A Note on a Greenhouse Evaluation of Wild *Arachis* Species for Resistance to *Athelia rolfsii*

R.S. Bennett*, A.D. Harting, C.E. Simpson, S.P. Tallury, A.B. Pickering, N. Wang, and J.C. Dunne[1]

ABSTRACT

Athelia rolfsii (Curzi) C.C. Tu & Kimbr. is the one of the most damaging pathogens of cultivated peanut, causing the soilborne disease known regionally as white mold, stem rot, or southern blight. Because the genetic base for cultivated peanut is narrow, wild *Arachis* species may possess novel sources of disease resistance. We evaluated 18 accessions representing 15 *Arachis* species (*batizocoi*, *benensis*, *cardenasii*, *correntina*, *cruziana*, *diogoi*, *duranensis*, *herzogii*, *hoehnei*, *kempff-mercadoi*, *kuhlmannii*, *microsperma*, *monticola*, *simpsonii*, *williamsii*) in the greenhouse for resistance to *At. rolfsii*. Assays were conducted on intact plants propagated from rooted cuttings inoculated with mycelial plugs, and lesion length and mycelial growth were measured at 4, 6, 10, and 12 days after inoculation. For lesion length, *Arachis batizocoi* (PI 468326 and PI 468327), and *A. kuhlmannii* PI 468159 were the most susceptible entries with a mean lesion length >50 mm at 12 days after inoculation. *Arachis microsperma* (PI 666096 and PI 674407) and *A. diogoi* PI 468354 had the shortest lesions with mean lengths ≤16 mm at 12 days after inoculation. *Arachis cruziana* PI 476003 and the two *A. batizocoi* PIs had the highest mean area under the disease progress curves (AUDPCs), and the lowest AUDPC was obtained from the *A. microsperma* PI 674407. Mycelial growth was correlated with lesion length in most species except *A. monticola* PI 497260. These results may be useful to peanut geneticists seeking additional sources of resistance to *Athelia rolfsii*.

Key Words: *Arachis batizocoi*, *Arachis benensis*, *Arachis cardenasii*, *Arachis correntina*, *Arachis cruziana*, *Arachis diogoi*, *Arachis duranensis*, *Arachis herzogii*, *Arachis hoehnei*, *Arachis kempff-mercadoi*, *Arachis kuhlmannii*, *Arachis microsperma*, *Arachis monticola*, *Arachis simpsonii*, *Arachis williamsii*, resistance assay, *Sclerotium rolfsii*, Section *Arachis*, southern blight, stem rot, white mold.

Cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid derived from a single recent hybridization event between *A. duranensis* and *A. ipaensis* (Kochert *et al*., 1996; Moretzsohn *et al*., 2013; Bertioli *et al*., 2015, 2019). Relative to some domesticated crops such as the common bean, *Phaseolus vulgaris* (Zizumbo-Villarreal *et al*., 2005), peanut cultigens possess little genetic variation (Kochert *et al*., 1991; Ferguson *et al*., 2004), even those collected from peanut's center of origin in South America (Halward *et al*., 1991; Moretzsohn *et al*., 2004). In contrast to the limited resistance to pests and diseases available within the germplasm pool for *Arachis hypogaea* (Stalker, 2017), many wild *Arachis* species possess high levels of genetic diversity, and importantly, resistance to various biotic stressors (Stalker *et al*., 2016; Stalker, 2017). Accessions from at least 30 species within Section *Arachis*, the secondary gene pool for cultivated peanut (Krapovickas and Gregory, 2007; Smýkal *et al*., 2015), have demonstrated resistance to diseases and insects (Stalker, 2017). The U.S. National Plant Germplasm System currently has approximately 500 available wild species accessions, approximately 200 of which are from Section *Arachis*.

Despite the potential of wild species for improving cultivated peanut, most cultivars in the U.S. do not have wild *Arachis* in their ancestries (Stalker, 2017). Nonetheless, the few examples descended from wild species have had significant impact. All nematode resistance in U.S. cultivars—i.e., COAN (Simpson and Starr, 2001), NemaTAM (Simpson *et al*., 2003), Tifguard (Holbrook *et al*., 2008), Webb (Simpson *et al*., 2013), Georgia-14N (Branch and Brenneman, 2015), and TifNV-High O/L (Holbrook *et al*., 2017)—is derived from TxAG-6, a complex interspecific hybrid generated from *A. cardenasii, A. diogoi* (formerly *A. chacoense*), and *A. batizocoi* (Simpson *et al*., 1993). *Arachis cardenasii* is also in the heritage of Bailey

[1]First and second authors: Research Plant Pathologist and Biological Science Technician, USDA-ARS, Wheat, Peanuts and Other Field Crops Research Unit, Stillwater, OK 74075; Third author: Professor Emeritus, Department of Soil and Crop Sciences, Texas A&M Agrilife, Stephenville, TX 76401; Fourth author: Geneticist, USDA-ARS, Plant Genetics Resources Conservation Unit, Griffin, GA 30223; Fifth and sixth authors: Undergraduate Student and Professor, Department of Biosystems and Agricultural Engineering, Oklahoma State Univ., Stillwater, OK 74078; Seventh author: Assistant Professor, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695.

