Postharvest Decay of Cantaloupe Caused by Epicoccum nigrum

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ABSTRACT

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A decay causing red discoloration in cantaloupe fruit was observed in postharvest storage studies and on occasion has been involved in load rejection of melons grown in southeastern Oklahoma. Epicoccum nigrum was consistently isolated from areas showing the red discoloration. The fungus was also pathogenic on fruit of cucumber, tomato, apple, and pear. Comparison of the cantaloupe isolate of E. nigrum with isolates from Pennisetum flaccidum and Pisum sativum indicated that all were similar if not identical, based on host range and decay characteristics on the previously mentioned fruit. Light and scanning electron microscopy revealed that sporodochia and conidia were typical of E. nigrum. Radial growth was greatest on potatodextrose agar at 20 C and limited at 1, 5, and 30 C. The fungus remained viable in screwcap culture tubes of soilless medium (potting mix) for 4 yr at about 20 C. A proposed common name for the disease is red rot.

Cantaloupe (Cucumis melo L. var. reticulatus Naudin) is a commercially important horticultural crop in Oklahoma. With improved market development, cantaloupe production could increase dramatically. At present, production is targeted for roadside stands and grocery stores within the state. Postharvest decays can be a major problem for shippers involved in interstate commerce (6,8). The principal postharvest decay-causing fungi of Oklahoma cantaloupe fruit are, in order of importance (B. D. Bruton, unpublished), species of Phomopsis, Fusarium, Alternaria, Cladosporium, and Rhizopus. Lasiodiplodia theobromae (Pat.) Griffon & Maubl.(= Botrvodiplodia theobromae Pat.), Erwinia carotovora subsp. carotovora (Jones) Bergey et al, and Macrophomina phaseolina (Tassi) Goidanich may occasionally cause decay in shipments but are relatively minor in importance (6). In 1989, a decay of cantaloupe fruit termed "red rot" was observed in postharvest storage studies and on occasion has been involved in load rejection of melons grown in southeastern Oklahoma. As recent as 1993, red rot was observed on cantaloupe fruit imported from Guatemala.

A preliminary report has been published describing the symptoms and identifying the fungus as *Epicoccum nigrum*

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Link (3). The present study was undertaken: 1) to describe the disease on cantaloupe fruit and compare a representative cantaloupe isolate with isolates from other hosts and 2) to determine the temperature range for optimum growth of the fungus in culture, which might aid in control during storage.

MATERIALS AND METHODS

Koch's postulates and host range. Pure cultures of the fungus were obtained by making a transverse section through a lesion, excavating a small portion of the affected tissue below the hypodermis, and placing the sample on potato-dextrose agar (PDA). Cultures were allowed to grow under laboratory conditions. Fruit of cantaloupe cv. Magnum 45 were used to determine the pathogenicity of six fungal isolates obtained from cantaloupe grown in southeastern Oklahoma. Fruit were washed in a solution of liquid detergent and 0.5% NaOCl and air-dried at 25 C for 2 hr. Each fruit was inoculated with an 8-mm-diameter plug of agar and mycelia of the test fungus from 10-dayold cultures grown on PDA. A single inoculation was made on each of 10 fruit per isolate by removing a plug 8 mm in

diameter and 5 mm deep with a cork borer. After the inoculum was added, the fruit section was returned and subsequently sealed with molten wax. Fruit were incubated at 20 C for 10 days. In a subsequent experiment, two studies consisting of 10 fruit each of cantaloupe, cucumber (Cucumis sativus L.), apple (Malus sylvestris Mill. 'Golden Delicious'), pear (Pyrus sp.), and tomato (Lycopersicon esculentum Mill.) were inoculated, as previously described, using E. nigrum isolates from leaves of Pennisetum flaccidum Griseb. collected in North Carolina, Pisum sativum L. (ATCC 34929), and C. melo (hereafter referred to as cantaloupe isolate ATCC 66091). Test fruit were incubated at 20 C for 7 days.

