Resistance of Strawberry Plants to *Colletotrichum fragariae* Affected by Environmental Conditions

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ABSTRACT

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Strawberry (*Fragariae* × *ananassa*) resistance to *Colletotrichum fragariae*, the causal agent of anthracnose crown rot (a new name proposed for this disease), is influenced by environmental conditions after inoculation. Strawberry plants were inoculated by spraying with conidial suspensions of two *C. fragariae* isolates, and subsequent disease development was rated on a severity scale of 0 (no symptoms) to 6 (plant dead). Disease severity ratings of plants inoculated with three concentrations of *C. fragariae* conidia ranging from 1.5×10^6 to 6.0×10^6 were not significantly different. Overall, plants incubated at a high temperature (35 C) for 48 hr in a dew chamber (relative humidity near 100%) had higher disease severity ratings than comparable plants incubated at 25 or 30 C. In two of three cultivars tested, inoculated plants maintained in a greenhouse at 32 C after the dew chamber incubation period developed more severe disease symptoms than similar plants held in a greenhouse at 25 C.

Colletotrichum fragariae Brooks, the causal agent of strawberry anthracnose, may attack any of the aboveground parts of the strawberry (*Fragaria* × *ananassa* Duch.) (2,3,7–9) but is most devastating when it invades the crown and causes a wilt and sudden death of the plant (7,11). To distinguish the disease caused by *C. fragariae* from anthracnose diseases caused by other *Colletotrichum* spp. (13), the name "anthracnose crown rot" is proposed. The crown rot phase of the disease is most severe during warm, humid conditions and frequently causes damage in both fruit production fields and summer nurseries in the Gulf Coast states and in summer nurseries in other southeastern states (11).

The importance of high temperature (> 25 C) and humidity (relative humidity [RH] near 100%) for disease development in strawberry plants infected with *C. fragariae* is well documented in the field (2,7) and greenhouse (2,4,7,18). Incubation in an atmosphere near 100% RH for at least 48 hr immediately after inoculation is necessary for consistent disease development (2,4,14,18). No reports were found on the influence of temperature during this initial incubation period on disease development. Delp and Milholland (4) suggested that an inoculum density of 10^6 conidia per milliliter and a greenhouse temperature of 25 C after removal of plants from the moisture chamber were optimal conditions for evaluating strawberry plant resistance based on petiole lesion size. Their results showed that plants held at a greenhouse temperature of 25 C or higher developed more severe petiole symptoms than plants held at 20 C or less but that, at a greenhouse temperature of 30 C, the resistance of the plants with which they were working was overcome.

The research reported in this paper was designed to define the inoculation conditions necessary to reliably assess resistance in strawberry plants to both petiole and crown infection by *C. fragariae*. The effects of inoculum concentration, dew chamber temperature, and greenhouse temperature on infection of three cultivars by two isolates of *C. fragariae* were evaluated.

MATERIALS AND METHODS

Cultivars. Three strawberry cultivars with different reported reactions to anthracnose (Sequoia [resistant], Cardinal [unknown], and Tioga [very susceptible]) were tested for their disease reactions to two isolates of *C. fragariae*. Plants were purchased as dormant crowns from a commercial nursery, potted in 10-cm plastic pots in a 1:1 (v/v) mixture of Jiffy-Mix (JPA, West Chicago, IL) and pasteurized sand, and grown for a minimum of 6 wk before inoculation. The plants were grown in a greenhouse at about 28 C with supplemental light to achieve a 16-hr photoperiod. Each pot received 0.25 g of Osmocote (14-14-14) (Sierra Chemical Co., Milpitas, CA) slow-release fertilizer every 8 wk beginning 6 wk after potting. Older leaves, runners, and flowers were removed 1–7 days before inoculation, and three or four young leaves remained on each plant at inoculation time.

