Comparison of North Carolina, Georgia, and Florida Isolates of *Cylindrocladium parasiticum*

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ABSTRACT

Thirteen isolates of *Cylindrocladium parasiticum* Crous, Wingfield & Alfenas from North Carolina and 11 from Georgia were grown on plates of PDA at 20, 25, and 30 C on a temperature-gradient plate. Culture diameters were measured daily for 6 d. Significant differences were not observed among isolates grown at 20 C and cultures was smaller as compared with those grown at 25 and 30 C. At 25 and 30 C, isolates varied consistently in growth. On average, Georgia isolates grew slightly less than North Carolina isolates, and all isolates grew better at the warmer temperature. Growth of three North Carolina isolates was compared to four Florida isolates in a second experiment. Florida isolates grew significantly faster than North Carolina isolates at all temperatures. Isolates from the three states were compared for their ability to cause root rotting on peanut at 25 and 30 C. Plants were grown in soil infested at a standardized inoculum density in temperature-controlled water bath tanks for 7 wk at which time roots were rated for Cylindrocladium black rot development. Georgia isolates caused more root rot than either North Carolina or Florida isolates at both temperatures and also caused more seedling disease. State effects were significant; Florida isolates caused less root rot than Georgia isolates. Temperature by state interactions were not significant which means that high temperature-tolerant isolates of *C. parasiticum* have not evolved from regional differences in soil temperature. Further, North Carolina field isolates do not appear to have changed in temperature optima since the 1970s.

Key Words: *Arachis hypogaea, Calonectria ilicicola, Cylindrocladium crotalariae*, groundnut.

Cylindrocladium parasiticum Crous, Wingfield & Alfenas infects peanut (*Arachis hypogaea* L.) roots, pegs, and pods, causing chlorosis and ultimately killing peanut plants. Although Cylindrocladium black rot (CBR) of peanut was first observed in Georgia in the early 1960s (Bell and Sobers, 1966), epidemics since the 1970s have been the most serious in North Carolina and Virginia where the disease often became a limiting factor in peanut production.

Regional differences in disease occurrence and progress of epidemics caused by *C. parasiticum* may be related to differences in cultivars, climate, and cultural practices which affect soil moisture or temperature early in the growing season (Sidebottom and Beute, 1989), or physiology of isolates. These factors may have prevented or delayed increases in *C. parasiticum* populations to damaging levels in Georgia and Florida soils. Previous studies have shown that the optimum temperature for vegetative growth of the fungus is between 26 and 28 C (Bell and Sobers, 1966).

In earlier studies with Georgia isolates, Bell (1967) showed plant damage increased as the soil temperature increased from 15 to 25 C; at higher temperatures, damage did not change significantly up to 40 C. With North Carolina isolates, essentially no growth occurred above 35 C, with optimum growth at 26 to 28 C (Rowe and Beute, 1975a). More root infection and rot occurred after 9 wk at 25 C and with a soil moisture near field capacity than in cooler or drier soils and no measurable disease developed at 35 C (Phipps and Beute, 1977). These results were supported by contemporary observations of disease distribution and led to the conclusion that severe outbreaks of CBR would be limited to cooler production regions. However, CBR incidence, severity, and damage have increased over time in Georgia and Florida (Kucharek and Atkins, 1993; Padgett *et al.*, 1995; Kucharek *et al.*, 1996). The increasing prevalence of CBR in the more southern production regions of the U.S. suggests that some pathogen populations may have been selected for greater growth or aggressiveness in warmer soils.

The potential for high temperature adaptation by *C. parasiticum* also could be of importance in North

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Carolina and Virginia because disease could progress more rapidly if infection and disease development occurs in warmer soils instead of being primarily restricted to the cooler soil temperatures in spring (Sidebottom and Beute, 1989). This study was initiated to determine whether isolates of *C. parasiticum* from different production regions differ in temperature optima for mycelial growth in culture or colonization of roots in the greenhouse.

