Alternaria alternataによるオクラ果実黒斑病(新称)

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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発行元	日本植物病理學會
巻/号	61巻4号
掲載ページ	p. 340-345
発行年月	1995年8月

日植病報 61：340-345 (1995)
Ann. Phytopathol. Soc. Jpn. 61：340-345 (1995)

A New Post-Harvest Disease of Okra Pods Caused by *Alternaria alternata**

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Abstract

Alternaria rot of okra pods caused by *Alternaria alternata* was described as a new post-harvest disease. The disease was frequently found in the market place of Japan. The optimal temperature for the mycelial growth of the fungus was more than 20°C. But expression of the disease symptoms were greatly promoted at low temperatures and became evident after prolonged exposure at 10°C in the laboratory. Chilling injury or "Alternaria rot" of okra pods which had previously recorded in the literature might be the same symptoms as seen in this study.

(Received November 21, 1994; Accepted February 27, 1995)

Key words: *Alternaria alternata*, okra, chilling injury, new disease.

INTRODUCTION

Consumer demand for unblemished agricultural products without residual chemicals has turned attention increasingly to post-harvest plant pathology. In Japan, however, post-harvest losses have been less extensively studied than pre-harvest losses in the field. In the course of a market survey of post-harvest disease in Kyoto, Japan, we noticed a unique disease of okra [*Abelmoschus esculentus* (L.) Moench] pods which had not been reported in the literature[2,6,10].

Young pods of okra are in rapidly increasing demand in Japan. The crop is produced primarily in the southwest of the Japanese archipelago, where the climate is suitable for the plant growth. Okra pods prepacked in plastic nets and enclosed in a master carton are usually transported by container truck at ambient temperature as a mixed consignment with other produce to urban markets. They are usually kept at about 10°C in cooled show-cases in the retail market.

In late summer of 1990, okra pods which were blemished with small black lesions were often observed in a supermarket in Kyoto, Japan. Our experiments revealed that the disfigurement was caused by an infectious fungus, *Alternaria alternata* (Fr.) Keissler, and it was common in all markets surveyed. We have made a preliminary report of the disease at the Meeting of the Kansai Division of the Phytopathological Society of Japan held at Shizuoka, October 25, 1991. Thus this disease has been recorded in the lists of economic plant diseases in Japan[3]. This paper described the details of symptoms, pathogen, and affected conditions of this new post-harvest disease of okra pods in Japan.

MATERIALS AND METHODS

Isolation and identification of the causal agents. Samples of affected pods were purchased in the markets of Kyoto and Kusatsu, located in the middle part of Japanese archipelago. Symptoms were recorded based on those observed under market conditions or after keeping 1-2 days in the laboratory at ambient temperature (20-30°C). Then they were incubated in a moist chamber to induce sporulation. Conidia produced on the diseased tissue were isolated monoconidially with the

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* Part of this paper was read at the Meeting of the Kansai Division of the Phytopathological Society of Japan held at Shizuoka, October 25, 1991.

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aid of a stereobinocular microscope onto PDA slant to obtain pure culture isolates of the causal fungus. They were cultured on PDA or V8-juice agar at 20–24°C in darkness. Mycelial growth of *A. alternata* was measured by colonial diameter on PDA after 7 days incubation in darkness.

Identification of *Alternaria* species was made by comparisons of cultural and morphological characteristics of their colony and conidia with those of standard isolates same as previous reports[13,14] and diagnostic keys provided by Ellis[5], Simmons[8] and Tohyama[12].

Pathogenicity tests. Conidial inoculum was prepared by growing monoconidial isolate of *A. alternata* from okra lesion (Miyazaki 1-1 or Kochi 1-1) on V8-juice agar for 12–14 days at 24°C in darkness. Conidial suspensions in sterile distilled water were adjusted to $2\text{–}3\times10^5$ conidia per ml.