Identification of the causal organism. Cultures of cantaloupe isolate ATCC 66091 were grown on PDA 30 cm below cool-white and warm-white fluorescent lights at about 20 C under a 12-hr photoperiod. Microscope slide mounts were prepared by inoculating pieces of PDA with fungal mycelium according to the method of Riddell (7). Pure cultures of cantaloupe isolate ATCC 66091 were fixed in glutaraldehyde, dehydrated through an ethanol series, critical-point dried with CO₂ as a transitional fluid, sputter-coated with 200 Å of goldpalladium, and examined by scanning electron microscopy.

For long-term storage, mycelia and conidia, including a small piece of agar (<2 mm), were introduced into screwcap culture tubes containing sterile potting mix and allowed to colonize. The cultures were maintained under laboratory conditions. Viability was determined annually by placing partial contents from the culture tube onto PDA.

Effect of temperature on growth. Glucose was incorporated into potato broth

Table 1. Extent of fruit decay caused by three isolates of Epicoccum nigrum

Test fruit	Area of decay (mm) per isolate source		
	Cucumis melo (ATCC 66091)	Pisum sativum (ATCC 34929)	Pennisetum flaccidum
Tomato	14.3 a ^z	8.0 b	11.5 ab
Pear	6.4 b	3.0 b	3.4 b
Cucumber	3.3 b	4.5 a	4.0 ab
Cantaloupe	2.4 a	1.0 b	3.3 a
Apple	1.2 a	1.2 a	0.6 b

External and internal sporulation of the fungus. Means followed by the same letter across columns are not significantly different (P = 0.05) according to Duncan's multiple range test.

(200 g/L of potato) by adding 20 g of glucose and 20 g of agar to make PDA. Approximately 10 ml of agar was poured into standard 90-mm-diameter petri dishes. Two studies of 10 replicates of cantaloupe isolate ATCC 66091 were incubated at 1, 5, 10, 15, 20, 25, or 30 C. Colony radial growth was measured daily for 6 days.

RESULTS AND DISCUSSION

Koch's postulates and host range. Symptoms of red rot developed in inoculated cantaloupe fruit after 10 days and were essentially identical to those in naturally infected melons. However, pigmentation of the epidermis seen on naturally infected melons was not normally observed on inoculated melons. The decaved area was similar in texture to that of healthy tissue. The rot did not have a dry-rot appearance and was not soft. The decayed tissue did not separate easily from the surrounding tissue. Lesions measured from 2.3 to 8.6 mm, but there was no significant difference among the isolates. Pure cultures of E. nigrum were subsequently reisolated from the red rot lesions, fulfilling Koch's postulates. Because the decay and growth characteristics were similar for all six cantaloupe isolates, isolate ATCC 66091 was selected for further studies.

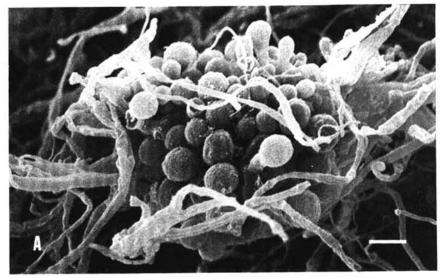
Inoculations demonstrated that isolates of E. nigrum from Pisum sativum and Pennisetum flaccidum were very similar if not identical to the cantaloupe isolate (Table 1). Although there was a significant difference (P = 0.05) between some isolates on a particular fruit, there was no consistency. The decay in a particular fruit was similar among the isolates and ranged from yellowish to orange to red. The decayed tissue was always firm and did not separate easily from surrounding healthy tissue. Tomato had substantially more decayed area than the other test fruit, and cantaloupe ranked next to last in amount of decay.

