New Sources of Cylindrocladium Black Rot Resistance among Runner-Type Peanut Cultivars

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ABSTRACT

Cylindrocladium Black Rot (CBR) caused by *Cylindrocladium parasiticum* Crous, Wingfield, & Alfenas syn. *C. crotalariae* (Loos) Bell & Sobers is a major disease problem in southeast U.S. peanut (*Arachis hypogaea* L.) production. Field trials were conducted for two-years (2008-09) at a test site (Gibbs Farm) that has a long history of continuous peanut production near the Univ. of Georgia, Coastal Plain Expt. Station, Tifton, GA to evaluate for CBR resistance among new runner-type peanut cultivars. All plots were artificially inoculated with microsclerotia of *C. parasiticum* after planting each year. Significant differences ($P \leq 0.05$) were found among the cultivars and advanced breeding lines for both CBR resistance and tomato spotted wilt virus (TSWV) resistance which was also present each year, but the predominant disease was CBR. Georgia Greener, Georgia-06G, Georgia-07W, Georgia-02C, and Carver were consistently found to be the most CBR resistant; whereas, C-99R and Tifguard were the most susceptible each year. In separate CBR tests conducted in 2009 and 2010 at a different location (Blackshank Farm), Georgia Greener also had the least difference, and Tifguard had the greatest difference, between *C. parasiticum* inoculated versus non-inoculated plots for pod yield. These combined test results demonstrate that useful levels of CBR resistance are currently available in promising new runner-type peanut cultivars.

Key Words: *Arachis hypogaea* L., groundnut, inoculated evaluation, disease incidence, pod yield, *Cylindrocladium parasiticum*, Crous, Wingfield, and Alfenas.

Cylindrocladium black rot (CBR) caused by the soilborne pathogen *Cylindrocladium parasiticum* Crous, Wingfield, and Alfenas, syn *C. crotalariae* (Loos) Bell and Sobers has long been a major disease problem in U.S. peanut (*Arachis hypogaea* L.) production. Consequently, new and improved CBR-resistant cultivars are continually needed to minimize damage, reduce chemical control costs, and increase yield, grade, and dollar value returns. These same cultivars also need to be resistant to tomato spotted wilt virus (TSWV) which is likewise another major peanut disease problem in the U.S.

In an earlier 3-yr extensive study under uniformly heavy disease pressure at the University of Georgia, Southeast Research and Education Center near Midville, GA (Branch and Brenneman, 2003), three CBR-resistant runner market type peanut cultivars, 'Georgia-01R' (Branch, 2002), 'Georgia-02C' (Branch, 2003), and 'Carver' (Gorbet, 2006) were all found to be similar in disease ratings as the CBR-resistant virginia-type checks, NC 3033 (Beute *et al.,* 1976), 'NC 8C' (Wynne and Beute, 1983), and 'Perry' (Isleib *et al.*, 2003), and produced significantly higher pod yields. However, 'C-99R' (Gorbet and Shokes, 2002), 'Southern Runner' (Gorbet *et al.,* 1987), and 'Florida MDR 98' (Gorbet and Shokes, 2002) had among the highest percentage of CBR disease incidence and were considered to be among the most CBR susceptible.

Since this earlier study, several newer runner-type peanut cultivars have been developed and released. Among these, the nematode-resistant, runner-type cultivar, 'Tifguard' (Holbrook *et al.,* 2008) was reported to be similar to Georgia-02C for CBR resistance in greenhouse and microplot studies (Dong *et al.,* 2009a). So, the objective of the present study was to determine if potentially new sources of CBR resistance exist among more recently released runner-type peanut cultivars under *C. parasiticum* inoculated field conditions.