Materials and Methods

Cultures. Cultures of *C. parasiticum* were obtained from North Carolina, Georgia, and Florida in 1996 from isolates collected in the field from 1989 to 1994 (Table 1). Most cultures were originally isolated from peanut. Isolates from Georgia and Florida received from collaborators were obtained from various tissues (Dr. T. Brenneman and Dr. R. Hoover, pers. communication). All North Carolina isolates were obtained by placing 1.0-cm^2 pieces of lightly washed pods onto semiselective medium (Phipps *et al.*, 1976). Fungal identifications were made after 5 d growth under lights. Isolates were transferred to potato dextrose agar (PDA) where they were maintained at 26 C until needed for growth studies on a temperature-gradient plate (Benson, 1982) and pathogenicity tests on plants growing at various soil temperatures. All isolates were of similar age except isolate 26 from Florida which was 5 yr older.

Table 1. Origin of *Cylindrocladium parasiticum* isolates from North Carolina, Georgia, and Florida.[a]

Isolate	State	Plant[b]	Plant part	Year isolated
1*	NC	Peanut	Pod	1994
2*	NC	Peanut	Pod	1994
3*	NC	Peanut	Pod	1994
4	NC	Peanut	Pod	1994
5*	NC	Peanut	Pod	1994
10	NC	Peanut	Pod	1994
11*	NC	Peanut	Pod	1994
12	NC	Peanut	Pod	1994
18	NC	Peanut	Pod	1994
19	NC	Peanut	Pod	1994
20	NC	Peanut	Pod	1994
21	NC	Peanut	Pod	1994
22*	NC	Peanut	Pod	1994
6*	GA	Beggarweed	Root	1993
7*	GA	Beggarweed	Root	1994
8*	GA	Peanut	?	1994
9	GA	Peanut	?	1994
13	GA	Soybean	Root	1994
14*	GA	Peanut	?	1994
15*	GA	Peanut	?	1993
16	GA	Peanut	?	1993
17*	GA	Peanut	?	1993
23	GA	Peanut	?	1993
24	GA	Peanut	?	1993
25	GA	Sicklepod	Root	1994
26*	FLA	Peanut	Stem	1989
27*	FLA	Peanut	Stem	1993
28*	FLA	Peanut	Stem	1993
29*	FLA	Peanut	Pod	1993

[a]All isolates were tested for mycelial growth, but isolate 17 was dropped from analysis because of contamination. Isolates with * were tested for pathogenicity.

[b]Peanut = *Arachis hypogaea* L., beggarweed = *Desmodium tortuosum* (Sw.) DC., soybean = *Glycine max* (L.) Merr, sicklepod = *Senna obtusifolia* (L.) Irwin & Barneby.

Growth in Agar Culture. Agar plugs (4 mm dia.) from PDA cultures of actively growing isolates of *C. parasiticum* were transferred to the center of 60-mm plates containing PDA at 20, 25, and 30 C on a temperature-gradient plate. Due to the size of the test and restrictions of the gradient plate, the test was divided into two parts. Thirteen North Carolina and 11 Georgia isolates were compared in the first test. Culture diameters were measured daily for 6 d. The isolates were divided into subsets, with three incomplete blocks per replicate, and were randomized within temperature whole plots. The experiment was conducted two times, and the combined data sets were analyzed.

A second test was conducted to compare three North Carolina isolates with four Florida isolates. Culture diameters were only taken for 5 d because growth reached the edge of plates more quickly for both groups of isolates. The experimental design was a split-plot with temperature as whole plots and four replicates in time.

Isolate Comparison In Vivo. Sixteen isolates of *C. parasiticum* previously compared for growth in culture were compared for their ability to cause root disease. Six isolates each from North Carolina and Georgia, and four from Florida were individually compared in soil at constant 25 and 30 C.

Isolates were grown on PDA for 6 wk in the dark. Cultures of each isolate were macerated in a blender and the suspension washed on nested 35- and 200-mesh sieves having openings of 500 and 75 µm, respectively. Microsclerotia on the 200-mesh sieve were quantified and a standard inoculum density of 5 microsclerotia/g soil was used to uniformly infest a pasteurized mixture of two parts sandy loam soil:one part sand (Black and Beute, 1984). Six-inch-diam. plastic pots fitted with drain tubes were placed in temperature-controlled water baths and each filled with 1.8 kg infested soil. Each pot was planted with two pregerminated seeds of NC 7 treated with Vitavax PC (45% captan, 15% PCNB, 10% carboxin; Gustafson, Inc., Dallas, TX) and watered by hand as needed for the next 7 wk. The experiment was arranged in a completely randomized split-plot design with temperatures as whole plots, isolates as subplots, and three replications. The experiment was repeated and the statistical analysis was conducted on pooled data.

