus persica* Batsch var. *vulgaris* Maxim. cultivar (cv.) Shimizu Hakuto) causing brownish to blackish brown spot with cracks and sunken lesions on fruits has occurred in one peach-growing area of Okayama prefecture. The symptoms were observed not only on fruits, but also on leaves and twigs. This disease was a serious problem for production of peach fruits. When the trees were severely infected, nearly all fruits on the trees were unsaleable, and most of the infected leaves dropped early.

This paper describes the new disease, black spot of peach, caused by *Alternaria alternata*. Some preliminary results have been reported elsewhere[2-4,8].

MATERIALS AND METHODS

Isolation of the causal fungus In 1995, a fungus was isolated from the margins of lesions on immature and mature fruits, twigs and leaves of the peach cv. Shimizu Hakuto. The plant tissues were surface-sterilized with sodium hypochlorite solution (2.0% as active chlorine) for 30 sec and placed on potato-sucrose agar (PSA) plates. The plates were incubated in the dark at 25°C for about 10 days. Monoconidial isolates were then transferred to PSA slants and kept in an incubator at 17°C until use.

Cultural and morphological characteristics An isolate No. 9544 obtained from affected immature peach fruit in May of 1995, an isolate of *A. alternata* Japanese pear pathotype (No. 15A) and reference isolate of *A. alternata* (IFO 32416) supplied by Nagoya University were used. The isolates were identified by comparing the cultural and morphological characteristics of their colonies and conidia with those of reference isolates of *A. alternata* grown on PSA and water agar medium at 25°C for 10 days.

Inoculation tests For cross-inoculation tests, two isolates (Nos. 9544, 9549) from peach fruits, two isolates (Nos. 9502, 9506) from peach twigs, two isolates (Nos. 9528, 9533) from peach leaves, two isolates (Nos. N-1-1, 15A) of *A. alternata* Japanese pear pathotype, and an isolate (No. B-2-2) of *A. alternata* apple pathotype were used. Isolate Nos. N-1-1 and B-2-2 were supplied by Tottori Prefectural Horticultural Experiment Station and Nagoya University, respectively.

Each isolate was cultured in liquid apricot medium (dry apricot fruit 30 g, sucrose 40 g, 1000 ml of distilled water, pH 6.5) at 25°C for 3 weeks. The mycelia growing on the surface of the medium were harvested and rinsed with water, and moisture was removed with tissue paper.

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The mycelia were then placed on sterilized filter papers in petri dishes at room temperature for 7 days to induce sporulation. Conidia were collected from the mycelial mats with a cotton-tipped applicator stick. The conidia were released from the cotton tip by stirring in distilled water containing 0.05% Tween-20. The concentration of conidia was adjusted to approximately 1×10^6/ml.

Healthy, intact, immature fruits of peach cvs. Hakuho and Shimizu Hakuto were inoculated in a field in the evening. A paper disk (8 mm in diameter, thick, ADVANTEC) was dipped in the conidial suspension and then placed on a fruit, which had been covered with paper bags to protect from diseases and insects. Ten to 17 fruits for each cultivar were inoculated with one isolate. Inoculated fruits were then covered with paper bags till harvest time. Pathogenicity was evaluated with a disease severity index as follows : 0, no symptoms ; 1, a few pale brown spots ; 2, many or fused brownish spots ; 3, enlarged or sunken brownish lesions at harvest. Pathogenicity of the *Alternaria* sp. reisolated from the lesions was confirmed by inoculating leaves of peach cv. Shimizu Hakuto as follows.

Detached, young intact leaves of peach cv. Shimizu Hakuto, Japanese pear cv. Nijisseiki and apple cv. Hokuto were cut into about 2×3 cm pieces. One drop of the conidial suspension was then put on each side of the midrib of the abaxial surface. A paper disk (6 mm in diameter, thin, ADVANTEC) was put on each drop to keep it in place. Seven leaf pieces for each plant were inoculated with an isolate. Pathogenicity was evaluated by rating necrosis on the leaves 7 days after incubation at 24-27°C in moist plastic trays.

RESULTS

Symptoms

In the field, black spot appeared on fruits, twigs and leaves. The type of symptom development was related to the stage of fruit maturity. On immature fruit, circular pale brown spots 2-3 mm in diameter were observed about 3 weeks after full bloom (Plate I-1). Then the lesions enlarged to about 5-10 mm long, and became brownish to blackish brown. Lesions on enlarging, maturing fruits were often sunken and developed cracking and gummosis (Plate I-2). In severe cases, the cracking sometimes extended from the lesion into healthy fruit tissues.