For inoculation tests superficially healthy pods purchased in the market were first used. Then young pods of several cultivars, namely Earlyfive and Greenrocket (Takii and Company Ltd., Japan) and Greenstar and Peakfive (Sakata Seed Co., Japan), grown in a vinylhouse on the field of the Agronomy Division of Shiga Junior College at Kusatsu, were employed. Seeds of these cvs. were purchased from a seed shop in Kusatsu. Harvested okra pods were kept at 4°C for 3 days prior to inoculation. They were then sprayed with the conidial suspensions and noninoculated control pods were sprayed with sterile distilled water. All pods were arranged in plastic trays, covered with clear plastic film, and maintained in a growth chamber at designated temperatures in darkness. Symptom development was observed and recorded after 2 days or designated periods of incubation.

Symptoms on the pods were assessed by the following criteria and recorded in Table 1.

− : Healthy, no lesion observed.
± : Small black lesions scattered on the pod surface.
+ : Black lesions covered less than half of the pod surface.
2+ : Pod surface blackened and mycelial mat of *Alternaria* recognizable, but a pod not decayed.
3+ : Pod blackened and decayed.

Except in Table 1, the severity of disfigurement of inoculated pods was expressed by the average percentage of surface area blackened on three or four inoculated pods.

Experiments conducted to find any relation between disease development and pod ages were separately done on late June and late July in 1991 for corresponding to early summer and mid-summer shipping seasons according by standard harvest times for okra pods in Japan[11]. Suitable maturity of the pods for those shipping seasons were 8–9 days and 4 days after anthesis, respectively. Okra pods with designated maturity measured by the days after anthesis were harvested and precooled at 4°C for 3 days. They were treated as mentioned above and checked for severity of disfigurement after keeping for 7–8 days at 15°C.

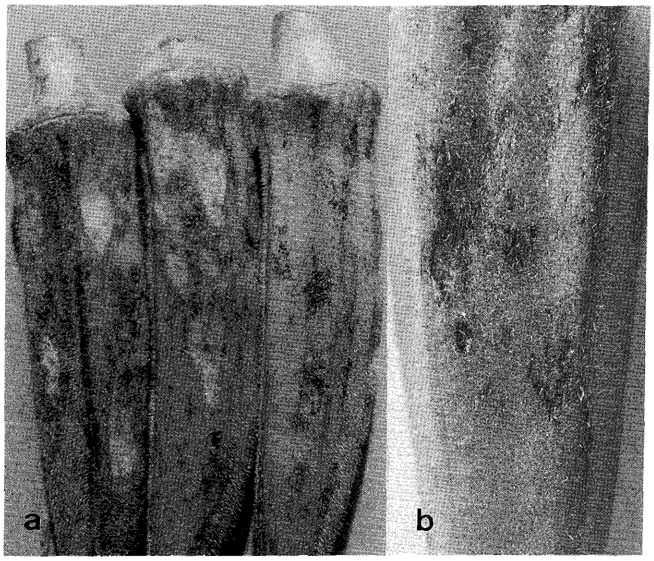

Fig. 1. Symptoms of Alternaria rot of okra pod. a: Symptoms on purchased okra pods. b: A close-up view of typical symptoms.

RESULTS

Symptoms and their occurrence under market conditions

When pods with minute brownish black lesions in the market were kept under humid conditions in the laboratory, severe symptoms developed in a few days. These were dark grey, slightly sunken irregular lesions, developing mainly lengthwise, which enlarged rapidly and often fused together as shown in Fig. 1. A grey cottony mycelial mat bearing conidia appeared on the surface of developing lesions. When the diseased pod was cut transversally or peeled off the surface part, blackened necrotic tissues were clearly observed under the surface lesions (Figs. 2a and 2b).

Symptoms observed in the market were usually less severe. Most were disfigured with black lesions which were difficult to distinguish from abrasion by the plastic net packaging. This might be due to removal of severely damaged pods from the show-case in the market. However, we occasionally found severe symptoms on old or unsuitably stored pods. Information obtained by inquiry from greengrocers supported this conclusion. These okra pods were observed primarily in the late summer to early autumn and in winter in Kyoto. The periods seem to correspond with the late season of crop production in the particular growing area or to correspond with seasonal need for long-distance transportation from the growing area.

Isolation and identification of the causal agents

A fungus identified as *A. alternata* (Fig. 3, Table 1) was consistently isolated from pods showing the above symptoms consigned from every producing district, namely, Miyazaki, Ohita, Kagoshima, Kochi, and Oki-

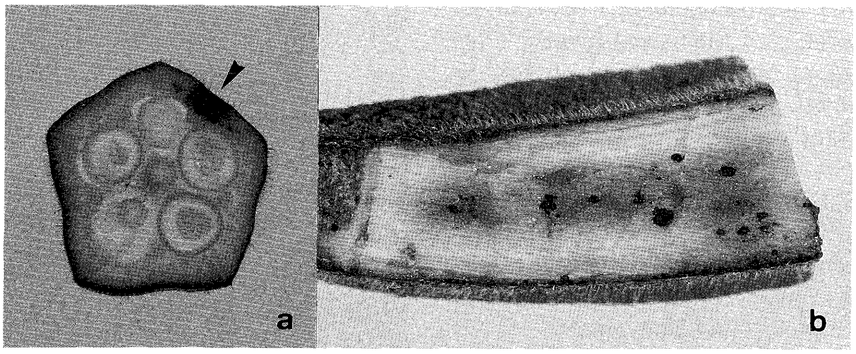

Fig. 2. Inner part symptoms of diseased okra pod. a: Necrotic tissues (arrow head) spreading into inside of a diseased pod invaded by *A. alternata*. b: Necrotic tissues under symptomatic lesions.

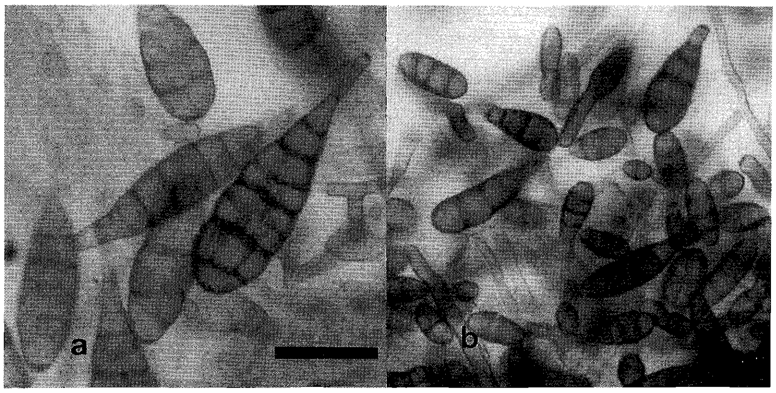

Fig. 3. Conidia of the causal agent, *A. alternata* (scale bar=20 μm). a: Conidia produced on okra pod kept at room temperature. b: Conidia of the isolate Miyazaki 1-1 on PDA slant kept at 24°C in darkness.

Table 1. Conidial measurements of *A. alternata* isolates from diseased okra pods