*Corresponding author's E-mail: rebecca.bennett@usda.gov

(Isleib *et al*., 2011), the popular Virginia cultivar with some resistance to early and late leaf spots, Sclerotinia blight, Cylindrocladium black rot, tomato spotted wilt virus, and *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. GP-NC WS 13, a North Carolina State University (NCSU) germplasm release generated from *A. hypogaea* and *A. cardenasii* (Stalker *et al*., 2002), is two breeding cycles removed from Bailey. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has also released several cultivars developed from interspecific crosses containing *A. batizocoi, A. cardenasii, A. duranensis, A. stenosperma,* and *A. villosa* with resistances to peanut rust, late leaf spot, and viruses (Gowda *et al*., 2002; Singh *et al*., 2003; Stalker, 2017).

Multiple studies have evaluated wild *Arachis* species for resistance to early and late leaf spots, rust, viruses, and nematodes (Subrahmanyam *et al*., 1983b; a, 1985, 2001; Nelson *et al*., 1989; Holbrook and Noe, 1990; Reddy *et al*., 2000; Pande and Rao, 2001; Sharma *et al*., 2017; Stalker, 2017). However, considerably less attention has been paid to soilborne disease resistance. An expansive evaluation by Tallury *et al*. (2013) evaluated 110 accessions from 23 *Arachis* species for resistance to Cylindrocladium black rot (CBR) and Sclerotinia blight. Considerable variation in resistance was found for CBR, but only *A. glandulifera* exhibited significantly more resistance than *A. hypogaea* for Sclerotinia blight (Tallury *et al*., 2013). Pande *et al*. (1994) included one each of *A. chacoense* (= *diogoi*, PI 276235), *monticola* (PI 497260), *A. stenosperma* (PI 497579), and an interspecific hybrid of *A. hypogaea* and *A. cardenasii* in a greenhouse resistance assay for *At. rolfsii*. Bera *et al*. (2016) at the Indian Council of Agricultural Research Directorate of Groundnut Research (ICAR-DGR) evaluated a total of 25 accessions of 11 species from four sections of *Arachis* for resistance to *At. rolfsii*. The most resistant accessions, *A. pusilla* DGR 12047 and *A. appressipila* ICG 8945, had 13 and 14% mortality, respectively. Researchers at ICAR-DGR also developed NRCG CS85, a multiple disease-resistant genotype derived from *A. kretschmeri*, to create a mapping population (Dodia *et al*., 2019) and to investigate mechanisms of resistance to southern blight (Bosamia *et al*., 2020).

The objective of this study was to evaluate a small subset of accessions within the Section *Arachis* for resistance to *At. rolfsii* in the greenhouse. Formerly known as *Sclerotium rolfsii* (Xu *et al*., 2010), this cosmopolitan pathogen is the most damaging soilborne disease of peanut in the U.S. (Backman and Brenneman, 1997). In Georgia alone, an estimated $91.4M was lost in yield and control costs from *At. rolfsii* in 2017 (Little, 2017). Disease-resistant peanut cultivars developed from resistant wild species would save growers millions of dollars and mitigate health impacts by reducing fungicide applications (Fisher *et al*., 2018).

Materials and Methods

A total of 18 PIs from the U.S. National Plant Germplasm System representing 15 section *Arachis* species (*batizocoi*, *benensis*, *cardenasii*, *correntina*, *cruziana*, *diogoi*, *duranensis*, *herzogii*, *hoehnei*, *kempff-mercadoi*, *kuhlmannii*, *microsperma*, *monticola*, *simpsonii*, *williamsii*) were screened in the greenhouse for resistance to *At. rolfsii* (Table 1). Resistant and susceptible reference genotypes, the runner cv. Georgia-03L (Branch, 2004), U.S. mini-core accessions CC650 (PI 478819), CC038 (PI 493581), and CC041 (PI 493631)(Bennett and Chamberlin, 2020) were included to compare the greenhouse system to a previous-used growth chamber system (Bennett, 2020). The susceptible Virginia cv. NC-V11 (Wynne *et al*., 1991), rated "30" for white mold resistance by Peanut Rx (Culbreath *et al*., 2010) was also included.

Plant Preparation.

