

Response to Oxalic Acid as a Resistance Assay for *Sclerotinia minor* in Peanut

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ABSTRACT

Response to oxalic acid was evaluated as an assay for screening peanut breeding lines for resistance to *Sclerotinia* blight caused by *Sclerotinia minor*. Detached stems of six runner- and seven spanish-type peanut genotypes, varying in resistance to *Sclerotinia* blight, were placed in 0, 20, and 50 mM oxalic acid. Wilt severity and length of stem discoloration were rated 12, 18, and 24 hr after immersion. Wilt and stem discoloration were not observed on stems placed in water (0 mM), but differences among entries were observed for some time intervals when placed in the 20 and 50 mM oxalic acid solutions. Susceptible runner cultivars exhibited the most severe wilt symptoms, but few differences in wilting were found among the spanish genotypes. More differences in stem discoloration were observed among the spanish peanuts than the runner entries, but length of discoloration did not appear to be correlated with field resistance. Oxalic acid appears to have some potential as a resistance assay for *Sclerotinia* blight in runner peanuts, but may have limited use for screening spanish peanuts. Rapid and accurate methods for screening peanut breeding lines for resistance to *Sclerotinia* blight would accelerate progress in breeding for disease-resistant peanuts.

Key Words: *Arachis hypogaea*, disease resistance, disease screening, *Sclerotinia* blight.

Sclerotinia blight, caused by *Sclerotinia minor* Jagger, is one of the most widespread and damaging peanut diseases worldwide. In the United States, *Sclerotinia* blight is a problem primarily in Virginia, North Carolina, Oklahoma, and Texas, capable of causing 50% yield loss in heavily infested fields (Porter and Melouk, 1997). For peanut growers, planting resistant cultivars is the most economical approach for managing this disease. Fungicides are frequently used to manage *Sclerotinia* blight in fields with high levels of disease, but treatments are costly. *Sclerotia* of *S. minor* are also viable in the soil

for four to five years without a host (Melouk and Backman, 1995); therefore, cultural practices such as multi-year crop rotations may be difficult economically for growers to implement (Damicone and Jackson, 1994). Peanut cultivars with significant resistance to *Sclerotinia* are available (Chappell *et al.*, 1995; Goldman *et al.*, 1995; Hollowell *et al.*, 2008), although none developed using traditional breeding methods are immune to the disease.

The time required to develop a new peanut cultivar may take more than ten years (Baring *et al.*, 2006; Smith *et al.*, 1991). Multiple years of field testing are needed to identify and verify disease resistance, but it may be possible to accelerate progress by augmenting field trials with greenhouse-based resistance assays. Greenhouse assays may be less limited by seasonality and physical space than field trials, and are thus suitable for screening a larger number of plants. Environmental conditions and pathogen inoculum levels, which frequently vary in the field (Hollowell *et al.*, 2008), may also be controlled more easily in the lab. Lab-based resistance assays for *Sclerotinia* blight using detached stems (Brenneman *et al.*, 1988; Melouk *et al.*, 1992), whole plants (Cruickshank *et al.*, 2002; Goldman *et al.*, 1995; Hollowell *et al.*, 2008), and leaflets (Hollowell *et al.*, 2003) have been developed. Unfortunately, many of these assays are better at identifying highly resistant and highly susceptible cultivars, and less capable of discriminating among moderately resistant cultivars (Faske *et al.*, 2006; Goldman *et al.*, 1995). In addition, results from greenhouse-based assays may not be completely congruent with results from field trials (Hollowell *et al.*, 2008, 2003). These assays cannot account for plant architecture characteristics that create favorable or unfavorable conditions for disease (Brenneman *et al.*, 1988; Hollowell *et al.*, 2008, 2003).

An alternative, potentially useful method for screening peanuts for resistance to *Sclerotinia* blight uses oxalic acid. Some fungal plant pathogens, including *Sclerotinia* spp. (Godoy *et al.*, 1990; Livingstone *et al.*, 2005) and *Sclerotium rolfsii* (Bateman and Beer, 1965; Kritzman *et al.*, 1977), produce oxalic acid to infect plants. Oxalic acid was used to screen several crops, including common bean (Kolkman and Kelly, 2000), scarlet runner bean (Chippis *et al.*, 2005), sunflower (Noyes and Hancock, 1981), and soybean (Cunha *et al.*,

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Table 1. Runner and spanish peanut genotypes used to evaluate oxalic acid assay.

Genotypes	Resistance to <i>Sclerotinia minor</i>	Reference
Runner		
Florunner	Susceptible	(Goldman <i>et al.</i> , 1995; Melouk <i>et al.</i> , 1992)
Okrun	Susceptible	(Melouk <i>et al.</i> , 2012; Woodward <i>et al.</i> , 2006)
Red River Runner	Moderate	(Melouk <i>et al.</i> , 2012)
Tamrun 96	Moderate	(Smith <i>et al.</i> , 1998)
ARSOK-R143-3	Moderate	
ARSOK-R35	Highly Resistant	
Spanish		
Pronto	Susceptible	(Goldman <i>et al.</i> , 1995)
Spanco	Susceptible	(Goldman <i>et al.</i> , 1995)
Tamnut OL06	Moderate	(Baring <i>et al.</i> , 2006)
ARSOK-S140-1OL	Moderate	
ARSOK-S1	Moderate	(Chamberlin <i>et al.</i> , 2014)
Tamspan 90	Highly Resistant	(Smith <i>et al.</i> , 1991; Woodward <i>et al.</i> , 2006)
ARSOK-S133-3	Highly Resistant	

2010; Wegulo *et al.*, 1995), for resistance to *S. sclerotiorum*. In these crops, response to oxalic acid exposure, as measured by wilt severity or discoloration in detached stems, discoloration in leaflets, or proportion of lysed cells, correlated with field resistance. However, the oxalic acid assay still shares the same disadvantage as other greenhouse-based assays in that it measures physiological resistance and cannot predict plant avoidance through canopy architecture (Brenneman *et al.*, 1988; Hollowell *et al.*, 2008, 2003). Response to oxalic acid was used to evaluate resistance in peanuts transformed with barley oxalate oxidase (Livingstone *et al.*, 2005); however, this method has not yet been evaluated as a resistance assay for wider selection of conventionally-bred peanuts. The objective of this project was to determine if detached stems of runner and spanish peanut cultivars known to be resistant or susceptible to *Sclerotinia* blight in the field differed in response to oxalic acid.

Materials and Methods

Six runner and seven spanish peanut genotypes varying in susceptibility to *Sclerotinia* blight were used to evaluate responses to oxalic acid (Table 1).

The following runner genotypes were included: susceptible cultivars Florunner (Norden *et al.*, 1969) and Okrun (Banks *et al.*, 1989); moderately resistant breeding line ARSOK-R143-3 and cultivars Red River Runner (Melouk *et al.*, 2012) and Tamrun 96 (Smith *et al.*, 1998); and the highly resistant breeding line ARSOK-R35. The following spanish peanut genotypes were used: susceptible cultivars Pronto (Banks and Kirby, 1983) and Spanco (Kirby *et al.*, 1989); moderately resistant breeding lines ARSOK-S140-1OL and ARSOK-S1 (PI 670132; Chamberlin *et al.*, 2014), and cultivars Tamnut OL06 (Baring *et al.*, 2006) and Tamspan 90 (Smith *et al.*, 1991); and the highly resistant breeding line ARSOK-S133-3. The relative resistance levels of the breeding lines were assessed on the basis of a minimum of 3 years of field evaluations.

Plants were grown in the greenhouse at 22 to 32 C in 15-cm pots filled with Metro-Mix 350 (Sun Gro Horticulture, Bellevue, WA). Supplemental lighting was supplied by 300-watt LED lights (Sunshine Systems, Wheeling, IL) set at a 14 hr photophase. After six weeks, the two lowermost lateral stems of each plant were cut near the main stem, and main stems were cut immediately below the third or fourth node. Cuts were made perpendicular to each stem using a razor blade, and detached stems were immediately placed in approximately 6 cm of reverse osmosis water. Stems were held in water 60 to 75 min until stems from all genotypes were prepared.

Detached lateral and main stems were rinsed with sterile reverse osmosis water, blotted dry, and placed individually into 5-ml tubes containing 3.5 ml of 0, 20, or 50 mM oxalate solution. Foam plugs were used to secure each stem so that at least 1.5 cm of the stem base was submersed in the solution. Tubes with detached stems were placed in the dark at room temperature (21 to 24 C). Plant response to oxalate exposure was rated after 12, 18, and 24 hr using a modification of the rating scale of Kolkman and Kelly (2000), where 1 = no wilt symptoms, 2 = one leaf wilted, 3 = two leaves wilted, 4 = one petiole collapsed, 5 = two petioles collapsed, and 6 = branch stem collapsed. The length of the bleached, lesion-like stem discoloration in response to oxalic acid exposure was also measured. Tubes were refilled with additional solution at 12 and 18 hr after immersion as needed.

Experiments evaluating the response of runner genotypes to oxalate solutions were conducted separately from the spanish genotypes. A randomized complete block design was used with three replications (three lateral stems and three main stems) per cultivar x oxalate concentration. Blocks were arranged so that the same replication for each

Table 2. Effects of oxalic acid concentration (mM), duration of immersion (TIME), and cultivar (cult) on wilt symptoms and length of stem discoloration on lateral and main stems of runner and spanish peanut entries.

Oxalic acid assay	Market type	Model effect ^a	Lateral stem ^b			Main stem ^b		
			df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Wilt symptoms	Runner	mM	1,101	45.80	<0.01	1,105	122.22	<0.01
		TIME	2,190	63.21	<0.01	2,183	110.96	<0.01
		mM*TIME	2,190	13.81	<0.01	2,183	32.24	<0.01
		cult	5,101	3.87	<0.01	5,106	1.99	0.09
		mM*cult	5,101	1.06	0.39	5,106	0.91	0.47
		TIME*cult	10,197	2.82	<0.01	10,191	1.51	0.14
		mM*TIME*cult	10,197	2.08	0.03	10,191	1.69	0.09
	Spanish	mM	1,107	22.35	<0.01	1,102	79.37	<0.01
		TIME	2,201	34.83	<0.01	2,188	70.70	<0.01
		mM*TIME	2,201	4.10	0.02	2,188	15.93	<0.01
		cult	6,107	0.66	0.68	6,101	4.89	<0.01
		mM*cult	6,107	1.40	0.22	6,101	0.79	0.58
		TIME*cult	12,208	1.01	0.44	12,196	1.49	0.13
		mM*TIME*cult	12,208	1.13	0.34	12,196	1.21	0.28
Stem discoloration	Runner	mM	1,89.3	161.74	<0.01	1,88.9	237.66	<0.01
		TIME	2,187	301.15	<0.01	2,180	449.08	<0.01
		mM*TIME	2,187	35.46	<0.01	2,180	58.38	<0.01
		cult	5,89.3	1.98	0.09	5,89.3	0.08	0.99
		mM*cult	5,89.3	1.06	0.39	5,88.8	0.80	0.56
		TIME*cult	10,190	0.79	0.63	10,182	1.48	0.15
		mM*TIME*cult	10,190	0.38	0.95	10,182	1.52	0.13
	Spanish	mM	1,102	127.87	<0.01	1,93.7	217.11	<0.01
		TIME	2,204	413.13	<0.01	2,190	228.95	<0.01
		mM*TIME	2,204	28.45	<0.01	2,190	42.59	<0.01
		cult	6,102	2.65	0.02	6,92.2	3.45	<0.01
		mM*cult	6,102	1.40	0.22	6,91.4	2.58	0.02
		TIME*cult	12,205	1.40	0.17	12,192	0.71	0.74
		mM*TIME*cult	12,205	1.15	0.32	12,192	1.39	0.17

^aRepeated measures ANOVA was used to analyze wilt symptoms and length of discoloration.

^b*F* test of fixed effects; df = degrees of freedom (numerator, denominator); *P* value.

cultivar was placed into the oxalic acid solution at approximately the same time. Test tube racks holding individual blocks were placed on separate trays. The experiment was conducted three times between December 2012 and February 2013. Separate analyses were conducted for each market type and stem type. Hypotheses regarding differences in lateral and main stem response (mean wilt scores and length of discoloration) of the differentially resistant genotypes to oxalate were tested using repeated measures ANOVA with an autoregressive covariance structure (TYPE=AR(1)) in SAS (v. 9.2, SAS Institute, Inc., Cary, NC). When a relevant interaction was significant, the SLICE option of the LSMEANS statement was used to examine simple effects.

Results and Discussion

No wilting or stem discoloration was observed at any time period for stems of either peanut

market type immersed in water (0 mM oxalic acid). Consequently, these data were removed from the analyses.

Wilt symptoms

The interaction between oxalic acid concentration and duration of exposure was significant for all stem and peanut market types (Table 2), indicating that the effect of one factor was dependent on the level of the other factor (Fig. 1). In addition, a significant three-way interaction among concentration, time, and cultivar was present for lateral stems of runner peanuts, which made interpretation of the main effects difficult. Cultivar did not have a significant effect on wilt responses of main stems of runners and of lateral stems of spanish genotypes (Table 2). Therefore, differences among cultivars in wilt response were not present or consistent over all concentrations and immersion times (Fig. 1). However, the effect of cultivar on wilt responses was significant in main stems of spanish (Table 2).

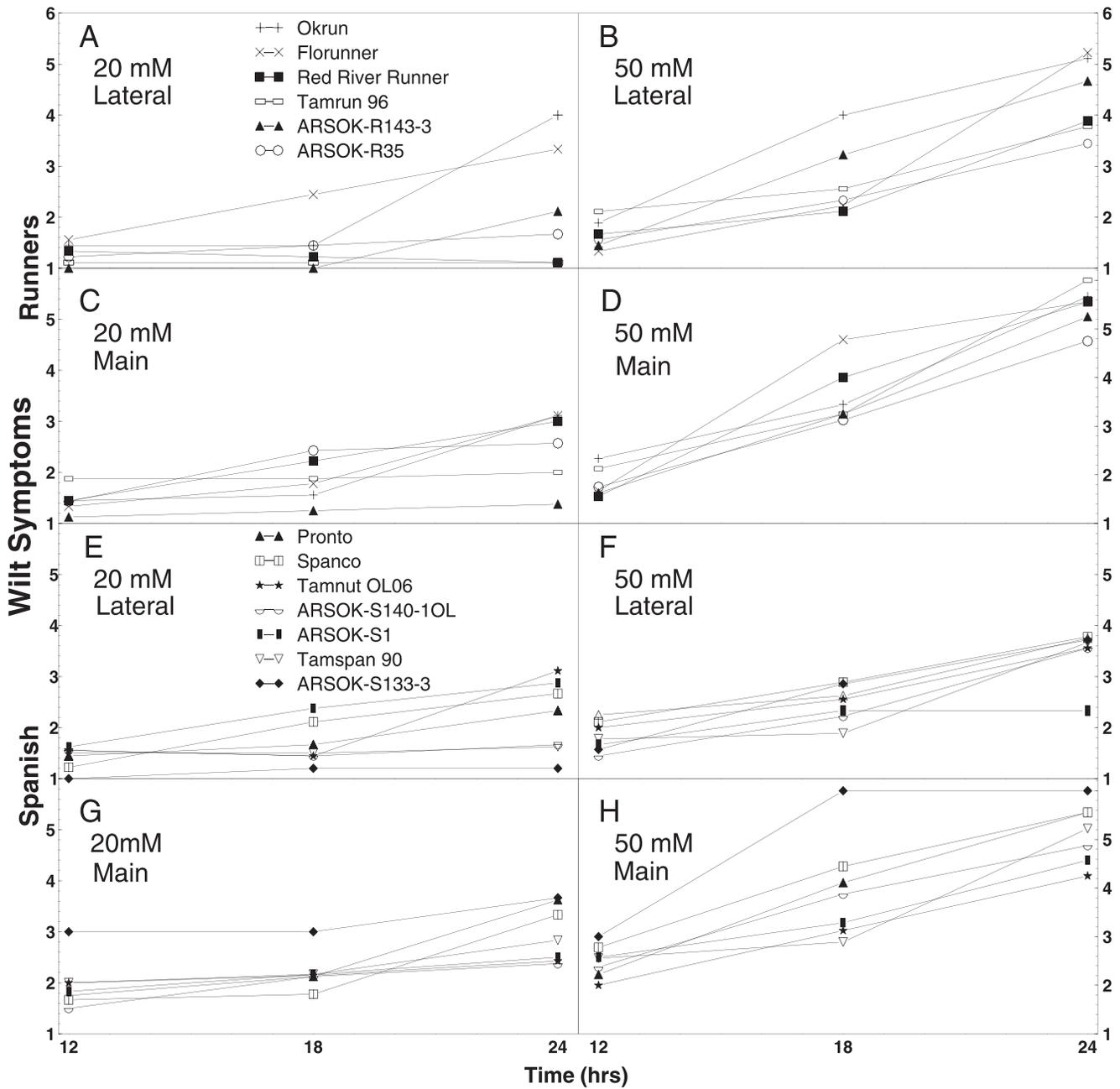


Fig. 1. Mean wilting score of runner peanut genotypes Okrun, Florunner, Red River Runner, Tamrun 96, ARSOK-R143-3, and ARSOK-R35 (A-D) and spanish genotypes Pronto, Spanco, Tamnut OL06, ARSOK-S140-1OL, ARSOK-S1, Tamspan 90, ARSOK-S133-3 (E-H). Wilt was rated from lateral and main stems of peanuts immersed in 20 mM or 50 mM oxalic acid for 12, 18, and 24 hr, using a modification of the rating scale of Kolkman and Kelly (2000), where 1 = no wilt symptoms, 2 = one leaf wilted, 3 = two leaves wilted, 4 = one petiole collapsed, 5 = two petioles collapsed, and 6 = branch stem collapsed.

To facilitate interpretation, the wilt responses were further examined so that cultivars could be compared by oxalic acid concentration and duration of exposure. Out of the twelve possible combinations of concentration (20 mM and 50 mM), time (12, 18, and 24 hr), and stem type (lateral and main), five tests among the runners and two tests among the spanish genotypes, resulted in significant differences among cultivars in wilt symptoms (Table 3). No significant differences in

wilt severity were observed among the genotypes at 12 hr of exposure. When mean comparisons were adjusted for type-I error, only two tests resulted in significant differences among cultivars: lateral stems of runners immersed in 20 mM for 24 hr, and main stems of spanish in 50 mM for 18 hr (data not shown).

Wilting appeared to be a better indicator of *Sclerotinia* blight resistance in runners than in spanish peanuts. Okrun and/or Florunner, cultivars

Table 3. Concentrations of oxalic acid and exposure times resulting in significant differences in wilt symptoms among runner and spanish genotypes immersed in oxalic acid.

Stem type	20 mM				50 mM				Wilt score
	Immersion time (hr)	Entry (n) ^a	Wilt score ^b	Stem type	Immersion time (hr)	Entry	Wilt score		
Runner									
Lateral	24	Okrun (9)	4.0 (0.8) ab	Runner Lateral	18	Okrun (9)	4.0 (0.7) a		
		Florunner (9)	3.3 (0.8) ab			ARSOK-R143-3 (9)	3.2 (0.8) ab		
		ARSOK-R143-3 (9)	2.1 (0.7) bc			Tamrun 96 (9)	2.6 (0.7) b		
		ARSOK-R35 (9)	1.7 (0.3) c			ARSOK-R35 (9)	2.3 (0.5) b		
		Red River Runner (9)	1.1 (0.1) c			Florunner (9)	2.2 (0.2) b		
		Tamrun 96 (9)	1.1 (0.1) c			Red River Runner (9)	2.1 (0.3) b		
Runner									
Main	24	Florunner (9)	3.1 (0.8) a	Lateral	24	Florunner (9)	5.2 (0.3) a		
		Okrun (9)	3.1 (0.7) a			Okrun (9)	5.1 (0.5) ab		
		Red River Runner (9)	3.0 (0.6) a			ARSOK-R143-3 (9)	4.7 (0.7) abc		
		ARSOK-R35 (7)	2.6 (0.6) ab			Red River Runner (9)	3.9 (0.7) bc		
		Tamrun 96 (8)	2.0 (0.3) bc			Tamrun 96 (9)	3.8 (0.6) c		
		ARSOK-R143-3 (8)	1.4 (0.2) c			ARSOK-R35 (9)	3.4 (0.7) c		
Spanish									
Lateral	24	Tamnut OL06 (9)	3.1 (0.7) a	Main	18	Florunner (9)	4.8 (0.6) a		
		ARSOK-S1 (8)	2.9 (0.7) a			Red River Runner (9)	4.0 (0.6) ab		
		Spanco (9)	2.7 (0.6) ab			Okrun (9)	3.4 (0.4) b		
		Pronto (9)	2.3 (0.5) a-c			ARSOK-R143-3 (8)	3.3 (0.6) b		
		ARSOK-S140-IOL (9)	1.7 (0.2) bc			Tamrun 96 (8)	3.3 (0.5) b		
		Tamspan 90 (8)	1.6 (0.2) bc			ARSOK-R35 (8)	3.1 (0.7) b		
		ARSOK-S133-3 (5)	1.2 (0.2) c			ARSOK-S133-3 (8)	6.0 (0.0) a		
						Spanco (9)	4.4 (0.6) b		
						Pronto (9)	4.1 (0.5) b-d		
						ARSOK-S140-IOL (8)	3.9 (0.7) b-d		
						ARSOK-S1 (7)	3.3 (0.7) b-d		
						Tamnut OL06 (8)	3.1 (0.6) cd		
						Tamspan 90 (9)	2.9 (0.5) d		

^an = number of stems assayed^bMean wilt score ± standard error in (). Wilt rated using a modification of Kolkman and Kelly (2000), where 1 = no wilt symptoms, 2 = one leaf wilted, 3 = two leaves wilted, 4 = one petiole collapsed, 5 = two petioles collapsed, and 6 = branch stem collapsed. Numbers with the same lowercase letter within each set are not significantly different ($\alpha = 0.05$).

Table 4. Concentrations and durations of exposure resulting in significant differences in length of stem discoloration among runner and spanish genotypes immersed in 50 mM oxalic acid.

Stem type	Time (hr)	Length of discoloration (cm) ^b		Stem type	Time (hr)	Entry (n)	Length of discoloration (cm)
		Entry (n) ^a	Mean				
Runners Lateral	24	ARSOK-R143-3 (9)	54.4 (5.4) a	Spanish Main	12	ARSOK-S133-3 (8)	38.5 (5.8) a
		Okrun (9)	47.9 (6.1) ab			ARSOK-S140-IOL (8)	37.4 (8.2) a
		Florunner (9)	47.4 (4.3) ab			Spanco (9)	30.7 (4.2) ab
		Tamrun 96 (9)	42.4 (7.5) bc			Pronto (9)	22.7 (2.9) bc
		Red River Runner (9)	41.8 (5.1) bc			Tamspan 90 (9)	20.4 (2.8) c
		ARSOK-R35 (9)	38.3 (5.9) c			Tamnut OL06 (8)	19.6 (2.8) c
						ARSOK-S1 (7)	17.5 (2.8) c
Spanish Lateral	18	Tamnut OL06 (9)	27.2 (3.5) a	Main	18	ARSOK-S140-IOL (8)	56.9 (11.1) a
		Pronto (9)	23.6 (2.1) ab			ARSOK-S133-3 (8)	53.5 (6.1) ab
		Spanco (9)	23.5 (2.8) ab			Spanco (9)	38.9 (4.8) c
		Tamspan 90 (8)	22.5 (2.6) ab			Pronto (9)	38.9 (4.8) c
		ARSOK-S140-IOL (9)	21.7 (2.0) bc			Tamspan 90 (9)	35.8 (5.0) cd
		ARSOK-S1 (9)	19.1 (2.1) bc			Tamnut OL06 (8)	32.4 (3.3) cd
		ARSOK-S133-3 (7)	18.6 (1.6) c			ARSOK-S1 (7)	31.9 (5.1) d
Lateral	24	Tamnut OL06 (9)	36.0 (4.5) a	Main	24	ARSOK-S140-IOL (8)	65.9 (10.2) a
		Spanco (9)	31.0 (3.2) b			ARSOK-S133-3 (8)	58.0 (5.7) ab
		Pronto (9)	30.1 (2.7) b			Spanco (9)	51.3 (5.3) bc
		ARSOK-S140-IOL (9)	28.7 (2.9) bc			Tamspan 90 (9)	49.4 (6.0) bc
		Tamspan 90 (8)	27.1 (2.6) b-d			ARSOK-S1 (7)	47.1 (4.4) bc
		ARSOK-S1 (9)	23.8 (2.4) cd			Pronto (9)	46.0 (4.8) c
		ARSOK-S133-3 (7)	22.7 (1.5) d			Tamnut OL06 (8)	44.6 (4.6) c

^an = number of stems assayed^bMean length of discoloration ± standard error in '()'. Numbers with the same lowercase letter within each set are not significantly different ($\alpha = 0.05$).

known to be highly susceptible to *Sclerotinia* blight, exhibited the most severe wilt symptoms in all of the statistically significant tests (Table 3). Wilting in response to oxalic acid was less consistent at discriminating the moderately resistant and highly resistant genotypes. In 20 mM oxalic acid, significant differences in wilting were observed at 24 hr after immersion among lateral and main stems of runner entries (Table 3; Fig. 1A). Lateral stems of Okrun and Florunner were significantly more wilted than lateral stems of ARSOK-R35, Red River Runner, and Tamrun 96. Main stems of ARSOK-R143-3 were less wilted than Okrun, Florunner, Red River Runner, and ARSOK-R35, but not significantly different than Tamrun 96 (Table 3; Fig. 1C). In 50 mM oxalic acid, significant differences in wilting among runner cultivars were observed at 18 and 24 hr after immersion for the lateral stems, and 18 hr after immersion for the main stems. Lateral stems of Okrun were more wilted than the lateral stems of other cultivars except ARSOK-R143-3 at 18 hr after immersion. After 24 hr of immersion, lateral stems of Okrun and Florunner exhibited more severe wilt than stems of Tamrun 96 and ARSOK-R35 (Table 3; Fig. 1B). For the main stems, Florunner had more wilt after 18 hr than all cultivars except Red River Runner.

No clear relationship between field resistance or susceptibility and wilting was observed among the spanish entries (Table 3). Significant differences in wilt symptoms among the spanish entries were observed only in lateral stems immersed for 24 hr at 20 mM and in main stems immersed for 18 hr at 50 mM (Table 3; Fig. 1E, 1H). Lateral stems of Tamnut 06 and ARSOK-S1 exhibited more wilt than stems of ARSOK-S140-1OL, Tamspan 90, and ARSOK-S133-3 when immersed at 20 mM for 24 hr. Main stems of ARSOK-S133-3 and Spanco immersed in 50 mM for 18 hr were more wilted than Tamnut OL06 and Tamspan 90.

Stem discoloration. A bleached, and occasionally reddish, area of discoloration typically appeared at the base of stems immersed in oxalic acid and lengthened up the stem over time, but only the white lesions were measured. In addition, the white areas on stems often became sunken and somewhat shriveled.

A significant interaction between oxalic acid concentration and duration of immersion was observed for both stem types in runner and spanish genotypes (Table 2). Cultivar did not have a significant effect on length of discoloration in lateral or main stems of runner peanuts. The effect of cultivar was significant for both spanish stem types, but for

main stems, this effect was dependent on the level of oxalic acid concentration (Table 2; Fig. 2).

Out of the twelve combinations of concentration, time, and stem type, one test among the runner and five tests among the spanish genotypes resulted in significant differences in stem discoloration (Table 4). All six tests were at the 50 mM concentration. Significant differences in length of discoloration in the runner entries were observed only on lateral stems immersed in 50 mM oxalic acid for 24 hr. ARSOK-R143-3, Okrun, and Florunner had significantly more discoloration than ARSOK-R35 (Table 4; Fig. 2B). Five tests of the spanish genotypes resulted in significant differences among cultivars in stem discoloration; however, severity of stem discoloration was less correlated with field resistance (Table 4). After 18 hr of immersion, lateral stems of Tamnut OL06 were more discolored than ARSOK-S140-1OL, ARSOK-S1, and ARSOK-S133-3. Similar results were obtained after 24 hr: Tamnut 06 had the greatest stem discoloration, and ARSOK-S1 and ARSOK-S133-3 had the least (Table 4; Fig. 2F). Significant differences in discoloration were observed for all times of exposure at 50 mM in the main stems. After 12 hr of immersion, main stems of ARSOK-S133-3, ARSOK-S140-1OL, and Spanco were more discolored than Tamspan 90, Tamnut 06 and ARSOK-S1. After 18 and 24 hr, ARSOK-S140-1OL had more stem discoloration than all other cultivars except ARSOK-S133-3 (Table 4; Fig. 2H).

Enzymes for breaking down oxalate are present in multiple cereal species, but they are absent from many other crops, including peanuts (Livingstone *et al.*, 2005). Several crop species have been transformed with oxalate-degrading enzymes to create plants resistant to *Sclerotinia* spp. (Cunha *et al.*, 2010; Dias *et al.*, 2006; