Growth, Sporulation, and Mucilage Production by *Ceratocystis fagacearum* at High Temperatures

F. H. TAINTER, Professor, Department of Forestry, Clemson University, Clemson, SC 29634-1003

ABSTRACT

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Ceratocystis fagacearum was grown at high temperatures and observed for growth and morphology. Germination of conidia of a South Carolina isolate was significantly greater at 32 than at 24 or 28 C, whereas linear growth rate of germ tubes at 32 C was less than 50% of the growth rate at 24 and 28 C. Scanning electron microscopy revealed profuse mucilage and conidia produced in colonies of South Carolina and Texas isolates at 24 C, whereas at 28 C, there were decreased mucilage and fewer conidia. At 32 C, there was little hyphal growth, few conidia, and virtually no mucilage. Similar behavior of South Carolina and Texas isolates within oak trees at high temperatures may help explain the slow progress or arrested symptoms of oak wilt caused by these isolates in nature.

Oak wilt, caused by *Ceratocystis fagacearum* (Bretz) Hunt, has caused serious losses (3) in the central and eastern United States, yet has not been discovered in forests of the extreme southeastern states where susceptible species of the red oak group (*Quercus*; subgenus *Erythrobalanus*) are abundant.

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South Carolina is on the southeastern periphery of the known range of oak wilt (7), but *C. fagacearum* has not had a significant impact on the state's oak timber resources. Artificially inoculated turkey oaks (*Q. laevis* Walt.; *Erythrobalanus*) were not quickly killed by *C. fagacearum* (9). Some even recovered, and the fungus could not be isolated from them 808 days after inoculation. Tainter and Ham (9) speculated that high summer temperatures may debilitate *C. fagacearum*, making it unable to initiate a severe enough wilt reaction to quickly kill the trees.

Because high temperatures have a negative effect on growth and viability of *C. fagacearum* (1,2,4,6), this research was undertaken to detect the effects of high temperatures on growth, morphology, and spore production of *C. fagacearum* in culture. Temperature-induced abnormalities might be a basis for apparent reduced virulence of the pathogen and survival of diseased trees in natural infections in South Carolina. A preliminary report has been published (8).

MATERIALS AND METHODS

The South Carolina isolate of *C. fagacearum* (SC-Camden) was obtained from a naturally infected turkey oak in Camden, SC. The Texas isolate (TX-Live Oak) from a wilting live oak (*Q. virginiana* Mill.) was provided by Robert Lewis (USDA Forest Service). Both cultures are maintained in the Clemson University, Department of Forestry, Mycology Research Culture Collection.

To detect the effects of temperature on germination and germ tube growth of the SC-Camden isolate, petri plates containing 3.9% potato-dextrose agar (Difco) were seeded with a conidial suspension prepared from a 10-day-old culture incubated at 24 C. The seeded plates were incubated at 24, 28, and 32 C and sampled at 0, 12, 24, and 36 hr. Four to six plugs (7 mm square) of agar were removed from

plates of each treatment and exposed to vapors of 1% osmium tetroxide for 36–48 hr, then air-dried for 24 hr, coated with gold, and viewed with an ETEC-Autoscan scanning electron microscope. Representative micrographs were made and percent germination and germ tube lengths measured from micrographs randomly selected from the entire collection, three per treatment. Only planar views were photographed. A 24-hr incubation period was selected because germ tubes were too short to measure at 12 hr and too long and intertwined to measure at 36 hr. Statistical analysis was done following an angular transformation of the percentage data.

Cultures derived from hyphal tips of both isolates were grown at 24 C for 7 days on agar plates prepared as described, then transferred to 24, 28, and 32 C, respectively, for an additional 3 days. Squares of agar with undisturbed mycelium were removed from the area of the colony adjoining the original inoculum plug and from the area of sparse and appressed mycelium that grew during the third day at the second temperature. Samples were prepared and viewed as described. This entire process was repeated three times with the SC-Camden isolate and once with the TX-Live Oak isolate. Observations of morphological differences were made and representative micrographs prepared.

RESULTS

Germination rates of 83% at 24 C and 79% at 28 C were not significantly different from each other but were significantly lower than the 92% rate that occurred at 32 C (Table 1). Conversely, 24 hr after germination, mean germ tube lengths of 25 μm at 24 C and 29 μm at 28 C were not significantly different but were significantly greater than the 12 μm at 32 C (Table 1).