Leaf symptoms were first visible as indistinct chlorotic or grayish necrotic spots, 5-10 mm in diameter (Plate I-3, white arrow). Later, the lesions became dark brownish necrotic spots leaving shot-holes. Often, irregular necrotic lesions expanded along veins during the rainy season (Plate I-3, black arrow). These infected leaves often dropped within several days after development of lesions.

Symptoms on twigs appeared as circular to oval, red or pale brown spots, 2 to 5 mm in diameter. The center of the spot became dark brown and sometimes had a red to purple outer margin (Plate I-4). With time, lesions developed gummosis and became zonate, sunken, with lengthwise cracks, and fused to extend several centimeters in length (Plate I-5). This necrosis usually extended into the xylem of twigs.

The disease occurred on all cultivars of peach, especially the cv. Shimizu Hakuto.

Isolation and identification of the causal fungus

An *Alternaria* sp. was the most frequently isolated fungus from the lesions of fruits, twigs and leaves. On PSA plates, the fungus developed aerial hyphae on grayish white, cottony colonies, which later turned olive-green to black. On water agar plates, conidiophores were simple or branched and several septate, straight or flexuous, pale olivaceous brown, smooth, 10-223 (avg. 33) μm long, with several conidial scars. Conidia formed in catenate, often branched chains, were obclavate or ovoid, with a pale brownish short conical or cylindrical beak (1.3-17.7 μm), buff to dark brown, with 0-10 transverse and 0-4

Table 1. Morphological comparison of *Alternaria* isolate from peach with reference isolates of *A. alternata*

Characters	Isolate from peach No. 9544	Isolate from Japanese pear No. 15A	Reference isolate of *A. alternata* IFO 32416[a)]	Description for *A. alternata* by Ellis[1)]
Conidia				
Total length (μm)	9-71 (avg. 32)	11-88 (avg. 31)	11-48	20-63 (avg. 37)
Width (μm)	6-18 (avg. 13)	5-16 (avg. 13)	6-20	9-18 (avg. 13)
Beak length (μm)	1.3-17.7	1.3-25	1.3-12.5	
Number of septa				
Longitudinal	0-4	0-5	0-3	
Transverse	0-10	0-10	0-7	<8
Conidiophores				
Length (μm)	10-233 (avg. 33)			<50

a) Isolated from *Datura metel* in India.

Table 2. Pathogenicity of *Alternaria alternata* isolates on peach fruits

Date of inoculation	Isolate		Peach cultivars inoculated			
			Hakuho		Shimizu Hakuto	
	No.	Source	Pathogenicity[a]	Disease index[b]	Pathogenicity	Disease index
1996 June 26	9544	Immature peach fruit	14/14	70	11/11	93
17	9549	Mature peach fruit	10/10	50	9/9	81
17	9502	Peach twig	7/8	31	9/9	53
26	9506	Peach twig	8/8	63	10/10	70
26	9528	Peach leaf	6/9	28	8/10	35
26	9533	Peach leaf	7/7	50	11/11	75
17	15A	Japanese pear	0/9	0	0/10	0
26	B-2-2	Apple	0/10	0	0/10	0

a) Number of diseased fruits/Number of fruits inoculated.

b) Disease index $=[\Sigma(X_i \cdot n_i)/3N] \times 100$, X_i = Index based on severity of the lesion (0, no symptoms ; 1, few pale brown spots ; 2, many or fused brownish spots ; 3, enlarged or sunken brownish lesions), n_i = Number of fruits expressed as X_i, N = Total number of fruits examined.

Table 3. Pathogenicity of *Alternaria alternata* isolates on leaves of peach, Japanese pear and apple

Isolate number	Source	Inoculated plant[a]		
		Peach cv. Shimizu Hakuto	Japanese pear cv. Nijisseiki	Apple cv. Hokuto
9544	Immature peach fruit	7/7	0/7	0/7
9549	Mature peach fruit	6/7	0/7	0/7
9506	Peach twig	7/7	0/7	0/7
15A	Japanese pear	0/7	7/7	0/7
N-1-1	Japanese pear	0/7	7/7	0/7
B-2-2	Apple	0/7	0/7	7/7
Distilled water		0/6	0/6	0/6

a) Number of diseased leaves/Number of leaves inoculated.

longitudinal or oblique septa, overall length 9-71×6-18 (avg. 32×13)μm (Plate I-6). The morphology of conidiophores and conidia of the isolates from peach coincided with that of the reference *A. alternata* isolates examined (Table 1).