Collection or Isolate	Total length Size (μm)	Body size (μm)		Number of septa		Beak length (μm)
		Length	Width	Vertical	Horizontal	
On okra pods[a]						
Miyazaki 1	20-100(49.7)	20-63(38.6)	9-19(13.2)	1-7(3.4)	1-10(5.4)	0-43(9.9)
Kochi 1	16- 64(40.9)	16-50(34.1)	8-15(11.2)	0-5(1.8)	1- 7(4.6)	0-12(6.9)
On V8-juice agar[b]						
Miyazaki 1-1	13- 45(22.2)	13-33(20.3)	4-11(7.8)	0-2(0.7)	1- 5(2.9)	0-25(1.7)
2	13- 40(25.7)	13-40(24.7)	7-15(10.6)	0-5(1.7)	1- 5(3.2)	0-10(1.1)
3	15- 35(25.1)	15-35(24.6)	6-18(11.7)	0-5(1.7)	1- 5(3.1)	0-11(0.5)
Kochi 1-1	13- 56(24.2)	8-36(20.4)	8-18(7.8)	0-3(0.9)	1- 5(2.3)	0-27(1.4)
2	14- 40(26.9)	14-37(26.0)	6-13(9.1)	0-4(1.5)	2- 5(3.5)	0-14(0.9)
Okinawa 1	13- 36(23.4)	13-36(22.3)	8-20(11.9)	0-5(1.6)	1- 4(2.6)	0-11(1.0)
2	18- 63(32.3)	18-58(29.1)	8-16(12.8)	0-6(2.7)	1- 8(4.1)	0-28(3.1)
Reference isolates of *A. alternata*						
15A[c]	15- 39(26.1)	15-39(25.2)	5-15(10.5)	0-4(1.5)	1- 5(3.2)	0-10(0.8)
TH-7[d]	15- 38(26.8)	15-38(26.2)	5-13(9.4)	0-3(0.8)	1- 6(3.2)	0- 5(0.1)

Mean values are indicated in the parentheses.
a) Conidia produced on purchased okra pods kept at 24°C.
b) Conidia produced on V8-juice agar for 7 days at 24°C.
c) Japanese pear isolate; Stock culture ex Plant Pathology Lab., Nagoya University, kindly provided by late Professor S. Nishimura.
d) Chinese radish isolate; Stock culture of Shiga Junior College.

nawa. The morphological characteristics of the fungus on diseased okra pods and cultural media concurred with those of diagnostic feature of *A. alternata* provided by Ellis[5] and Tohyama[12] and those of standard isolates[13,14] used for reference. Colonies on PDA and V8-juice agar are grayish turning olive green to black.

Table 2. Results of inoculation tests with *A. alternata* to surface sterilized okra pods purchased in the market

Treatment	Lesions detected	Inoculation of *A. alternata* (Isolate used)	Incubation for 2 days				Incubation for 7 days			
			−−±	+	2+	3+	−−±	+	2+	3+
Surface sterilized	−	−	4	0	0	0	0	2	2	0
	−	+(Miyazaki 1-1)	0	0	4	0	0	0	0	4
	−	+(Kochi 7)	0	0	4	0	0	0	0	4
Control	−	−	0	0	2	1	0	0	0	3
	+	−	2	2	0	0	2	2	0	0

Figures in the table indicate the numbers of pods used.
Criteria for symptoms on the pods (disease severity) was shown in the text.

Table 3. Differences in symptom development induced by incubation at different temperatures after inoculation on okra pods with *A. alternata*

Cultivar	Inoculation	Incubation temperature (°C) 10	15	20	25	30
Greenstar	+	57.2± 7.9	28.3±24.2	16.7±4.0	8.3±4.4	2.3±2.1
Earlyfive	+	50.0± 6.7	15.0±18.6	13.5±5.5	6.7±4.8	1.7±2.7
Greenrocket	+	56.7±10.9	24.2±12.2	11.7±6.4	5.9±2.7	5.2±3.0
Greenrocket	−	13.3±16.2	0.0	0.3±0.5	0.0	0.0

Figures are percentages of pod surface covered by lesions (±95% confidence interval).
Incubation for 4 days at designated temperatures after inoculation with conidial suspensions.

Table 4. Symptom development at low temperature incubation of okra pods after inoculation with *A. alternata*

Cultivar	Inoculation	Incubation temperature 4°C	10°C
Peakfive	+	0.0	21.5±5.7
	−	0.0	5.3±4.3
Earlyfive	+	0.0	31.5±17.5
	−	0.0	4.8±8.2

Figures: see text and Table 2.
Incubation for 4 days after inoculation with conidial suspensions.

