Characterization of Growth and Sclerotial Production of *Sclerotinia minor* Isolated from Peanut in Texas

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ABSTRACT

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Five isolates of *Sclerotinia minor* (Behrens, Charlotte, Dirk, Eppler, and TAES#1) were collected from diseased peanut (*Arachis hypogaea*) plants obtained from four commercial peanut production areas in Texas. The isolates were grown on potato-dextrose agar (PDA), cornmeal agar (CMA), and Czapek-Dox agar (CDA) in a controlled environment at temperatures ranging from 0 to 36 C in 2-degree increments. The optimum temperature for radial mycelial growth (RMG) of each isolate varied with the growth medium. Optimum RMG occurred at 24–26 C on PDA for the five isolates and at 18–22 C on CMA and CDA. RMG of all isolates on PDA was about double that on CMA or CDA at the optimum temperature and in the mid-temperature range. Optimum sclerotial production on PDA occurred at 20–24 C for all five isolates, with a high of 1,219 sclerotia of Eppler per 9-cm culture plate at 24 C. The maximum sclerotial production on CMA was 68 per 9-cm plate for Eppler at 26 C; the other isolates produced their maximum numbers of sclerotia at 18 or 22 C. The maximum sclerotial production on CDA was 212 per 9-cm plate for Dirk at 20 C; sclerotial production peaked in the 18–20 C range in three of the isolates but at 26 C in the other two isolates. The maximum weight of sclerotia produced on PDA per 9-cm petri dish occurred in isolates grown at 22 C for all isolates except Behrens, which reached its maximum sclerotial weight at 28 C. The maximum weight of sclerotia on CMA occurred at different temperatures in the 18–28 C range for all isolates. For four of the five isolates, the maximum sclerotial weight on CDA occurred in the 6–12 C range. Sclerotia produced on any medium at temperatures of 14–26 C generally had 50–100% myceliogenic germination on water agar at 21 C.

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* Jagger, was first reported in the United States in Virginia in 1971 and North Carolina in 1972 (8). This disease was also reported in Oklahoma in 1972 (10). Symptoms of the disease were found in a peanut field in Mason County, Texas, in the fall of 1981. *S. minor* was isolated from and identified in diseased peanut pods and stems. Since that time, Sclerotinia blight of peanut has been observed in 10 commercial peanut-producing counties in Texas and is now considered to be a major soilborne disease of peanut in the state. *Sclerotinia* spp. are known to infect susceptible hosts over a range of temperatures from 0 to 25 C, with an optimum at 15–20 C (2); however, optimum temperatures for growth and production of sclerotia by a Virginia peanut isolate have been reported to be 20–25 C in vitro and in field culture (4). The average soil temperature in Texas peanut fields during most of the growing season is well above 15–20 C and above 25 C for much of this time. Field observations in central and south central Texas peanut-growing areas indicate that *S. minor* is very active at soil temperatures near 25 C.

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The objective of this study was to characterize and compare mycelial growth and sclerotial production, over a range of temperatures and on different growth substrates, of isolates of *S. minor* from every peanut production area of Texas where Sclerotinia blight has been reported. A preliminary account of this work has been published (14).

MATERIALS AND METHODS

Isolates. *S. minor* isolates were obtained by culturing sclerotia collected from diseased peanut pods and stems. The isolates used were Charlotte (Atascosa County), Dirk (Comanche County), TAES#1 (Erath County), and Behrens and Eppler (Mason County). The isolates are named for the vicinity or producer from which the organisms were obtained and represent the major areas of Texas where Sclerotinia blight of peanut occurs. Isolates were selected from representative cultures from each geographical area and were maintained on potato-dextrose agar (PDA) (Difco) throughout the study.