Plants that died before the end of the experiment were removed from the pots and plated on semiselective medium (Phipps *et al.*, 1976) to confirm that *C. parasiticum* was responsible for disease. At the end of 7 wk, all remaining plants were removed from soil and roots were rinsed and rated on a scale of 0-5 where 0 = healthy and 5 = rotted (Rowe and Beute, 1975b). These roots were not plated because visual observation showed evidence of decay by *C. parasiticum*.

Data Analysis. In all experiments, state and temperature effects were considered fixed and isolate ef-

fects random in the ANOVA models. The analyses on the data from the *in vitro* experiments were conducted using the SAS (Cary, NC) proc mixed procedure. The analysis for the *in vivo* experiments was conducted using the SAS general linear model procedure for mixed models (Steel *et al.*, 1997).

Results and Discussion

Growth in Agar Culture. For all isolates, colony diameter was measured until the entire agar surface in the Petri plate was covered at 5 or 6 d at 30 C. Means for colony diameter of all isolates across three temperatures showed an increase with time (Fig. 1). Mean colony diameter for North Carolina isolates was not significantly different from diameter for Georgia isolates on each day ($P > 0.70$). Mean colony diameter for Florida isolates was greater than for North Carolina isolates ($P \leq 0.01$). All isolates grew slower at 20 C as compared to 30 C (Fig. 2). Isolates within North Carolina and Georgia varied significantly ($P = 0.005$; Fig. 3A), but there were no significant differences in temperature responses of growth between geographic sources. As a group, Georgia isolates grew slightly less rapidly than North Carolina isolates at 25 and 30 C (Fig. 2A); generally all isolates grew faster at the warmer temperature. This was contrary to our original expectation that Georgia isolates would grow faster at the higher temperature. Increasing CBR incidence in Georgia does not appear to be related to high temperature adaptation in the vegetative growth rate of the isolates tested. Differences in virulence or aggressiveness, inoculum buildup, or the susceptibility of cultivars are other possibilities for increased disease incidence in Georgia.

Colony diameters of North Carolina and Florida isolates were compared at 4 d (Fig. 1B). Florida isolates grew faster than North Carolina isolates at all temperatures ($P \leq 0.01$; Fig. 2B). There were no significant isolate within state effects (Fig. 3B).

***Isolate Comparison* In Vivo.** *Cylindrocladium parasiticum* was isolated from all plants that died in less than 7 wk. Isolations were obtained from seed, fibrous roots, and tap roots of these dead plants. Most seedling disease (45 of 66 dead plants) was caused by Georgia isolates. Although seed transmission of *C. parasiticum* has been reported at low rates (Randall-Schadel *et al.*, 1993), seedling disease in these experiments differed among isolate sources. This suggests that infections originated from the inoculum placed in soil because the seeds came from the same source in all treatments.

State main effects on root rot rating were significant at $P = 0.06$; Georgia isolates always caused the greatest root rot, and Florida isolates the least (Table 2). The contrast of Georgia vs. Florida isolates was significant at $P = 0.03$. Georgia isolates tended to cause more root rot than North Carolina isolates at both temperatures, but the contrast was not significant (Table 2). There was significant variability in the pathogenicity of isolates from the same state ($P = 0.02$). On the average, the root rot rating at 25 C was nearly double that at 30 C; the temperature