Because of limited seed and inconsistent germination of most wild *Arachis* accessions, they were vegetatively propagated from May to September 2019 to obtain plants for the experiments. Mother plants were maintained in pots (33-cm-top diam., 20-cm-bottom diam., 15-cm tall) filled with the soilless mix BM7-35 (Berger). Greenhouse temperatures were set at 22 to 32 C. A small amount of *Rhizobium* inoculant (Guard-N; Verdesian Life Sciences) was applied by dipping the seed into the inoculant powder immediately before planting or by placing ca. 3 mm^3 of the inoculant into the planting hole.

Cuttings were taken from branches with 3 to 4 nodes, and leaves, pegs, and flowers were removed from the lowermost 1 to 2 nodes. The bottom nodes were dipped in rooting powder (Garden Safe TakeRoot; Spectrum Brands Holdings) before placing in 9.5-diam. pots filled with a soilless propagation mix (Sunshine Redi-Earth Plug and Seedling; Sungro). *Rhizobium* inoculant was applied to the planting hole as previously described before cuttings were inserted into the potting mix. Potted cuttings were placed in an 0.9-m-wide x 3.3-m-long x 0.9-m-high enclosed humidity chamber constructed from 2.54-diam. polyvinyl chloride piping and 6-mil Solar-Ice polyethylene film (now Luminance, RPC BPI Agriculture). The humidity

Table 1. Entries used in this study.

Entry[a]	PI No.	Resistance[b]	Life Cycle[c]
A. batizocoi	PI 468326	—	Perennial/Biennial
A. batizocoi	PI 468327	—	Perennial/Biennial
A. benensis	PI 475878	—	Annual
A. benensis	PI 475879	—	Annual
A. cardenasii	PI 475994	—	Perennial
A. correntina	PI 681079	—	Perennial
A. cruziana	PI 476003	—	Annual
A. diogoi	PI 468354	—	Perennial
A. duranensis	PI 219823	—	Annual
A. herzogii	PI 476008	—	Perennial
A. hoehnei	PI 666086	—	Perennial
A. kempff-mercadoi	PI 468333	—	Perennial
A. kuhlmannii	PI 468159	—	Perennial
A. microsperma	PI 666096	—	Perennial
A. microsperma	PI 674407	—	Perennial
A. monticola	PI 497260	—	Annual
A. simpsonii	PI 688958	—	Perennial
A. williamsii	PI 688988	—	Annual
CC650	PI 478819	Resistant	—
Georgia-03L	PI 634333	Resistant (10)	—
NC-V11	PI 540461	Susceptible (30)	—
CC038	PI 493581	Highly Susceptible	—
CC041	PI 493631	Highly Susceptible	—

[a]U.S. mini-core accessions are listed by core collection number.

[b]Relative resistance of reference entries to *Athelia rolfsii*. Qualitative and numerical estimates for cvs. Georgia-03L and NC-V11 are from the 2010 Peanut Rx (Culbreath *et al*., 2010). Susceptibilities of CC038, CC041, and CC650 from Bennett (2020).

[c]Life cycle of species from Krapovickas and Gregory (2007).

chamber was partially covered with 50% Aluminet (Ecologic Technologies, Inc.) to further reduce heat from infrared radiation. Overhead misting lines (Orbit Irrigation Products) provided high humidity by misting approximately 5 sec every 5 min. After four weeks, cuttings were monitored weekly for root development by gently pulling on the stem. Rooted cuttings were removed from the chamber and placed in shade for 2-3 weeks to acclimate to lower humidity. Cuttings were then transplanted into 15-cm-diam. pots filled with BM7-35 soilless mix and moved into full light on greenhouse benches. A 46-cm-long x 6.35-diam. acrylic rod (McMaster-Carr) was inserted into each pot, and top-heavy and trailing plants were tied to the rod with paper wire twist ties. In order to prolong the life of biennial and annual species (Kvien and Ozias-Akins, 1991), the vegetatively propagated plants were monitored weekly to remove any pegs that had developed. Plants were also pruned as needed to keep branches from extending ca. 30 cm beyond the top of the acrylic rod.

For the resistant and susceptible *A. hypogaea* reference genotypes, three seeds were planted with *Rhizobium* inoculant in 15-cm-diam pots filled with the BM7-35 potting mix. Pots were planted eight weeks before inoculation and thinned to one plant after emergence. All wild and cultivated plants were fertilized with 15 mL of NPK 14-14-14 slow-release product (Osmocote Smart Release Flower and Vegetable; ScottsMiracle-Gro). In addition, micronutrients were applied by adding Fertileader Vital (300 mL/379 L water; Timac Agro USA) to the irrigation water until a minimum of 2 weeks before inoculation.