Pathogenicity

Representative isolates (Nos. 9544, 9549, 9502, 9506, 9528, and 9533) which were isolated from lesions on fruits, twigs and leaves of peach cv. Shimizu Hakuto were tested for their pathogenicity to immature peach fruits in the field. The six isolates were all pathogenic to fruits of peach cv. Hakuho and Shimizu Hakuto (Table 2). The symptoms developed were the same as those caused by natural infection (Plate I-7). Each isolate could be reisolated from the inoculated fruits. However, the Japanese pear isolate (No. 15A) and the apple isolate (No. B-2-2) did not cause any symptoms on peach fruits.

Cross-inoculation tests on leaves of peach, Japanese pear and apple showed that peach isolates (Nos. 9544, 9549, and 9506), two Japanese pear isolates (Nos. 15A, N-1-1) and an apple isolate (No. B-2-2) had distinct specificity for their respective hosts (Table 3).

DISCUSSION

Alternaria spp. on peach have been reported mainly as saprophytes on wounded, mature fruits during storage[6,11,12]. In China[13], peach black spot caused by *A. alternata* has been reported. A yellow to red nipple formed on the fruit apex and zonate black rings appeared after wounding of the nipple tissue during harvest or storage. However, these symptoms did not develop on the fruits described in this paper, even though both of these diseases are caused by *A. alternata*. Information about the incidence of lesions on leaves and twigs was not included in other papers. In India, a leaf spot disease of peach caused by *A. alternata* was reported[7], but no information was given about the pathogenicity to fruits and twigs. No previous reports have been made on a disease affecting peach fruits, as well as leaves and twigs caused by *A. alternata* during the growing period.

The morphology of conidiophores and conidia of the peach isolates is indistinguishable from those of the tested reference *A. alternata* isolates and those in other reports[1]. The peach isolates produced conidia with long

chains, often branched on water agar plates, and developed grayish white, cottony colonies with aerial hyphae on PSA plates. Only *A. alternata* and *A. brassicicola* producing long chains are listed as members of the *Longicatenatae*[10]. But *A. brassicicola* produces sooty black colonies and differs from the peach isolates[10]. Therefore, the peach isolates were identified as *A. alternata* (Fr.) Keissler.

The present isolates of *A. alternata* are only pathogenic to peach. This is the first report of host-specificity of an *A. alternata* isolated from peach. We propose that this new disease should be called black spot of peach ('Kokuhanbyo' in Japanese). Nishimura[9] proposed that host specific toxin-producing isolates of *A. alternata* should be called distinct pathotypes. Results from cross-inoculation tests indicated that the present isolates in Okayama might be a new pathotype and distinct from other isolates of *A. alternata* (black spot of Japanese pear and Alternaria blotch of apple).

Host-specific toxins are involved in at least seven plant diseases caused by *A. alternata* as pathogenicity factors. The *A. alternata* isolates from peach produced phytotoxin(s) in culture fluid, which induced necrosis on injured peach leaves[5]. Whether this phytotoxin satisfies the conditions for a host-specific toxin, however, needs to be examined.

Black spot of peach is now found only in one peach-growing area of Okayama prefecture. However, this disease might spread by airborne propagules to other districts. Countermeasures against this disease must be quickly established.

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Explanation of plate

Plate I

1. Sunken lesions on immature peach (cv. Shimizu Hakuto) fruit after natural infection.
2. Lesions with cracking and gummosis on mature peach (cv. Shimizu Hakuto) fruit after natural infection.
3. Circular grayish spot (white arrow) and necrotic area along vein (black arrow) naturally occurring on leaf (cv. Shimizu Hakuto).
4. Brown, sunken spot with red spindle zone, the initial symptom on a twig (cv. Shimizu Hakuto) after natural infection.
5. Severe cracking and necrosis with gummosis on naturally infected twig (cv. Shimizu Hakuto).
6. Conidia of *Alternaria alternata* peach isolate (No. 9544) formed on liquid apricot medium (scale is 30μm).
7. Sunken lesion with cracking on mature fruit (cv. Shimizu Hakuto) after inoculation of immature fruit with conidial suspensions of *A. alternata* on a paper disk.

Plate I