Materials and Methods

During 2008 and 2009, field tests were conducted at the Gibbs farm and in 2009 and 2010 at the Blackshank farm near the UGA, Coastal Plain Experiment Station, Tifton, GA. Each year, several different genotypes were evaluated at these two locations for CBR resistance compared to the previously found CBR-resistant check (ck) cultivars, Georgia-01R, Georgia-02C, Carver, Perry and the CBR-susceptible check (ck) cultivar, C-99R. The Gibbs farm test site has a long history of

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continuous peanut production, and the soil type at both locations was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults).

Planting dates at the Gibbs Farm were April 15, 2008 and April 17, 2009, and the seeding rates were six seed per 30.5-cm of row. Recommended cultural practices with irrigation were used throughout each growing season, except no fungicides were applied with activity against CBR. All plots were artificially inoculated with microsclerotia of *C. parasiticum* in early June at approximately 50 days after planting (DAP) each year to avoid summer heat stress during the inoculation process.

Several isolates of *C. parasiticum* collected the previous year were grown for 4–6 weeks on 90-cm-dia petri plates with potato dextrose agar. Approximately 2 plates per plot were ground in a Waring blender with water and the slurry poured through cheese cloth. Enough water was added to give a total volume of 100 ml per plot, and this suspension of microsclerotia and hyphae was sprayed on the plant crowns with a pump-up garden sprayer. All plots were irrigated following inoculation.

A randomized complete block design was used each year with six replications. Plots consisted of two rows 6.10 m long × 1.83 m wide (0.81 m within and 1.02 m between rows on adjacent plots). Percentages (0–100%) of early-season TSWV disease incidence were scored at approximately 60 days after planting (DAP) when TSWV is usually the only disease occurring. Since some symptoms of CBR and TSWV are very similar, both mid-season TSWV + CBR were evaluated at ca. 100 DAP, and again just prior to harvest. The symptoms of CBR are most evident on plant roots, so CBR disease was also rated immediately after digging each year. A disease hit equaled one or more symptomatic plants within a 30.5-cm section row. Individual plots were harvested near optimum maturity according to visual above-ground disease pressure in conjunction with the hull-scrape maturity method from adjoining border plots (Williams and Drexler, 1981). Plots were mechanically harvested, and pods were dried with forced warm air to 6% moisture content, then hand-cleaned over a screen table before weighing for pod yield. Data from each test was subjected to analysis of variance. Waller-Duncan's T-test (k-ratio = 100) was used for mean separation of significant differences ($P \leq 0.05$).

Planting dates at the Blackshank Farm were May 20, 2009 and May 20, 2010, and the seeding rates were five seed per 30.5-cm of rows. Several weeks before planting, the field was treated with methyl bromide and chloropicrin at a rate of 448 kg/ha. A plastic tarp was used to contain the gas for several days and was then removed. This treatment will result in the elimination of most soilborne pathogens. Recommended cultural practices with irrigation were used throughout each growing season, and the only fungicide applied was chlorothalonil to control foliar diseases, but it has no activity against CBR. Inoculated plots were artificially inoculated with microsclerotia of *C. parasiticum* also in early June at approximately 15 DAP each year as described previously to avoid summer heat stress during the inoculation process. A split-plot design was used with genotypes as whole plots and non-inoculated vs. inoculated with *C. parasiticum* as sub plots with four replications. Plots consisted of two rows 7.62 m long × 1.83 m wide with 0.91 m spacing between rows. The CBR disease incidence was scored immediately after digging each year. A disease hit equaled one or more symptomatic plants within a 30.5-cm section of inverted row. All plots were dug at the same time, 152 DAP in 2009. However in 2010, digging was delayed until 166 DAP due to extremely hot conditions during the summer which inhibited CBR development until temperatures declined in the fall. Plots were mechanically harvested, and pods were dried with forced warm air to 6% moisture content, then hand-cleaned over a screen table before weighing for pod yield. Data from each test was subjected to analysis of variance, and least significant differences (LSD) was used for mean separation ($P \leq 0.05$).