The two isolates of *C. fagacearum* grew similarly in culture. Cultures maintained at 24 C grew at a steady rate across the agar surface during the entire 10-day incubation period (Fig. 1). Growth slowed abruptly after the cultures were placed at the higher temperatures. A narrow ring of extremely appressed hyphal growth was evident during the first 24 hr at the higher temperature (Fig. 1). During the subsequent 48 hr, growth recovered somewhat and aerial hyphae increased (more at 28 than at 32 C). Hyphae on the colony edge were sinuate and appeared identical at all temperatures. Cultures maintained at 24 C showed no change in colony growth between days 7 and 10.

Abundant aggregations of mucilagelike material were observed around the inoculum plug in cultures maintained at 24 C (Fig. 2A). Higher magnification revealed that mucilage aggregations also often contained large numbers of conidia (Fig. 2B). Mucilage was copious and conidia were abundant in both isolates.

In cultures transferred to 28 C, scanning electron microscopy revealed less mucilage (Fig. 2C,D) than in cultures incubated at 24 C, and fewer conidia were present in the mucilage.

In cultures transferred to 32 C, very little mucilage was evident (Fig. 2E,F), and the few conidia present were shrunken and deformed. Some plates of the TX-Live Oak isolate had more mucilage and conidia present at 32 C than did the SC-Camden isolate, but no attempt was made to quantify the difference because it was noticeably smaller than differences between temperature treatments.

Table 1. Percentage germination and germ tube length at 24 hr of the SC-Camden isolate of *Ceratocystis fagacearum* incubated at different temperatures

Temperature (C)	Mean germination (%)	No. measured	Germ Tube length (μm)	No. measured
24	83 a*[z]	147	25 c**	16
28	79 a*	166	29 c**	13
32	92 b*	267	12 d**	21

[z] Means within columns not followed by the same letter differ significantly at * = $P = 0.05$ and ** = $P = 0.01$.

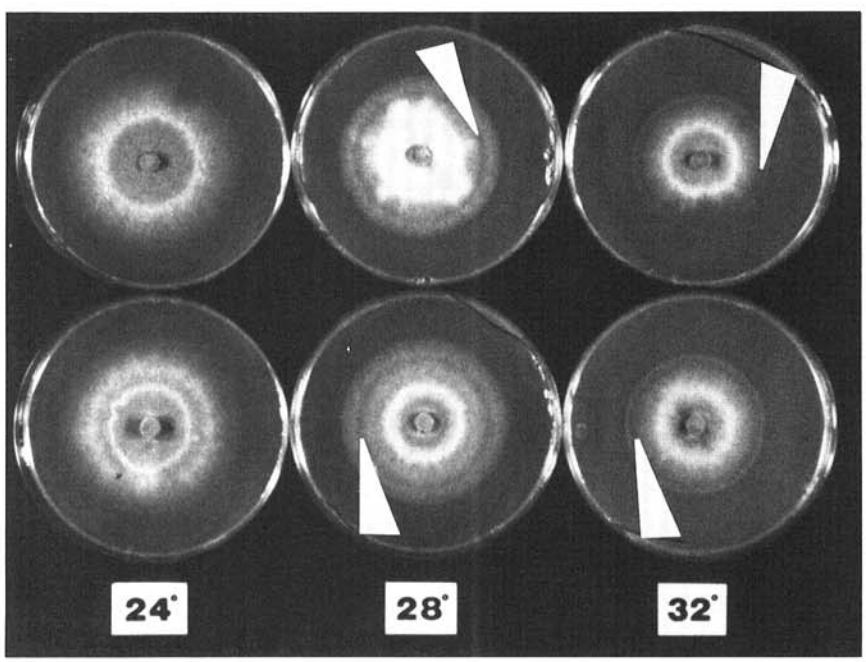

Fig. 1. Cultures of SC-Camden isolate of *Ceratocystis fagacearum* grown at 24 C for 7 days, then for an additional 3 days at 24, 28, and 32 C, respectively. Arrows mark the zones resulting from change in temperature.

DISCUSSION

Previous work by W. Witcher (*personal communication*) showed that his South Carolina isolates did not significantly differ in growth rate from northern isolates. F. H. Tainter and T. A. Lomax (*unpublished*) found the SC-Camden isolate used in the present study as effective as northern isolates in causing wilt and death of artificially inoculated live oak and scarlet oaks (*Q. coccinea* Muenschh.).

In infected northern pin oak (*Q. ellipsoidalis* E. J. Hill) (2,10), northern red oak (*Q. rubra* L.) (5), and chestnut oak (*Q. prinus* L.) (5), the fungus was distributed rapidly and generally throughout the tree. Production of fewer conidia during early stages of infection could lessen the deleterious effect on the host. The relatively few conidia produced would presumably be at a survival disadvantage at higher temperatures. Fergus (2) observed that after 170 hr, spores of *C. fagacearum* kept at 32 C had contents that were granular and appeared plasmolyzed. Exposure at 36 C for 34 hr and at 40 C for 24 hr killed all conidia. In the present study, the SC-Camden isolate had a higher rate of germination at 32 C, but germ tube elongation was much less than at lower temperatures.