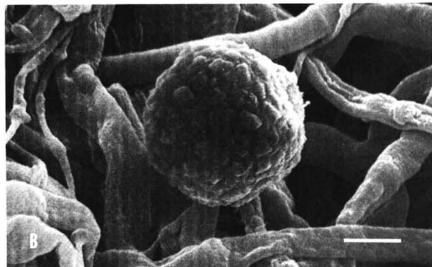
Identification of the causal organism. Sporulation of cantaloupe isolate ATCC 66091 was sparse on PDA. The production of sporodochia (Fig. 1A) and conidia (Fig. 1B) was facilitated by allowing Riddell slide mounts to desiccate or by transferring the fungus from PDA to water agar. The aggregations of conidiogenous cells and regions of conidial detachment were prominent (Fig. 1C), typical of E. nigrum (2). Inoculation of tomato fruit resulted in extensive sporulation of the fungus on the epidermis and within the locules. On PDA, colonies were initially yellow, changing to red. Sporulation was observed within 25-30 days at 22-25 C. Cultures of the fungus remained viable in sterile potting mix for at least 4 yr.

Effect of temperature on growth. Radial growth for cantaloupe isolate 66091 of *E. nigrum* occurred between 1 and 30 C, with an optimum at 20 C (Fig. 2).

Little growth occurred at 1, 5, and 30 C. Cantaloupe fruit are typically shipped and stored at temperatures from 3 to 6 C. Proper storage temperatures may contribute greatly to restricting the development and expansion of lesions on the fruit.

Epicoccum spp. are primarily saprophytes or opportunistic plant pathogens on several hosts, including Cucurbita





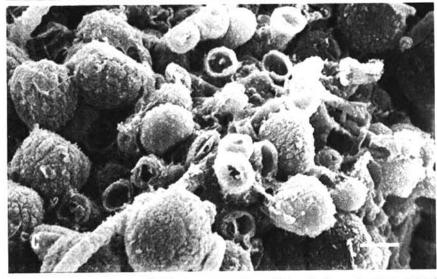


Fig. 1. Epicoccum nigrum: (A) Pulvinate sporodochium with terminally produced monoblastic conidia at several stages of development. Scale bar = $10 \mu m$. (B) Verrucose outer wall of mature conidium. Scale bar = $5 \mu m$. (C) Aggregations of conidiogenous cells after conidial liberation. Scale bar = $5 \mu m$.

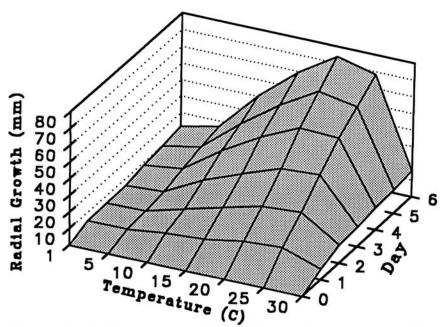


Fig. 2. Growth of *Epicoccum nigrum* (ATCC 66091) on potato-dextrose agar at different temperatures.

maxima Duchesne (1,4). The ubiquitous nature of E. nigrum as well as its biology and physiology have been extensively reviewed (4). On cantaloupe, the fungus appears to be a weak parasite, similar to Alternaria spp. and Cladosporium spp. (6,8). When red rot is observed, Alternaria and Cladosporium rot are also generally present.

There is no evidence that disease spreads from fruit to fruit through contact. Red rot disease of cantaloupe has been observed only after harvest and is associated with melons approaching the

end of their shelf life or those stored for 10-14 days. Lesions rarely extend beyond 3 cm in diameter and 2 cm into the rind. As the lesions approach a diameter of 2-3 cm, they often become sunken.

It is possible that the postharvest decay caused by *E. nigrum* can be confused with decays caused by certain *Fusarium* spp. (6,8). Several *Fusarium* spp. produce a red to purple pigmentation in the affected tissue with no pigmentation of the epidermis. Conversely, red rot does impart a visible red pigmentation to the

epidermis. Lesions caused by the Fusarium spp. tend to be much larger than those of red rot, and the advancing lesion typically has a prominent white halo around its perimeter internally. In addition, Fusarium rot lesions are easily separated from the surrounding healthy tissue, whereas red rot does not separate easily. E. nigrum has not been previously reported on Cucumis spp. (5). A proposed common name for the disease is red rot.

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