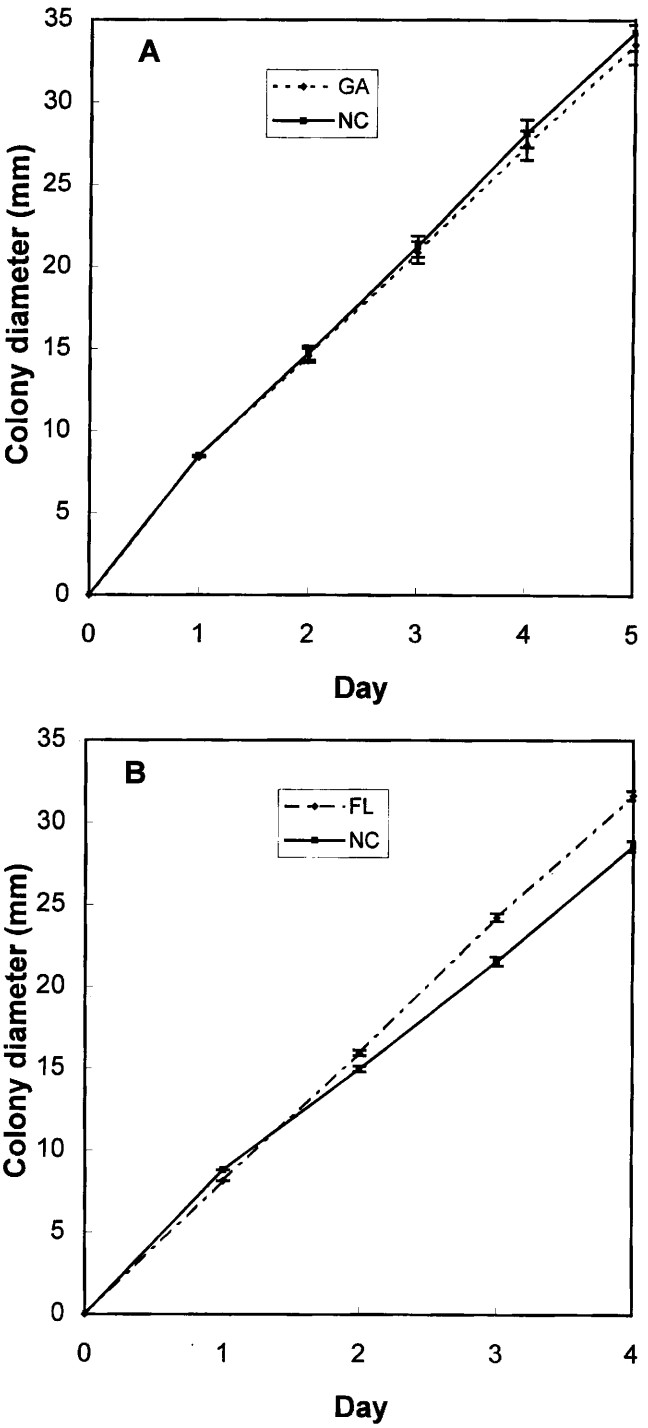

Fig. 1. Colony diameter (mm) increase with time for isolates of *Cylindrocladium parasiticum* on potato dextrose agar. Points represent means for A) 11 Georgia isolates compared to 13 North Carolina isolates, and B) four Florida isolates compared to three North Carolina isolates (2, 3, and 4) across three temperatures. Differences between means on each day were not significant for North Carolina and Georgia isolates ($P > 0.70$). Means for North Carolina and Florida isolates were significantly different on days three and four. Bars indicate standard errors.

main effect was significant at $P = 0.02$. Temperature-by-state interactions were not significant, indicating that state differences were consistent at both tem-