Results and Discussion

During 2008 at the Gibbs Farm (Table 1), 'Georgia-06G' (Branch, 2007) had the lowest early-season TSWV disease incidence, but it was not significantly ($P \leq 0.05$) different from 'Georgia Greener' (Branch, 2007), Georgia-02C, 'Georgia-07W' (Branch and Brenneman, 2008), and 'Georgia-10T' (Branch and Culbreath, 2011). At both mid-season and late-season, TSWV and CBR incidence was also lowest for these same five cultivars, and GA 052524 and GA 052527, two sister lines of Georgia-10T. However after digging, CBR incidence was the lowest with Perry, the virginia-type resistant check; whereas, CBR incidence was highest (most susceptible) with C-99R. These findings agree with the previous report by Branch and Brenneman (2003). However, both Georgia-06G and Georgia Greener were also found in this 2008 study to have CBR resistance and high yields, and the late-maturing genotypes, Georgia-01R, Georgia-10T, GA 052524, GA 052527, and the medium-maturing cultivar, Tifguard were all found to be CBR susceptible. These findings with

Table 1. TSWV and CBR Disease Incidence and Pod Yield among 16 Peanut Genotypes when Inoculated with *C. parasiticum* in Field Trials at the Gibbs Farm near Tifton, GA, 2008.

Peanut genotype	TSWV[a] (%)	TSWV + CBR[b] (%)	CBR + TSWV[b] (%)	CBR[c] (%)	Yield (kg/ha)
Georgia Greener	8.8 def*	13.8 e	19.6 f	29.6 fgh	4516 a
Georgia-02C (ck)	8.3 ef	15.0 e	27.5 c-f	37.5 ef	4378 ab
Carver (ck)	14.2 b	24.2 bc	34.6 cd	35.8 ef	4376 ab
Georgia-06G	6.7 f	13.3 e	19.6 f	23.8 gh	4318 ab
Perry (ck)	14.6 b	29.6 b	32.9 cde	18.3 h	4252 abc
Georgia-08V	10.0 de	22.5 cd	32.5 cde	34.2 efg	4206 abc
Georgia-07W	8.8 def	13.3 e	26.7 c-f	35.4 ef	3925 bc
Georgia-03L	10.8 cde	19.6 cde	30.0 cde	52.9 cd	3885 bc
AP-3	13.3 bc	23.3 bcd	35.4 c	43.8 de	3736 cd
Georgia-05E	10.8 cde	25.0 bc	46.2 b	58.8 c	3352 de
GA 052524	10.8 cde	13.3 e	19.2 f	61.7 c	2896 ef
Georgia-01R (ck)	11.2 cd	25.8 bc	30.8 cde	79.6 a	2750 f
GA 052527	10.4 de	17.1 de	25.4 def	74.6 b	2546 f
Georgia-10T	9.2 def	13.3 e	24.2 ef	79.2 ab	2518 f
Tifguard	11.2 cd	43.8 a	65.4 a	75.4 b	2460 f
C-99R (ck)	18.8 a	40.0 a	58.3 a	88.8 a	1405 g

*Within columns, means followed by the same letter are not significantly different at P≤0.05.

[a]Percent of 30.5-cm row sections with one or more plants showing symptoms of TSWV on foliage when evaluated at ca. 60-DAP.

[b]Percent of 30.5-cm row sections with one or more plants showing symptoms of TSWV and CBR when evaluated at ca. 100-DAP and ca. 130-DAP, respectively.

[c]Percent of 30.5-cm row sections with one or more plants showing symptoms of CBR on roots and pods when evaluated after the plots were dug and inverted.

Tifguard and Georgia-01R disagree with earlier reports (Dong *et al.,* 2009a and Branch and Brenneman, 2003), where both cultivars had previously shown some CBR resistance. Since the tests in this study were inoculated, it is understandable that later-maturing genotypes, such as Georgia-01R, were exposed longer to high-levels of CBR before digging as compared to non-inoculated evaluations. However, this would not be the same for Tifguard which is reported to be a medium-maturing cultivar (Holbrook *et al.,* 2008), and as such was dug earlier than Georgia-01R.