Inoculum. *C. fragariae* isolates CF-1 (obtained from N. L. Horn, Louisiana) and CG-164 (obtained from C. M. Howard, Florida) were grown on a 1:1 (v/v) mixture of Difco oatmeal agar and Difco potato-dextrose agar in petri dishes for 7–14 days at room temperature (about 25 C) under continuous fluorescent

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light. Conidia were washed from cultures with distilled water containing two drops of Tween 20 per liter, and the desired conidial concentration was obtained by dilution. The inoculum was sprayed uniformly over the foliage of the plants with a hand-pump sprayer to the point of runoff. Immediately after inoculation, the plants were placed in a dew chamber to maintain free water on the foliage during a 48-hr incubation period, then transferred to the greenhouse for the remainder of the study.

Dew chamber construction. The dew chamber consisted of a 0.6-m^3 (1.4 m long × 0.6 m wide × 0.7 m high) black polyethylene enclosure placed inside an unlighted growth chamber. The thermostat controlling the growth chamber was placed inside the dew chamber to provide a constantly regulated air temperature. Water in a shallow pan in the bottom of the dew chamber was maintained at 5–7 C higher than the air temperature with an immersion heater.

Disease severity rating (DSR) scale. Disease development was rated 10, 20, 30, and 50 days after inoculation on a DSR scale modified from that of Delp and Milholland (4) to include a category that recognizes symptoms of crown infection in live plants. Rating categories were: 0 = healthy plant with no visible lesions; 1 = plant with petiole lesions < 3 mm long; 2 = plant with petiole lesions 3–10 mm long; 3 = plant with petiole lesions > 10–20 mm long, usually girdling the petiole; 4 = plant with petiole lesions > 20 mm long to entire petiole necrotic; 5 = plant whose youngest leaf was wilted, indicating a crown infection with or without petiole lesions; and 6 = dead plant with necrotic crown. Plants with a DSR ≤ 2 30 days after inoculation were considered resistant, those with a DSR of 2.1–3.9 were considered intermediate, and those with a DSR ≥ 4 were considered susceptible to anthracnose crown rot.

Inoculation conditions. Plants of the three cultivars were tested for their disease reactions to two isolates of *C. fragariae* under various postinoculation environmental conditions. Four plants of each cultivar were used in each treatment. Five concentrations of inoculum (0, 7.5×10^5, 1.5×10^6, 3.0×10^6, and 6.0×10^6 conidia per milliliter) and three dew chamber temperatures (25, 30, and 35 C) during the first 48 hr after inoculation were tested for their effects on subsequent disease development. The effect of environmental temperature after dew chamber incubation was tested by maintaining the inoculated plants in greenhouses at temperatures of 25 ± 3 C and 32 ± 3 C.

Data analysis. The SAS statistical package (6) was used to analyze data. Mean separation was by Duncan's multiple range test, least significant difference, or the Waller-Duncan test.