Table 5. Results of inoculation tests with *A. alternata* conidia onto okra pods with different ages

Cultivar	Inoculation	Days after anthesis <8-9>	10-12	13-16
Greenrocket	+	14.5±7.4	11.4±5.6	33.6±8.6
Greenstar	+	10.6±4.2	21.9±6.3	32.2±23.4
Greenrocket	−	0.4±0.4	0.6±1.2	2.0±1.5
Greenstar	−	1.8±1.8	1.8±1.8	3.5±2.0

(Experiment 1, correponding to early summer shipping.)
Figures: see text and Table 2. < >: Optimal harvest time.
Stored at 4°C for 3 days before inoculation and incubated at 15°C for 8 days after inoculation.

Conidiophores are simple, straight or curved, sometimes branched and 1-several septate. They are yellow brown to medium golden brown in color. Conidia with transverse and longitudinal and also oblique septa, pale to dark brown in color, are produced in short chains. The morphology of conidia (Fig. 3) is indistinguishable from those of typical *A. alternata* isolates examined. They are commonly observed among the cottony mycelium covering the lesions on okra pods or on agar media.

The conidial measurements of the fungus produced on diseased okra pods from Miyazaki and Kochi, those of representative isolates obtained from them, and reference isolates of *A. alternata*, 15A (Japanese pear isolate) and TH-7 (Chinese radish isolate), were given in the Table 1.

Pathogenicity tests

A trial was made with pods purchased in the market. When okra pods were kept at ambient temperature (20-30°C) prior and after inoculation of the fungus, the experiment gave inconsistent results and sometimes failed. Experiments using refrigerator stored pods and keeping at low temperature after inoculation were consistently successful (data not shown). Symptom development on market purchased okra pods during low temperature storage (10°C) with or without inoculation was next examined. Because the symptoms of Alternaria rot developed on the market purchased control pods without inoculation of the fungus, surface sterilization was made with sodium hypochlorite solution (a.i., 1.0% chloride, soaking for 2-3 min treatment) before inoculation. Typical symptoms developed on inoculated pods and the severity of disease reaction was more pronounced on 7 days incubation than 2 days incubation (Table 2).

In the following experiments young pods of designated cvs. from pilot field were used.

The severity of symptoms developed on inoculated pods varied with temperature of storage after inoculation from almost negligible to blackening over half of

Table 6. Results of inoculation tests with *A. alternata* conidia onto okra pods with different ages

Cultivar	Inoculation	Days after anthesis				
		3	<4>	5	6	7
Greenrocket	+	5.0±3.3	7.5±2.2	15.0±5.6	18.6±10.0	28.5±5.9
Earlyfive	+	1.9±2.7	3.3±2.7	10.0±5.3	11.0±5.2	16.7±5.5
Greenstar	+	8.2±7.0	9.5±6.4	16.0±4.0	15.0±5.5	16.8±7.8
Peakfive	+	1.8±1.8	2.3±2.2	10.0±7.9	9.5±10.5	21.7±16.5
Greenrocket	−	0.0	0.0	2.5±2.0	0.3±0.6	1.7±2.7
Earlyfive	−	0.0	0.0	0.0	0.3±0.7	0.3±0.8
Greenstar	−	0.6±0.6	1.6±2.8	—	—	—
Peakfive	−	0.3±0.5	0.3±0.5	—	—	—

(Experiment 2, correponding to early summer shipping.)
Figures: see text and Table 2. < >: Optimal harvest time.
Stored at 4°C for 3 days before inoculation and incubated at 15°C for 6 days after inoculation.

the surface (Table 3). It was highest at 10°C and decreased with increasing temperature (Table 3) and no symptoms were observed at 4°C (Table 4).

The difference of susceptibility between cultivars was not significant (Tables 3 and 4). All cultivars used in this study developed typical symptoms on storage at temperatures in the range of 10-20°C.

The relations between severity of symptoms and the periods after anthesis are shown in Tables 5 and 6. The harvesting date in Experiment 1 was 26 June and that of Experiment 2 was 27 July, respectively. The susceptible reaction of the pods was already pronounced 8-9 days and 4 days after anthesis in each experiment. Those days corresponded to standard harvest times decided by the pod length for early and mid-summer shipping in Japan[11]. In general, pods left longer on standing plants after anthesis had developed more clear and severe symptoms with *A. alternata* inoculation.