During 2009 at the Gibbs Farm (Table 2), Georgia Greener, Georgia-06G, Georgia-10T, Georgia-07W, GA 042634-11, and 'Georgia-05E' (Branch, 2006) had the lowest TSWV incidence, and Perry had the highest TSWV incidence earlier in the season. These results agree with a previous 3-yr maximum and minimum production input study for TSWV disease resistance (Branch and Fletcher, 2010). Again at both mid and late-season, TSWV and CBR incidence was found to be the lowest with Georgia-07W, Georgia-06G, Georgia Greener, and Georgia-10T. After digging, CBR incidence was again lowest with Perry, Georgia Greener, Georgia-07W, and two advanced Georgia breeding lines, GA 042634-6 and GA 042634-11. However, high CBR incidence and low-pod yield was again found for C-99R, Georgia-01R, and Tifguard which agrees with the 2008 results.

At the Blackshank Farm in 2009 (Table 3), non-inoculated vs. *C. parasiticum* inoculated plots showed significant differences between many of these same peanut genotypes. Georgia Greener, Florida-07, and SEQ 910 were found to have similar CBR resistance, comparable to Georgia-02C; whereas, Tifguard was found to have the highest CBR incidence after inoculation and low pod yield. Pod yield for Tifguard was very high in non-inoculated plots. However, Georgia Greener had the same yield in both non-inoculated and inoculated field trials, and Tifguard had the greatest yield difference (2333 kg/ha), again reflecting its susceptibility to CBR.

When the *C. parasiticum* inoculated vs. non-inoculated tests were repeated in 2010 at the Blackshank Farm (Table 4), similar results were found with Georgia Greener showing CBR resistance and Tifguard having CBR susceptibility. Yields were not significant in the 2010 inoculated tests because of delaying harvest until very late fall in an attempt to allow some disease to develop. Overall there was more variability in this test, and disease development was greatly delayed due to the extremely hot, dry weather in 2010. Although significant disease levels eventually developed;

Table 2. TSWV and CBR Disease Incidence and Pod Yield among 16 Peanut Genotypes when Inoculated with *C. parasiticum* in Field Trials at the Gibbs Farm near Tifton, GA 2009.

Peanut genotype	TSWV[a] (%)	TSWV + CBR[b] (%)	CBR + TSWV[b] (%)	CBR[c] (%)	Yield (kg/ha)
Georgia-07W	9.6 cde*	17.9 g	26.7 h	17.5 fg	4127 a
Georgia Greener	7.9 e	24.2 ef	31.7 fgh	16.7 fg	3987 ab
Georgia-08V	10.4 cde	31.2 cd	55.0 bc	32.9 de	3936 ab
Georgia-06G	8.8 de	21.2 fg	36.7 ef	24.2 ef	3861 ab
Georgia-10T	9.2 de	22.5 fg	28.3 gh	44.6 bc	3820 ab
Carver (ck)	11.7 bcd	32.1 bc	48.3 cd	28.8 de	3775 abc
Georgia-02C (ck)	11.2 bcd	29.6 cde	40.0 e	30.8 de	3576 bcd
AP-3	12.5 bc	24.2 ef	35.8 efg	38.3 cd	3573 bcd
Georgia-03L	10.0 cde	25.8 def	35.4 efg	30.0 de	3536 bcd
Georgia-01R (ck)	11.2 bcd	33.3 bc	41.7 de	67.5 a	3332 cde
Georgia-05E	9.6 cde	29.6 cde	58.3 b	49.6 b	3287 de
Tifguard	10.0 cde	26.2 def	62.5 ab	50.4 b	3266 de
GA 042634-11	9.2 de	29.2 cde	39.2 ef	14.6 fg	2977 e
GA 042634-6	10.0 cde	30.4 cd	39.6 ef	7.5 g	2916 ef
C-99R (ck)	13.8 b	37.5 b	62.1 ab	74.2 a	2472 fg
Perry (ck)	20.8 a	48.8 a	68.3 a	12.9 g	2357 g

*Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

[a]Percent of 30.5-cm row sections with one or more plants showing symptoms of TSWV on foliage when evaluated at ca. 60-DAP.