Growth rates. Growth rate studies were conducted on PDA, cornmeal agar (CMA) (Difco), and Czapek-Dox agar (CDA) (Difco) in a controlled environment chamber (Freas 818, GCA/Precision Scientific) at temperatures ranging from 0 to 36 C in 2-degree increments. Isolates were maintained on PDA and then transferred to petri plates (9 cm in diameter) containing 25 ml of fresh PDA, in which they were grown at 21 C. When mycelial growth approached the edge of a plate, usually after 2–3 days, a cork borer (0.5 cm in diameter) was used to cut disks from the edge of the colony. A single disk of an individual isolate was placed (mycelia side down) in the center of a 9-cm petri dish containing either PDA, CMA, or CDA. There were five replications of each isolate for each growth medium at each temperature. The treatment plates were incubated for 120 hr in continuous darkness, except for a brief period every 24 hr when they were removed from the chamber so that mycelial growth could be marked. A wax pencil was used to outline the edge of mycelial growth at 24-hr intervals. After 120 hr, the plates were moved to a laboratory bench for an additional growth period of 7 days. Growth during each 24-hr period was recorded as one-half the mean of two perpendicular diameters minus one-half the diameter of the inoculum plug (0.25 cm). The plates were arranged in a completely randomized design in the controlled environment chamber. The growth rate study was conducted over a period of 18 wk, with a new temperature increment beginning on the same day of each week.

Production of sclerotia. After 7 days of growth at ambient temperature (20–21 C), sclerotia were counted in each culture plate. Only sclerotia maturing within the growth area attained in the incubator (during the first 120 hr) were counted. Total sclerotial production was counted on plates containing fewer than 100 sclerotia. For plates with more than 100 sclerotia, a template marked with a 1.0-cm^2 grid was placed over the plate, one-fourth of the plate production was counted, and the result was multiplied by four.

Weight of sclerotia. After the sclerotia were counted, the cultures were dried at ambient lab temperature for 4 wk. By this time sclerotia were dry enough to pop free from the surface with no agar clinging to them. They were then collected and weighed to the nearest 0.1 mg. The average sclerotium weight was calculated from the weight of 100 sclerotia or, for plates producing fewer than 100 sclerotia, from the weight of the total sclerotial production divided by the number produced. Sclerotial weight per 9-cm plate was determined by multiplying the average sclerotium weight by the number of sclerotia produced in each plate.

Viability of sclerotia. Sclerotia produced at each temperature were tested for viability 4 wk after being weighed. They remained at ambient temperature for the period between postincubation and viability testing. Sclerotia were surface-disinfected for 5 min in 0.5% sodium hypochlorite, blotted dry on sterile paper towels, and plated in 9-cm petri dishes containing 2.0% water agar. Five sclerotia were spaced equidistantly, 1.0 cm from the edge of a petri dish. There were four replications per treatment. Test plates were completely randomized and incubated for 10–14 days at 26 C and in 24-hr darkness. After the incubation period, germinated sclerotia were counted. The test was repeated over a range of temperatures in 4-degree increments, with similar results for corresponding temperatures.

RESULTS

Radial mycelial growth. Means for radial mycelial growth (RMG) of five Texas peanut isolates of *S. minor* on PDA, CMA, and CDA, recorded over a range of temperatures from 2 to 34 C in 2-degree increments, are presented in Fig. 1. There was no growth at either 0 or 36 C. RMG ranged from 0.3 cm in 48 hr on all three media to 4.3 cm in 48 hr on PDA (4.3 cm was the highest RMG in this test). On PDA, optimum RMG occurred at 26 C for Charlotte (3.5 cm) and at 24 C for the other four isolates (Behrens, 4.1 cm; Dirk, 3.3 cm; Eppler and TAES#1, 4.3 cm). On CMA, optimum RMG occurred at 18 C for Behrens (1.9 cm), 20 C for Dirk (1.9 cm), and 22 C for Charlotte (1.7 cm), Eppler (1.9 cm), and TAES#1 (2.0 cm). On CDA, optimum RMG occurred at 22 C for TAES#1 (1.6 cm) but at 20 C for the other isolates (Behrens, 1.8 cm; Charlotte, 1.7 cm; Dirk and Eppler, 1.6 cm).

Production of sclerotia. The number of sclerotia per 9-cm plate on all media ranged from 0 to a maximum of 1,219, produced by Eppler at 24 C on PDA (Fig. 2). On PDA, sclerotia were produced in the temperature range of 4–30 C, but none were produced at 32 or 34 C. Maximal sclerotial production on PDA occurred at 20 C for Charlotte (1,007 sclerotia) and Dirk (1,084), 22 C for Behrens (1,145) and TAES#1 (1,031), and 24 C for Eppler (1,219). On CMA, sclerotia were produced at temperatures from 4 to 32 C, but none were produced by any isolate at 34 C (Fig. 2). The number of sclerotia on CMA ranged from 0 to a maximum of 68 per 9-cm plate for Eppler at 26 C. Maximal sclerotial production on CMA occurred in the temperature range of 18–22 C for the other isolates. On CDA, sclerotial production occurred at all temperatures from 4 to 34 C (Fig. 2). The number of sclerotia on CDA ranged from 0 to a maximum of 212 per 9-cm plate for Dirk at 20 C. Maximal production occurred at temperatures in the 18–20 C range for all isolates except Charlotte and TAES#1, which peaked at 26 C.