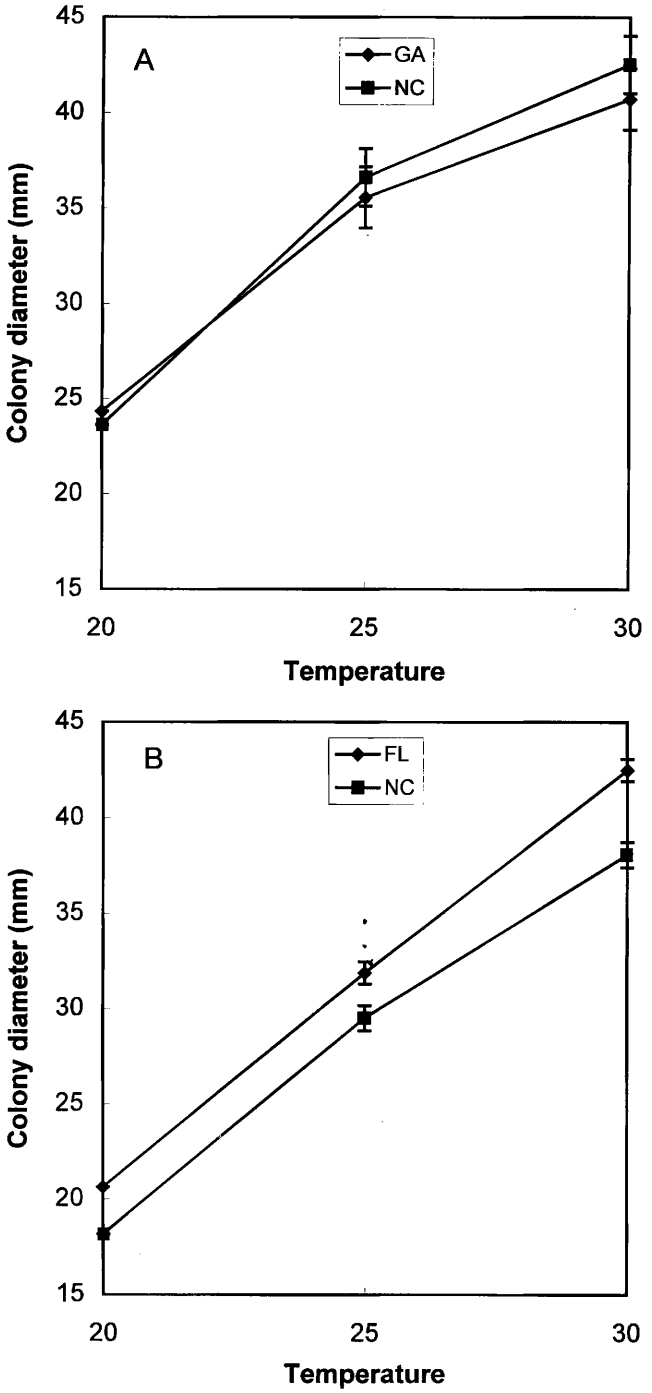

Fig. 2. Mean diameters of isolates of *Cylindrocladium parasiticum* grown 5 or 6 d on potato dextrose agar at three temperatures. A) Mean colony diameters for 13 North Carolina and 11 Georgia isolates were not significantly different, and B) mean diameters of four Florida isolates were significantly greater ($P \leq 0.01$) than mean diameters of three North Carolina isolates (isolates 2, 3, and 4). Bars indicate standard errors.

peratures. Root weight data corresponded with the root disease ratings (data not shown).

Previous research in North Carolina indicated that 25 C was most conducive for development of CBR on plant roots (Phipps and Beute,1977). North Carolina field isolates did not appear to have changed in tem-