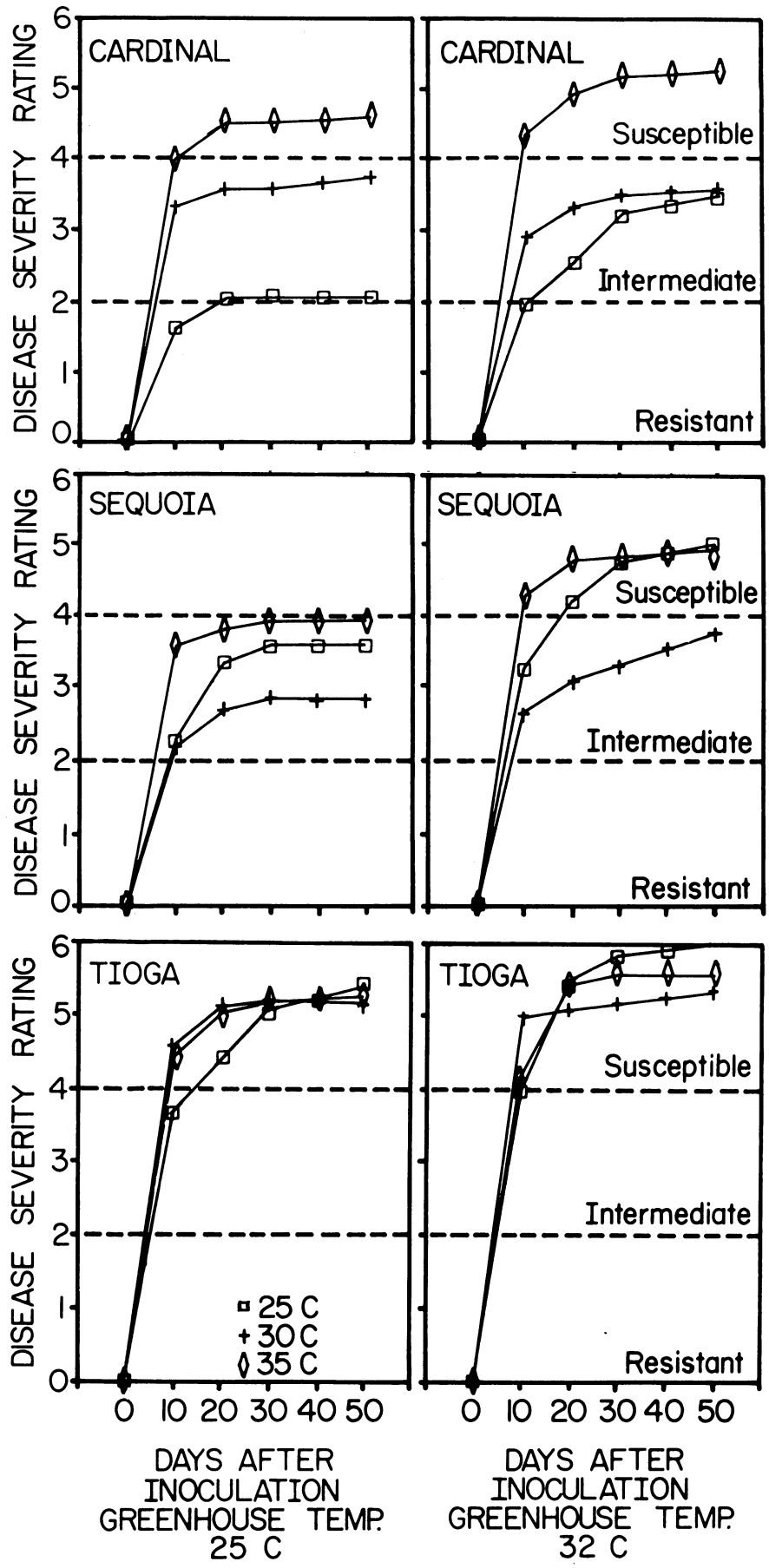

Fig. 1. Disease response curves of three strawberry cultivars to plant spray inoculation with *Colletotrichum fragariae* isolate CG-164. Plants held in a dew chamber at 25, 30, or 35 C for 48 hr after inoculation, then transferred to a greenhouse and held at 25 or 32 C for 50 days. Disease severity rating ≤ 2 = resistant, 2.1–3.9 = intermediate, and ≥ 4 = susceptible.

RESULTS

Plant response to each isolate was similar. (Data presented in Figure 1 are for isolate CG-164 only.) On all three cultivars inoculated with either isolate, petiole lesion size increased steadily and rapidly during the first 20 days after inoculation, gradually during the next 10 days, and very slightly if at all during the next 20 days (Fig. 1). During the first 10 days, lesion development was most rapid on the very susceptible cultivar, Tioga, and slowest on Cardinal incubated in the dew chamber and greenhouse at 25 C. Thirty days after inoculation was considered the best time to make disease severity assessments, and all subsequent data are reported at 30 days after inoculation.

There were no significant differences in the overall DSRs after inoculation with the three higher inoculum levels of isolate CF-1 or with all four inoculum levels of isolate CG-164 (Fig. 2). Therefore, data from the three higher inoculum concentrations were combined to give a total of 12 plants per treatment per isolate in the dew chamber and greenhouse studies.