***Athelia rolfsii* Inoculations.**

Athelia rolfsii mycelial plugs were prepared and plant inoculations were conducted as described in a previous study (Bennett, 2020) with the exception that plants were inoculated on the main stem, usually at the 2nd node, approximately 30-60 mm from the soil line. Because *A. microsperma* PI 674407 had a particularly bushy growth habit, the impeding branches were tied upwards with string to facilitate inoculation and data collection. Plants were placed inside the humidity chamber, where high humidity was maintained for part of the day with an ultrasonic transducer fogging system on each end of the chamber. The fogging system, hooked up to a water hose, was constructed using a 39 cm x 30 cm x 17 cm project box, a water tank float to maintain proper water level, a 24 V

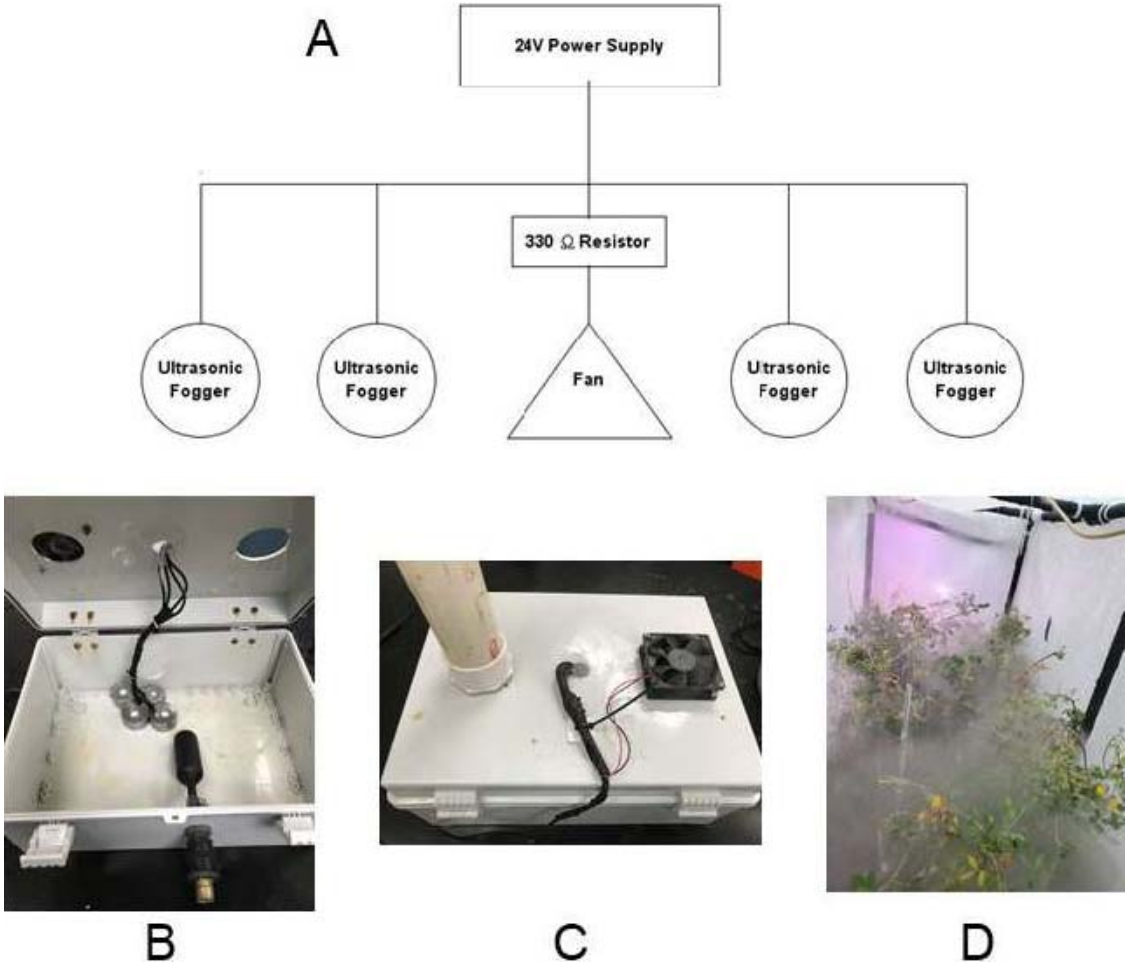

Fig. 1. A) Point-to-point wiring of the ultrasonic fogging system used to maintain humidity for inoculated plants; B and C) Inside and top views; D) Interior of humidity chamber with fogger.

computer fan, four 24 V ultrasonic transducers, and a 24 V power supply (Figure 1). Each ultrasonic transducer was able to diffuse between 200-400 mL of water per hour. The fogging systems operated 9 hr per day, with one running between 0600 and 1500 hr, and the other between 1100 and 2000 hr. Temperature and relative humidity were monitored each hr with three HOBO U23 data loggers (Onset Computer Corp.) starting 25 Feb. 2020. Main stem lesion length was measured 4, 6, 10, and 12 d after inoculation using a digital caliper (Mitutoyo America). Because mycelium frequently obscured lesions, it is often faster to measure mycelium than to measure lesions. Thus, mycelium measurements were taken to see if they could be substituted for lesion measurements. If the plant died before the end of the experiment, measurements for remaining days were recorded as missing data. The experiment (trial) was conducted 11 times between 9 Feb. 2020 to 29 Apr. 2020.

Data Analyses.

Plant pots were arranged inside the chamber in a randomized complete block design with two replications. Data were analyzed using SAS Version 9.4 (SAS Institute). Differences among entries in lesion length and mycelial growth were determined using repeated measures ANOVA in PROC MIXED with TOEP covariance structure. Trial and block(trial) were used as random variables in the model. Differences among and within entries at 4, 6, 10, and 12 d after inoculation were analyzed using the SLICE option. Correlation analysis between lesion and mycelium lengths were conducted using PROC CORR. Area under the disease progress curves (AUDPCs; Shaner and Finney, 1977) for lesion length were estimated and analysed using PROC GLIMMIX. AUDPC means were compared using a split-plot design with trial as the whole plot and entry as the subplot, and the SLICE option was used to examine differences among trials and entries. All pairwise comparisons were

Table 2. Lesion length and mycelial growth at 6, 10, and 12 days after inoculation with *Athelia rolfsii*.

Entry[a]	PI Number	No. Plants[b]	P (All Days)[c]	Lesion (mm)[d]			Mycelium (mm)[d]		
				Day 12	Day 10	Day 6	Day 12	Day 10	Day 6
A. batizocoi	PI 468327	18	<0.01	56.3 a	41.0 a	19.1 ab	68.1 a-c	57.0 a	36.2 ab
A. batizocoi	PI 468326	11	<0.01	51.9 a	37.4 a-c	18.4 ab	49.8 b-e	42.0 ab	23.1 ab
A. kuhlmannii	PI 468159	22	<0.01	50.1 a	34.3 a-c	18.4 ab	62.0 a-d	48.7 ab	25.8 ab
A. cruziana	PI 476003	12	<0.01	49.2 ab	40.8 ab	29.8 a	72.7 a-c	63.0 a	54.9 a
A. kempff-mercadoi	PI 468333	18	<0.01	44.3 ab	28.7 a-d	13.2 ab	94.1 a	52.5 a	27.8 ab
A. hoehnei	PI 666086	15	<0.01	43.7 ab	26.9 a-d	15.5 ab	78.0 ab	54.5 a	32.1 ab
A. herzogii	PI 476008	20	<0.01	40.8 ab	26.0 a-d	12.8 ab	44.3 b-e	31.4 ab	20.8 ab
A. monticola	PI 497260	22	<0.01	37.8 a-c	25.0 a-d	9.4 ab	69.4 a-c	55.3 a	39.1 ab
A. benensis	PI 475878	20	<0.01	36.4 a-d	19.5 a-d	7.9 ab	56.8 b-e	41.0 ab	25.0 ab
A. williamsii	PI 688988	21	<0.01	35.5 a-d	27.8 a-d	7.3 ab	60.2 a-d	47.8 ab	28.6 ab
A. simpsonii	PI 688958	22	<0.01	33.8 a-d	23.7 a-d	7.6 ab	60.3 a-d	49.4 a	30.8 ab
A. benensis	PI 475879	15	<0.01	33.4 a-d	20.7 a-d	8.0 ab	53.3 b-e	38.0 ab	24.2 ab
A. duranensis	PI 219823	17	<0.01	28.5 a-d	20.2 a-d	8.5 ab	50.4 b-e	40.4 ab	20.5 ab
A. correntina	PI 681079	10	0.23	26.4 a-d	18.6 a-d	13.5 ab	59.4 a-e	45.1 ab	34.1 ab
A. cardenasii	PI 475994	22	<0.01	23.0 b-d	14.0 b-d	4.2 ab	46.8 b-e	33.5 ab	22.9 ab
A. diogoi	PI 468354	22	0.04	16.0 cd	13.0 b-d	4.5 ab	33.3 c-e	28.1 ab	16.5 ab
A. microsperma	PI 666096	22	0.12	13.8 d	10.7 cd	3.4 ab	30.0 de	23.5 ab	14.1 ab
A. microsperma	PI 674407	21	0.09	12.3 d	4.7 d	1.1 b	21.2 e	14.7 b	8.2 b
			F	6.54	3.69	1.63	5.61	2.88	1.69
			df	17, 527.1	17, 523.7	17, 522.4	17, 548.1	17, 543.3	17, 542.2
			P	<0.01	<0.01	0.05	<0.01	<0.01	0.04
CC038	PI 493581	22	<0.01	26.1 a	19.8 a	7.0 a	39.8 a	30.8 a	11.5 a
CC041	PI 493631	22	<0.01	25.3 a	15.6 b	5.3 a	35.5 a	30.6 ab	15.6 a
NC-V11	PI 540461	22	<0.01	17.6 ab	11.9 a-c	6.3 a	25.9 ab	21.0 a-c	13.4 a
Georgia-03L	PI 634333	22	0.02	11.8 bc	8.2 bc	3.8 a	18.5 b	16.2 bc	11.0 a
CC650	PI 478819	22	0.23	6.9 c	5.2 c	2.0 a	12.7 b	9.1 c	5.2 a
			F	9.80	4.71	0.57	9.41	6.44	1.1
			df	4, 152.9	4, 152.4	4, 151.7	4, 150.0	4, 150.0	4, 150.0
			P	<0.01	<0.01	0.68	<0.01	<0.01	0.36

[a]Assay conducted on rooted cuttings for wild species and on 8-wk-old plants for control genotypes. U.S. mini-core accessions are listed by core collection number. Entries sorted from largest to smallest lesions on d 12.

[b]Number of plants used in experiments.

[c]P values for *F* tests comparing lesion lengths within entry among d 4 to 12.

[d]Length (mm) of lesion and mycelial growth on the main stem. Lesion SE ±5.1-7.0 (wild *Arachis*), ±2.7 (control genotypes); mycelium SE ±7.8-11 (wild *Arachis*), ±3.8 (control genotypes). Multiple comparisons adjusted for Type I error, and numbers with the same lowercase letter within column are not significantly different ($P \leq 0.05$). *F* test of fixed effects; df = degrees of freedom (numerator, denominator); P value.

adjusted for Type I error with the ADJUST = TUKEY option at $\alpha = 0.05$.

Results and Discussion

Some *Arachis* species were easier to propagate vegetatively than others, so there were fewer plants for some species, e.g. *A. correntina*, *A batizocoi* PI 468326, and *A. cruziana* (Table 2). During the experiments, the temperature inside the humidity chamber varied from 20 to 37 C but reached maximum temperatures of 27 C or greater on 90% of the days recorded by the data loggers. Temperatures between 27 and 30 C are considered to be optimal for *At. rolfsii* growth (Punja, 1985). In addition, higher temperatures were observed in April than in March and February. Median and maximum temperatures in April were 0.9 C and 6.8 C greater, respectively, than those observed in February. On 64% of the logged data times, relative humidity inside the chamber was ≥ 90%, and daily maximum relative humidity reached 98% or greater on 61 of the 63 logged days.

Lesion Length and Mycelial Growth.

In the repeated measures analyses of lesion length, the interaction between entry and time was significant, indicating that differences among entries depended on the day of observation ($F = 1.44$; df = 51, 827; P = 0.03). Lesion lengths among entries differed on d 6, 10, and 12 (Table 2) but not on d 4 ($F = 0.63$; df = 22, 693.1; P = 0.91). On d 12, the longest lesions (> 50 mm) were found on the

Table 3. Mean area under the disease progress curve (AUDPC) values for lesion length (mm).

Entry[a]	PI Number	Trials 1-5[b]		Trials 1-11[b]	
A. cruziana	PI 476003	216.0	a	—	
A. batizocoi	PI 468326	206.6	ab	—	
A. batizocoi	PI 468327	206.5	ab	—	
A. hoehnei	PI 666086	188.0	a-c	—	
A. monticola	PI 497260	172.1	a-c	142.9	ab
A. kuhlmannii	PI 468159	154.7	a-c	217.1	a
A. williamsii	PI 688988	143.0	a-c	—	
A. benensis	PI 475879	137.6	a-c	—	
A. benensis	PI 475878	125.4	a-c	—	
A. herzogii	PI 476008	118.9	a-c	—	
A. kempff-mercadoi	PI 468333	113.4	a-c	—	
A. correntina	PI 681079	112.9	a-c	—	
A. cardenasii	PI 475994	97.3	a-c	78.5	bc
A. duranensis	PI 219823	84.2	a-c	—	
A. diogoi	PI 468354	75.3	a-c	57.2	bc
A. simpsonii	PI 688958	68.7	a-c	130.4	ab
A. microsperma	PI 666096	42.6	bc	57.9	bc
A. microsperma	PI 674407	21.9	c	26.1	c

Model Effect	*F*	df	P	*F*	df	P
Trial	2.43	4, 3.94	0.21	1.18	10, 9.4	0.41
Entry	3.05	17, 82.28	<0.01	8.06	6, 62.8	<0.01
Trial*Entry	0.88	68, 82.17	0.70	1.2	60, 62.6	0.24

[a]Entries sorted by highest to lowest AUDPC from trials 1-5.

[b]Multiple comparisons adjusted for Type I error, and numbers with the same lowercase letter within column are not significantly different ($P \leq 0.05$). *F* test of fixed effects; df = degrees of freedom (numerator, denominator); P value.

two *A. batizocoi* accessions, and *A. kuhlmannii* (Table 2). The shortest lesions ($\leq$ 23 mm) were found on *A. cardenasii, A diogoi,* and *A. microsperma* PI 674407 and PI 666096. When sliced by entry, lesions lengths were significantly different over time for all entries except *A. correntina*, and the two *A. microsperma* PIs (Table 2).

The interaction between entry and time ($F = 1.41$; df = 51, 834; $P < 0.03$) was also significant for mycelial growth. Significant differences among entries were found for all days except d 4 ($F = 0.53$; df = 17, 541.3; $P = 0.94$). Mycelial growth occasionally decreased on some plants between d 10 and 12, but entries with the most mycelium (> 52 mm) for both days were *A. kempff-mercadoi*, *A. hoehnei*, *A cruziana*, *A. monticola,* and *A. batizocoi* PI 468327 (Table 2). These entries sustained significantly more mycelial growth than both *A. microsperma* entries by d 12. The correlation between mycelial growth and lesion length over time was considerable ($r = 0.74$; $P < 0.01$), but not as strong as in a previous study consisting only of *A. hypogaea* entries ($r = 0.92$; $P < 0.01$; Bennett, 2020). When the relationship was examined by day, the best correlation was found on d 10 ($r = 0.76$; $P < 0.01$), followed by d 12 ($r = 0.73$; $P < 0.01$), d 6 ($r = 0.52$; $P < 0.01$), and d 4 ($r = 0.36$; $P < 0.01$). Most entries had strong correlations ($r > 0.70$) between mycelial growth and lesion length except *A. monticola* ($r = 0.40$; $P < 0.01$), *A. cruziana* ($r = 0.61$; $P < 0.01$), *A. diogoi* ($r = 0.62$; $P < 0.01$), and *A. kempff-mercadoi* ($r = 0.69$; $P < 0.01$). Interestingly, *A. monticola* supported substantial mycelial growth but had comparatively smaller lesions on d 10 and 12. Thus, it appears that mycelial growth may not be a suitable indicator of resistance for comparisons among *Arachis* species.

Area Under the Disease Progress Curve (AUDPC).

Area under the disease progress curves were also examined. Missing data for the 11 entries with fewer plants resulted in non-convergence of the model, so data from all entries in trials 1 to 5 were used in one analysis, and data from the seven entries used in all trials were analyzed separately (Table 3). For both analyses, the effects of trial (trials 1-5, $P = 0.21$; trials 1-11, $P = 0.41$) and trial x entry ($P > 0.24$) were not significant. However, the effect of entry was significant ($P < 0.01$). In the first five trials, the highest AUDPC was in *A cruziana*, followed by the two *A. batizocoi* accessions; the two *A. microsperma* accessions had the lowest AUDPCs. When the seven entries were compared over all trials, *A. kuhlmannii* had the highest mean AUDPC, which was significantly greater than those of *A. cardenasii, A. diogoi,* and both *A. microsperma* PIs. To note, *A. simpsonii* was among