Weight of sclerotia. On PDA, sclerotial weight peaked at 22 C for all isolates except Behrens; the range was 0–377 mg per 9-cm plate, with Charlotte grown at 22 C producing the greatest weight (Fig. 3). Charlotte also had the highest sclerotial weight on CMA, with 46 mg per 9-cm plate at 22 C (Fig. 3). Each isolate produced a maximum weight at a different temperature, and only Charlotte was significantly different ($P = 0.05$) from the other isolates over the entire temperature range on CMA. On CDA, Behrens at 6 C had the highest sclerotial weight of all isolates, with 76 mg per 9-cm plate (Fig. 3). Four of the five isolates produced maximum sclerotial weight at the lower end of the temperature scale.

Viability of sclerotia. Germination of sclerotia produced at the extreme temperatures of the test range was highly

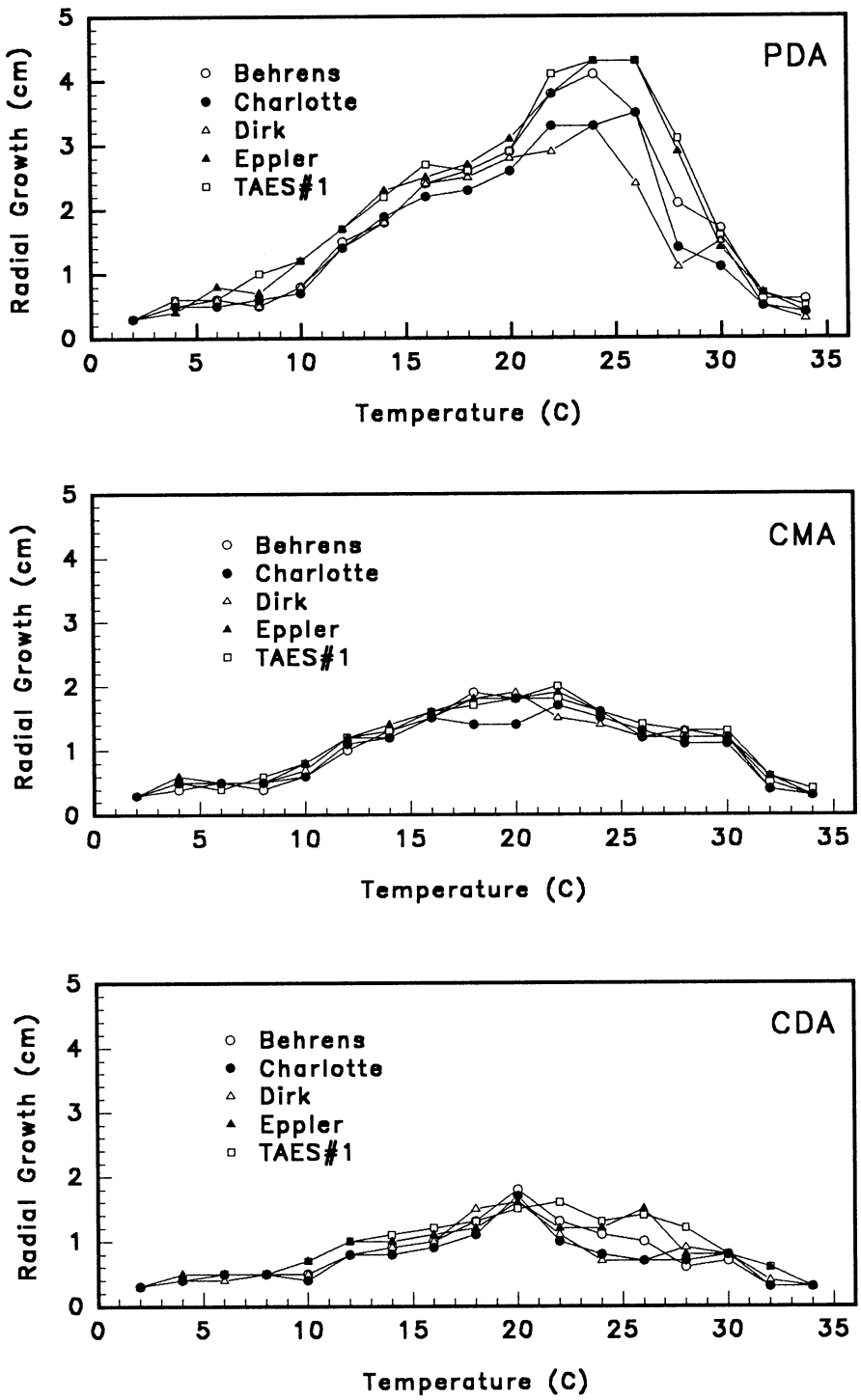

Fig. 1. Radial mycelial growth of five peanut isolates of *Sclerotinia minor* after 48 hr on potato-dextrose agar (PDA), cornmeal agar (CMA), and Czapek-Dox agar (CDA) at temperatures ranging from 2–34 C in 2-degree increments.

variable on PDA, CMA, and CDA. Generally, between 50 and 100% of sclerotia produced by all isolates in the temperature range of 14–26 C germinated on all three media (data not shown).

DISCUSSION

At optimum RMG for all the isolates of *S. minor* on PDA, CMA, and CDA, a randomly arranged pattern (11) of sclerotia was observed. The small, irregularly shaped, black sclerotia presented a striking contrast against a solid white mycelial background. Although the isolates were morphologically indistinguishable, results from this study demonstrated physiological differences in isolates of *S. minor* as well as isolate-by-medium and isolate-by-medium-by-temperature interactions. The cardinal temperatures were 2, 24–26, and 34 C on PDA. These results correspond closely to cardinal temperatures reported earlier for isolates of *S. minor*: 3, 22, and 35 C by Goidanich (5) and 0, 20–25, and 30 C by Keay (7). They also correspond to the optimum of 22–25 C and maximum of 30 C reported by Sereni (9). More recently, Dow et al (4), working with *S. minor* isolates from horsenettle (*Solanum carolinense* L.), soybean (*Glycine max* (L.) Merr.), and peanut, reported mycelial growth after 3 days on PDA to be optimum at 20–25 C for all isolates. Imolehin