[b]Percent of 30.5-cm row sections with one or more plants showing symptoms of TSWV and CBR when evaluated at ca. 100-DAP and ca. 130-DAP, respectively.

[c]Percent of 30.5-cm row sections with one or more plants showing symptoms of CBR on roots and pods when evaluated after the plots were dug and inverted.

Table 3. CBR Disease Incidence and Pod Yield among 12 Peanut Genotypes when Compared under Non-inoculated vesus *C. parasiticum* Inoculated Field Trials at the Blackshank Farm near Tifton, GA, 2009.

	Non-inoculated		Inoculated	
Peanut genotype	CBR[a] (%)	Yield (kg/ha)	CBR[a] (%)	Yield (kg/ha)
Georgia Greener	0.0	3024	39.6	3024
SEQ 925	0.0	3580	43.7	2944
Georgia-02C (ck)	0.0	3798	23.9	2930
Florida Fancy	0.0	4069	42.9	2930
AP-4	1.7	3364	46.2	2889
Florida-07	7.4	3567	23.1	2795
SEQ 910	0.0	3758	24.8	2713
Georgia-07W	0.0	4042	50.3	2333
Georgia-06G	0.8	3255	61.9	2008
Tifguard	0.0	4110	65.2	1777
Georgia Green	2.5	2360	47.9	1710
Georgia-08V	0.0	3459	53.6	1668
LSD ($P \leq 0.05$)	6.4	1109	22.3	1019

[a]Percent of 30.5-cm row sections with one or more plants showing symptoms of CBR on roots and pods when evaluated after plots were dug and inverted.

Table 4. CBR Disease Incidence and Pod Yield among 13 Peanut Genotypes when Compared under Non-inoculated versus *C. parasiticum* Inoculated Field Trials at the Blackshank Farm near Tifton, GA, 2010.

	Non-inoculated		Inoculated	
Peanut genotype	CBR[a] (%)	Yield (kg/ha)	CBR[a] (%)	Yield (kg/ha)
SEQ 910	10.0	4801	35.0	4666
Tifguard	0.6	3880	34.4	4630
Georgia-09B	3.8	4232	30.0	4354
Georgia-10T	0.0	3731	25.6	4327
Georgia-06G	2.5	4774	21.9	4164
Georgia-08V	0.6	4178	22.5	4138
Georgia-07W	0.0	3527	12.5	4138
Florida Fancy	5.6	4042	36.3	3960
SEQ 925	0.6	3431	45.0	3771
Georgia Greener	0.0	3907	9.4	3703
Florida-07	0.6	3907	19.4	3703
Bailey	0.0	4056	11.3	3567
Georgia-02C (ck)	0.0	2875	16.7	3382
LSD ($P \leq 0.05$)	2.6	1435	14.0	1440

[a]Percent of 30.5-cm row sections with one or more plants showing symptoms of CBR on roots and pods when evaluated after plots were dug and inverted.

symptoms were primarily pod discoloration and had little effect on yield.

Summary

Results from three years (2008-10) at two Tifton, GA locations (Gibbs Farm and Blackshank Farm) clearly show new sources of CBR resistance with Georgia Greener being the most consistent in low disease incidence and high pod yield. However, these findings also show that Tifguard is highly susceptible to CBR, and is not CBR resistant as previously reported from microplot studies by Dong *et al.,* 2009a. However as Dong *et al*., (2009b) concluded, CBR screening of peanut genotypes are most reliably done in the field with inoculation or using uniformly infested field sites, as previously described by Branch and Brenneman (2003).

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