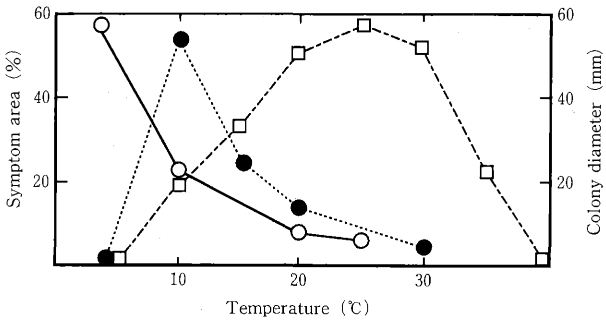

Fig. 4. Relationships between temperature and of mycelial growth of the causal agent, symptom development on inoculated okra pods, and susceptibility of okra pods. □------□ Mycelial growth of *A. alternata* (Miyazaki 1-1) on PDA medium after 7 days incubation in darkness. ●·········● Symptom development of okra pods after inoculation. ○——○ Susceptibility of okra pods pre-stored at different temperatures.

DISCUSSION

This study showed that *A. alternata* caused post-harvest deterioration of okra pods in Japan. Low temperature greatly promoted expression of symptoms caused by *A. alternata* in all cultivars used in this study. Symptoms became evident after prolonged exposure at 10°C in the laboratory.

Unfortunately, there has been no report on okra pods caused by *Alternaria* species in our literature survey. The only reported *Alternaria* diseases of okra are the foliar disease caused by *Alternaria brassicae* (Berk.) Sacc. and by an unidentified *Alternaria* species under field conditions in the U.S.A.[6] Okra pods have also been reported to suffer chilling injury in the form of minute black spots[1]. Baxter and Walters[4] have described the "Alternaria rot" of okra pods during their experiment on CA storage of the crop. These might be the same symptoms as seen in our study and the same Alternaria rot as that affecting crop reported in this paper. But unfortunately the authors gave no further details. Therefore, we consider the disease to be a new post-harvest disease of okra pods. This disease should be called Alternaria rot of okra pods[3].

Various commodities of tropical ancestry may suffer chilling injury at temperatures between 0-15°C[9,10]. But okra is believed not to be injured at 10°C and the recommended storage temperature is 7°C[7,10]. However, fresh okra pods have a short post-harvest life, being prone to physical and physiological changes that reduce quality. Thus post-harvest treatments such as precooling and modification of atmosphere (MA) have been used to extend the post-harvest life and maintain the quality of fresh okra[4].

Our results indicate that okra pods kept at low temperature are prone to infection with the fungus and to develop dark lesions. When these pods are transferred to ambient or high temperature, mycelium of *A. alternata* will cover the lesions or pod. These findings indicate the importance of temperature in relation to disease development. Low temperature may induce the physiological changes that allow infection by *A. alternata*. Differences in temperature dependence of mycelial growth (measured by colonial diameter on PDA after 7 days incubation in darkness), symptom development, and susceptibility of pods are summarized in Fig. 4.

The severe incidence of this deterioration of okra pods might be specific to Japan, due to climatic characteristics and/or cultural practices such as cultivation in vinyl-houses. The next task is to examine pre- and post-harvest factors affecting the disease development.

We are indebted to the owners of vegetable shops and supermarkets who kindly give us information on okra pods in the market.

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和　文　摘　要

遠山　明・林　邦博・谷口尚樹・成瀬知詠子・小沢佳子・獅山慈孝・津田盛也：*Alternaria alternata* によるオクラ果実黒斑病（新称）

Alternaria alternata によるオクラ果実黒斑病（新称）を記載した。本病は流通市場でよく検出された。病原菌の生育適温は20°C以上であったが，本病の病徴は低温下でよく発達した。また，接種により品種との関係を調べた結果，どの品種でもよく発生が認められた。従来オクラの低温障害として記載記録されてきた品種低下の一部は本病による可能性が示唆された。