This research shows that high temperatures affect the amount of mucilage, conidia, and mycelium produced by *C. fagacearum* in culture. The net effect is that the pathogen develops slowly. In nature, this may allow an enhanced effectiveness of host resistance responses, partially explaining why red oaks in South Carolina and some live oaks in Texas recover from infection.

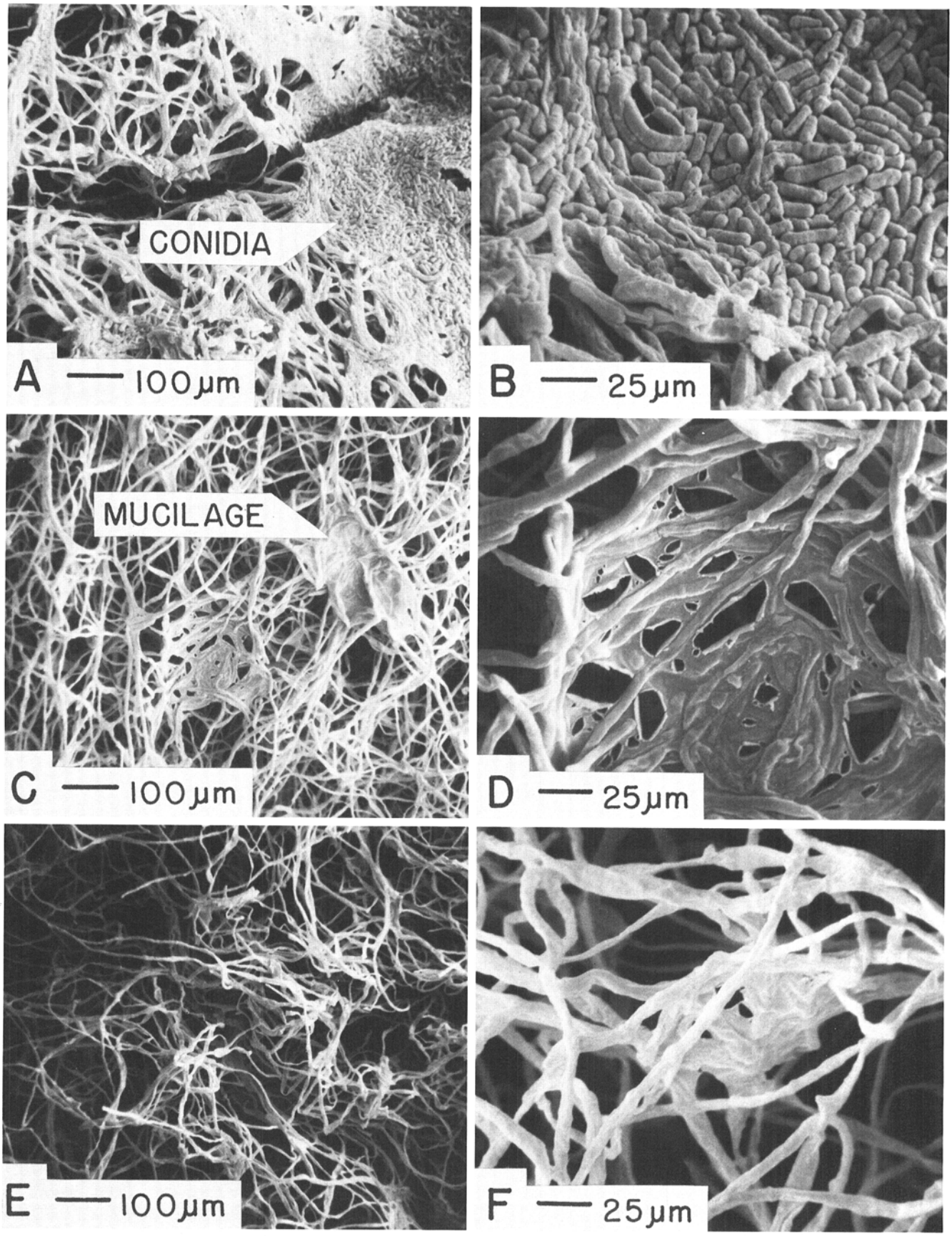

Fig. 2. Scanning electron micrographs of SC-Camden isolate of *Ceratocystis fagacearum* grown at **(A and B)** 24 C, **(C and D)** 28 C, and **(E and F)** 32 C. Note the decrease of mucilage and conidia at temperatures higher than 24 C.

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