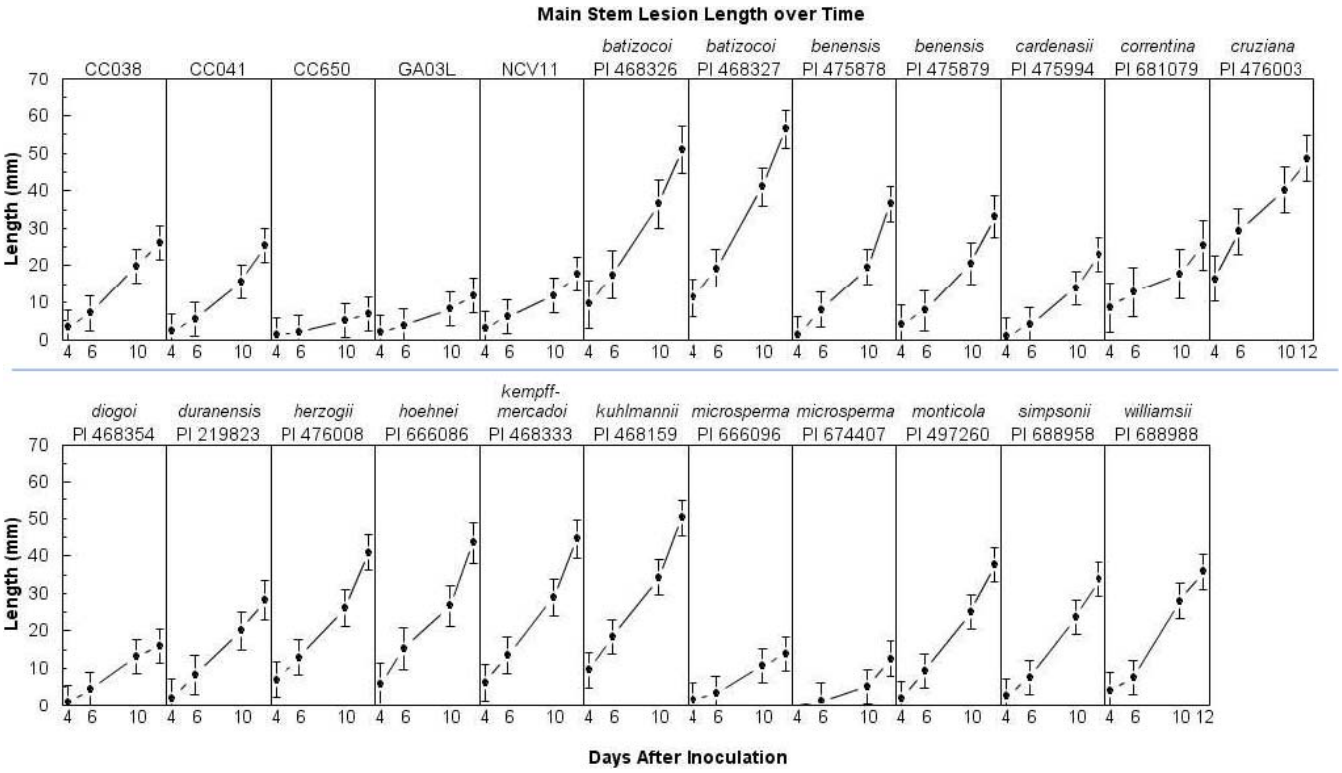

Fig. 2. Lesion length (±SE) over time in wild *Arachis* species and reference genotypes inoculated with *Athelia rolfsii*.

the more resistant entries in trials 1-5 but appeared considerably more susceptible in the analyses of all trials. Similarly, the AUDPC of *A. kuhlmannii* increased by 62 mm when the later trials were added. These changes in susceptibility may be due to the higher temperatures observed in the later trials, and *A. simpsonii* susceptibility to *At. rolfsii* may be particularly temperature sensitive. Pande *et al.* (1994) also observed that plant mortality to *At. rolfsii* increased with higher temperatures in some genotypes.

Compared to a previous study (Bennett, 2020), the greenhouse humidity chamber used here resulted in less severe disease, especially among the susceptible control genotypes CC038 and CC041. The difference in results is likely due to the constant optimum temperature maintained by growth chambers in that study. However, CC038 and CC041 were significantly more susceptible to *A. rolfsii* than the resistant genotypes Georgia-03L and CC650 (Table 2), consistent with recent laboratory and field studies (Bennett, 2020; Bennett and Chamberlin, 2020). The susceptible cultivar NC-V11 was numerically intermediate in lesion length and mycelial growth to the susceptible and resistant genotypes and differed statistically only from CC650 in lesion length on d 12. Both the greenhouse and growth chamber assays were better at identifying highly susceptible and some highly resistant genotypes than discriminating among intermediate entries (Bennett, 2020). Despite this limitation, laboratory assays are less likely to be influenced by canopy microclimates than field evaluations because high levels of humidity are easier to maintain. *Arachis* spp. vary considerably in canopy architecture, e.g. ranging from the relatively short and ramose *A. microsperma* to the little-branched, 1-m-tall mainstem (and up to 4-m-long lateral branches) of *A. batizocoi* (Krapovickas and Gregory, 2007).

Comparisons between the reference *A. hypogaea* genotypes and wild species were not made due to differences in plant age at inoculation. Several studies have observed that susceptibility to *At. rolfsii* decreases with plant age (Pande *et al.*, 1994; Pratt and Rowe, 2002; Bekriwala *et al.*, 2016), yet the younger *A. hypogaea* entries used in this study had smaller lesions than most of the wild species. Additional work is needed to determine if vegetative propagation enhanced disease susceptibility. In future studies, an ordinal rating ranking, such as the Florida scale for leaf spot (Chiteka *et al.*, 1988), will added to accommodate informative qualitative characters such as wilting and death. Nonetheless, there were significant differences among the wild *Arachis* accessions in susceptibility to *At. rolfsii* as indicated by lesion length, mycelial growth, and AUDPCs. Within the wild species, *A. microsperma*

PI 674407 and PI 666096 exhibited small lesions, mycelial growth, and AUDPC. Both accessions have previously shown resistance to Cylindrocladium black rot (Tallury *et al*., 2013). *Arachis cardenasii* PI 475994 and *A. diogoi* PI 468354 also had relatively small lesions. PI 475994 has high resistance to *Meloidogyne javanica* race 3 (Sharma *et al*., 2002), and PI 468354 is resistant to tomato spotted wilt virus (Lyerly *et al*., 2002; Wang *et al*., 2009). Since the three species have an A genome, facilitating introgression into cultivated peanut, these four accessions may merit additional evaluation as candidates for pre-breeding.

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