et al (6) found isolates of *S. minor* from lettuce (*Lactuca sativa* L.) in New York and California to have optimum growth on PDA after 48 hr at 12–24 C, but after 72 hr optimum growth of all isolates occurred at 18 C. Optimum sclerotial formation was at 12 C (6). Willetts and Wong (12) reported optimum growth and sclerotial formation of *S. sclerotiorum, S. trifoliorum,* and *S. minor* at 15–22 C. This is a lower range for optimum RMG than we found but is near the range we reported for sclerotial production.

Wong (13) reported a range of 1,000–3,000 sclerotia per plate on PDA and an average dried sclerotium weight of 0.05 mg. Our optimum sclerotial production on PDA was in this range, but our average dried sclerotium weight was much higher for PDA, at about 0.6 mg (data not shown). This difference may have resulted from different drying methods; however, differences between isolates cannot be discounted.

Sclerotia produced on PDA, CMA, or CDA in this test, at all except the low and high temperatures of the range, generally germinated at rates greater than 50% on water agar at 21 C. Coley-Smith and Cooke (3) referred to three types of sclerotial germination: myceliogenic, sporogenic, and carpogenic. We observed only myceliogenic germination. Two types of myceliogenic germination, hyphal and eruptive, have been described (1). No data were recorded on the mode of germination in our experiment, but hyphal germination of sclerotia appeared to dominate, although both types were observed.

Mycelial growth of *S. minor* on all media in this study was limited above 30 C, although it peaked at 24–26 C on PDA. The average daily soil temperature at a depth of 5 cm in Texas peanut fields is normally above 28 C from late spring until peanut foliage laps the row middle (unpublished data). If the in vitro data from this test correlates with the peanut field environment, manipulating such parameters as date of planting, irrigation schedule, and choice of plant architecture (upright instead of runner) can affect soil temperature and perhaps aid in controlling Sclerotinia blight. Imolehin et al (6) reported sclerotial germination and mycelial growth of *S. minor* on PDA to be optimum at 18 C, as was infection of lettuce by hyphae from germinating sclerotia. A peanut isolate of *S. minor* in Virginia had optimum RMG at 20–25

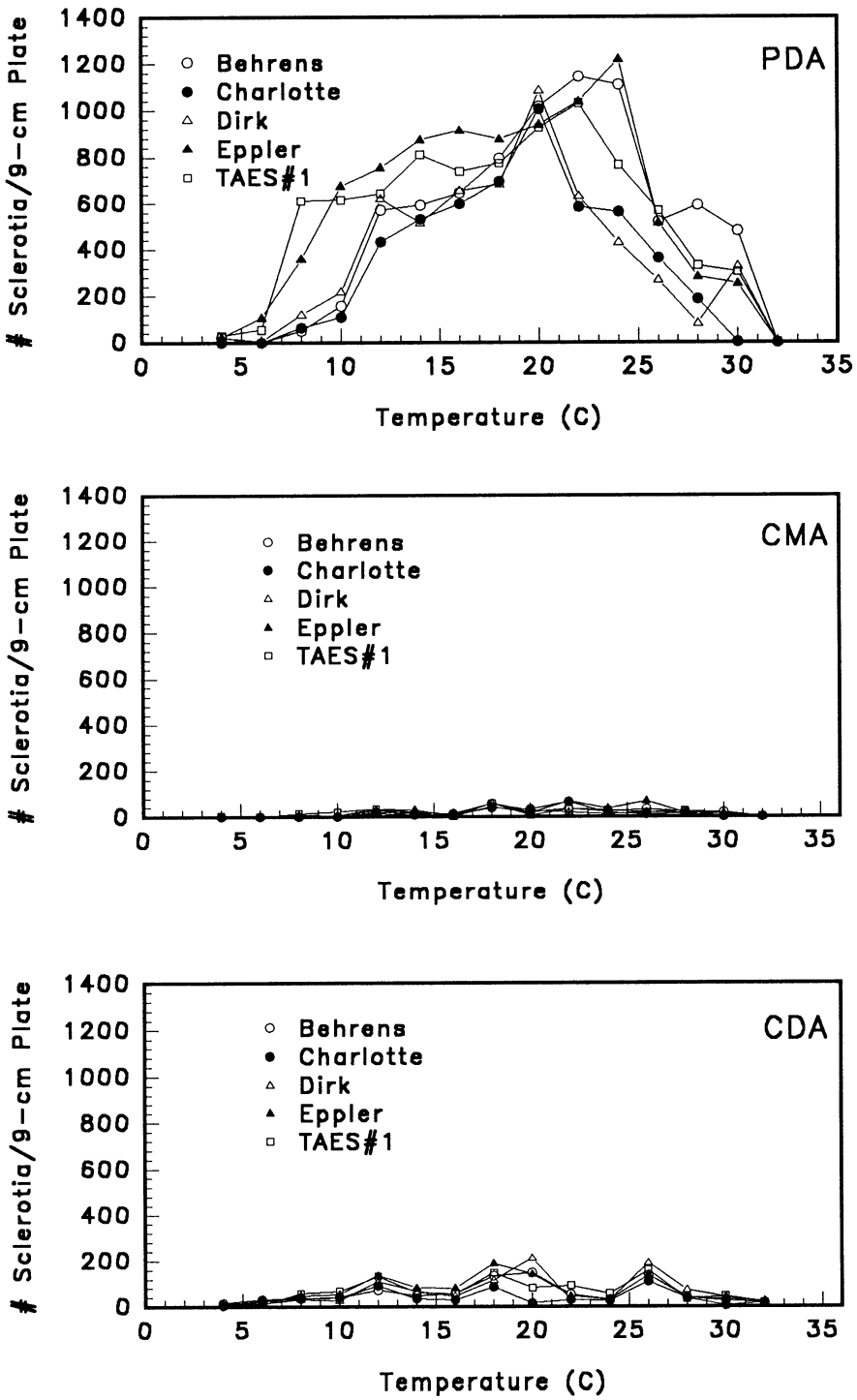

Fig. 2. Number of sclerotia produced by five isolates of *Sclerotinia minor* on potato-dextrose agar (PDA), cornmeal agar (CMA), and Czapek-Dox agar (CDA) for 120 hr at temperatures ranging from 4–32 C in 2-degree increments.

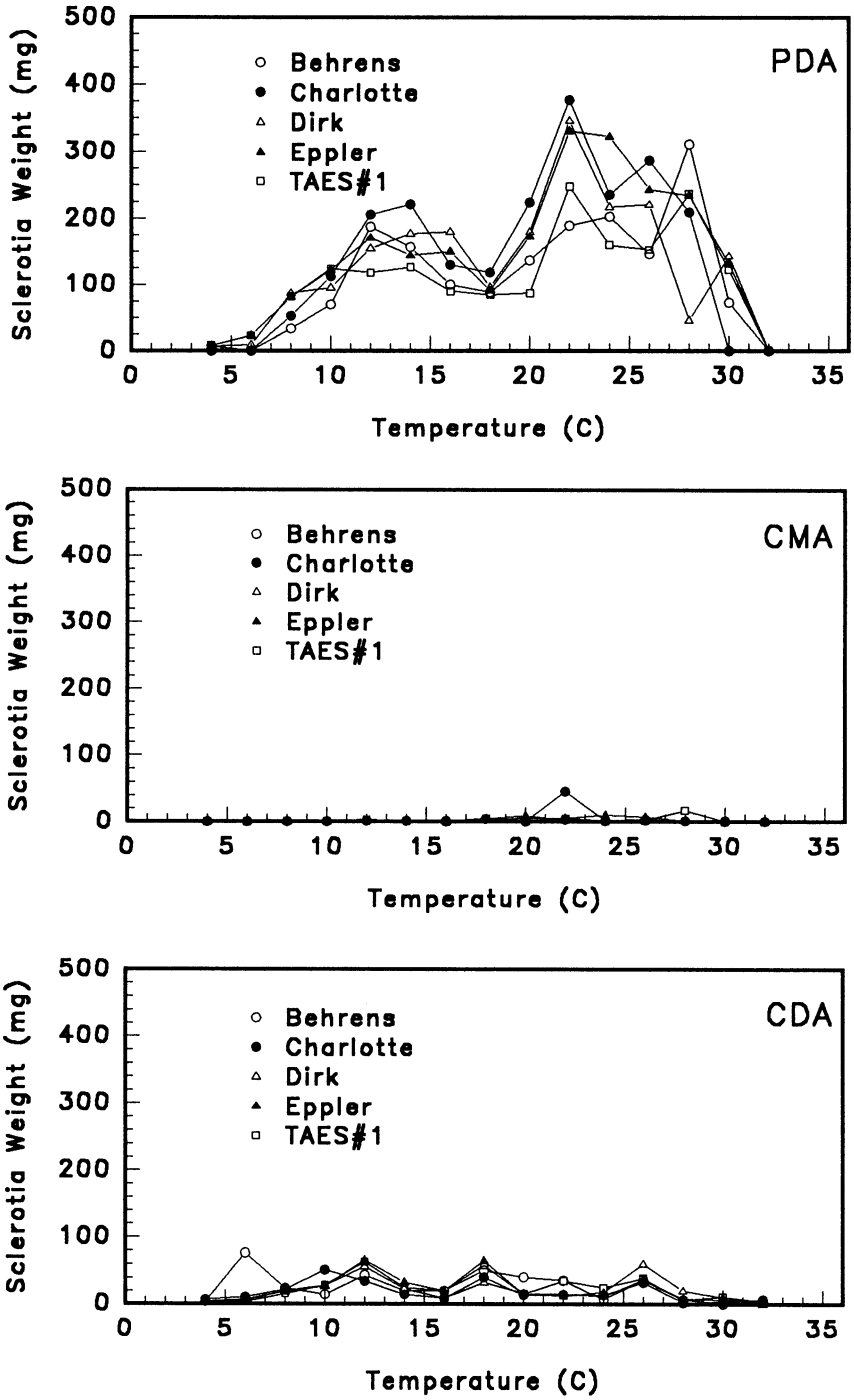

Fig. 3. Weight of sclerotia produced per 9-cm petri dish by five isolates of *Sclerotinia minor* on potato-dextrose agar (PDA), cornmeal agar (CMA), and Czapek-Dox agar (CDA) at temperatures ranging from 4–32 C in 2-degree increments.

C on PDA, with the optimum temperature for infection and colonization of peanut tissue being the same (4).

Differences in the growth of *S. minor* isolates on PDA, CMA, and CDA suggest that modification of the field substrate may affect the growth and reproduction of the organism. For example, incorporation of sucrose (the carbon source in CDA) into *S. minor*–infested soil or rotating corn (*Zea mays* L.) after *S. minor*–infested peanuts may alter the growth and reproduction of the pathogen.

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