At the day-30 rating, there was a significant interaction between dew chamber temperatures and cultivar responses, indicating that dew chamber temperature affected cultivars differently. Dew chamber temperature had no effect on the disease response of Tioga plants, which were rated susceptible (DSR of 4–6) to both *C. fragariae* isolates at all three dew chamber temperatures (Fig. 1). DSRs of Cardinal plants inoculated with each fungal isolate increased as dew chamber temperatures increased, resulting in an intermediate disease response at 25 and 30 C and a susceptible response at 35 C. Surprisingly, the DSRs of Sequoia plants were significantly lower for both fungal isolates on the plants held in the dew chamber at the middle (30 C) temperature than on plants held in the dew chamber at either lower (25 C) or higher (35 C) temperatures. The DSRs on Sequoia plants held in dew chambers at 25 or 35 C after inoculation with either isolate of the fungus were not significantly different.

Inoculated plants of each of the three cultivars held in the greenhouse at 32 C had higher DSRs than similar plants held at 25 C (Fig. 1); however, the DSR of Tioga inoculated with CG-164 was not significantly different at 25 and 32 C. No significant interaction occurred between greenhouse temperature and cultivar response.

When DSRs were averaged over all treatment variables, the DSR of the Tioga plants was significantly higher than those of Sequoia or Cardinal. Both *C. fragariae* isolates responded similarly to all variables; however, isolate CG-164 caused a slightly higher DSR on plants in most treatments and a higher overall DSR than isolate CF-1.

DISCUSSION

We propose that the common name of the disease caused by *C. fragariae* be changed from anthracnose to anthracnose crown rot. This common name will better describe the disease as it is seen in hot, humid environments, where it is of the most economic importance, and will reduce confusion of this disease with other fungal diseases of strawberry. When Brooks (2) first described *C. fragariae* and named the disease anthracnose , the primary symptoms were black, sunken lesions on the runners. Later, wilt and sudden death of plants were reported in summer nurseries in Florida (3) and in fruiting fields in Louisiana (7), with both reports referring to this phase of the disease as crown rot. Crown rot, however, has been used to refer to at least two strawberry diseases caused by other fungi, *Phytophthora cactorum* (Leb. & Cohn) Schroet. (15) and *P. nicotianae* B. de Haan var. *parasitica* (Dastur) Waterh. (13). Anthracnose diseases of strawberry are also caused by several fungi other than *C. fragariae*. These include *C. acutatum* Simmonds (16,17), *C. dematium* (Pers. ex Fr.) Grove (1), *Glomerella cingulata* (Stonem.) Spauld. & Schrenk (10), and *Gloeosporium* spp. (12,19,20).

Inoculum concentrations ranging from 1.5×10^6 to 6.0×10^6 conidia per milliliter of *C. fragariae* were equally effective for evaluating anthracnose crown rot resistance in strawberry plants. Concentrations of fewer than 10^6 conidia per milliliter may allow some plants to escape infection (4).

Dew chamber temperature during the initial 48-hr incubation period and the greenhouse temperature at which inoculated plants were held subsequently had significant effects on DSRs. Plants incubated at a dew chamber temperature of 35 C were generally more susceptible than comparable plants held at either 25 or 30 C, but the level of effect of dew chamber temperature differed among cultivars. For example, the DSR for Cardinal was almost doubled when the plants were incubated in the dew chamber at 35 C rather than at 25 C, changing this cultivar's response from intermediate (nearly resistant) to very susceptible. On the other hand, Tioga was susceptible at all three dew chamber temperatures. The anthracnose crown rot response of Sequoia ranged from intermediate to susceptible. Sequoia plants held at the lowest (25 C) and the highest (35 C) dew chamber temperatures had higher DSRs than those held at the middle (30 C) dew chamber temperature. Strawberry plants held in a greenhouse at 32 C after inoculation developed significantly more severe anthracnose crown rot symptoms than those held at 25 C; the only exception was Tioga plants inoculated with isolate CG-164, which was equally severe at both temperatures.