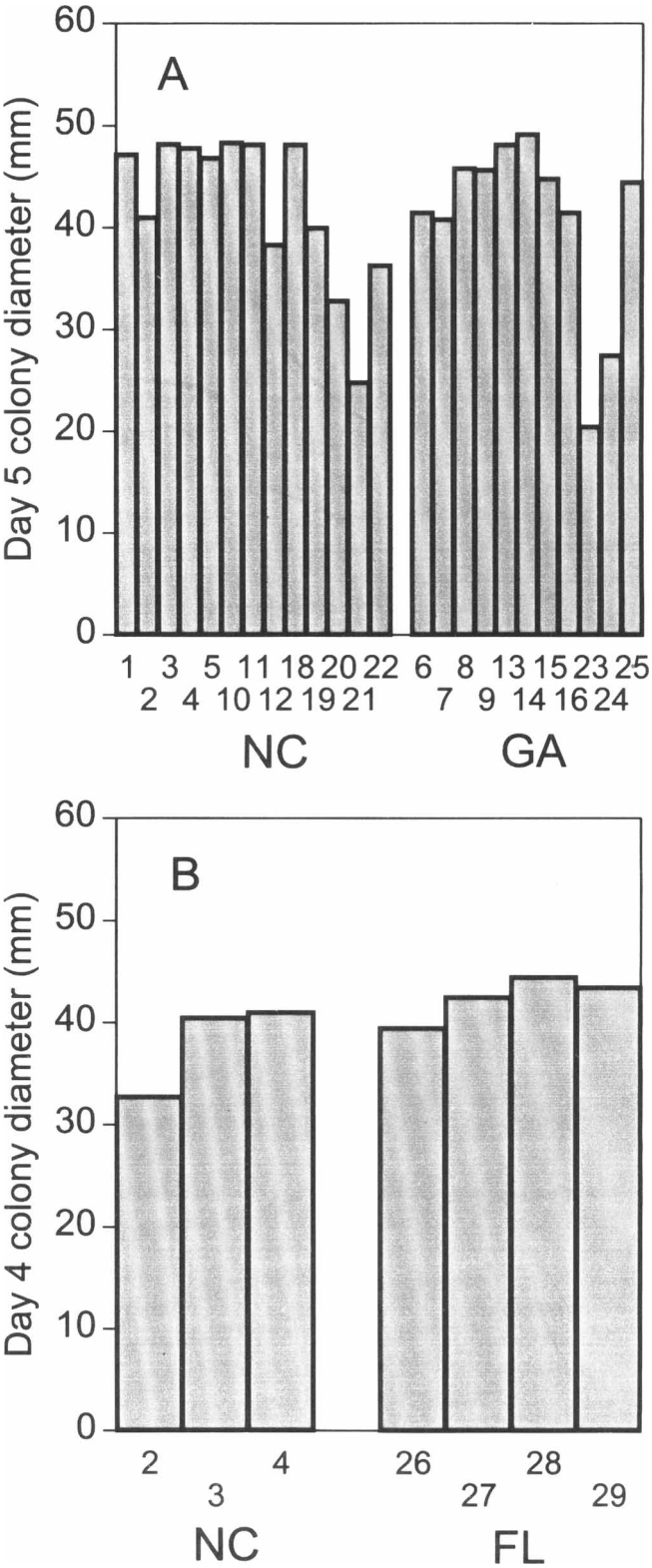

Fig. 3. Mean colony diameters (mm) of A) 13 North Carolina isolates and 11 Georgia isolates or B) three North Carolina isolates and four Florida isolates of *Cylindrocladium parasiticum* grown on potato dextrose agar at 30 C.

perature optima since the 1970s; results with our field isolates were very similar to those of Phipps and Beute (1977). Similarly, Georgia and Florida isolates

Table 2. Mean root rot rating of NC 7 peanut grown at constant soil temperature of 25 or 30 C in soil infested with isolates of *Cylindrocladium parasiticum* from different states[a].

State	Soil temperature		Mean
	25 C	30 C	C
GA	3.9[b]	2.2	3.1[c]
NC	2.5	1.4	1.9
FL	1.9	0.7	1.3
Mean	2.8	1.4	2.1

[a]Test included six isolates from Georgia (GA), six from North Carolina (NC), and four from Florida (FL).

[b]Roots were rated on a scale of 0 = no visible lesions; 1 = few lesions on secondary roots and/or a few small lesions on the taproot; 3 = many lesions on secondary roots, many lesions on the taproot, and with several secondary roots missing; 5 = completely rotted roots; and 2 and 4 = intermediate levels of severity.

[c]Significance levels: Temperature main effect, P = 0.02; state main effect, P = 0.06; NC vs. GA contrast, P = 0.09; GA vs. FL contrast, P = 0.03; NC vs. FL contrast, P = 0.32.

did not appear to show different temperature optima for pathogenicity in soil than North Carolina isolates. The more vigorous growth of Florida compared to North Carolina isolates in culture did not appear to increase pathogenicity *in vivo* in a warmer soil temperature. As previously described by Phipps and Beute (1977), soil temperature lower than 30 C was more favorable for disease development than the higher temperature. The reason for increased expression of CBR in the Southeast remains unknown, but may include an increase in inoculum densities in soil, the increased susceptibility of new cultivars now grown, the possibility of seed transmission, an increase in irrigation, and/or an increase inoculum dispersal by farm equipment traffic from field to field.

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