Environmental conditions play an important role in the development of anthracnose crown rot. Controlled, defined environmental conditions are mandatory for evaluating plant responses to anthracnose crown rot. The independent way in which cultivars in the current study responded to different dew chamber temperatures, i.e., the temperature and high humidity during the initial stages of infection, may explain some of the variation in disease severity reported for certain cultivars at different locations (5,13). All three cultivars in this study were susceptible to both isolates tested when incubated initially in the dew

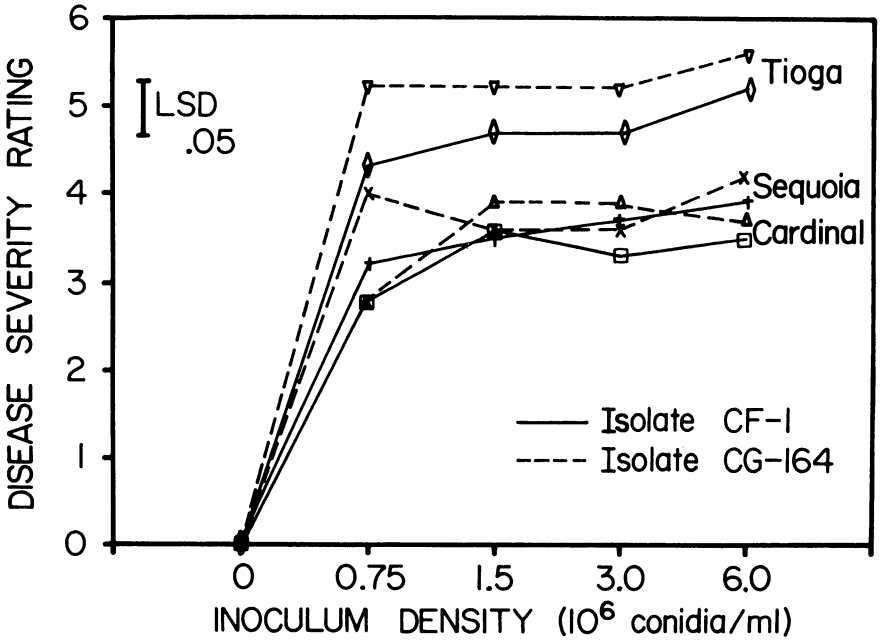

Fig. 2. Disease response curves of three strawberry cultivars to plant spray inoculation with various inoculum densities of two *Colletotrichum fragariae* isolates. Values are means of four replicates, six plants per replicate. Disease severity rating scale: 0 = no symptoms to 6 = plant dead.

chamber at 35 C and held in the greenhouse at 32 C after inoculation. Studies at some locations have shown Sequoia to be resistant to anthracnose crown rot (5,13); however, greenhouse (17) and field studies (*unpublished*) conducted at Poplarville during 1984 showed Sequoia to be susceptible at this location.

The 30-yr average maximum temperature for the 5-mo period of May through September at the South Mississippi Branch Experiment Station, Poplarville, is 32 C (experiment station weather records). Because anthracnose crown rot is most severe in the field during hot summer months in the southeastern United States, evaluation of resistance at the higher temperatures would be more likely to result in the selection of resistance that would persist under field conditions in this region. Similar inoculation studies with other cultivars and breeding clones have shown that there are plants available that express resistance at the higher temperature (17).

To identify levels of resistance to anthracnose crown rot in strawberry plants that are likely to be useful in the Gulf Coast states, inoculum levels and environmental conditions conducive to severe disease development should be used. Results of this study suggest that 1) the inoculum level should be 10^6 conidia per milliliter or greater; 2) plants should be held at 30–35 C in a chamber with 100% RH for 48 hr after inoculation; 3) after removal from the humidity chamber, the plants should be maintained at 32 C during the remainder of the study; and 4) disease assessment should be made about 30 days after inoculation.

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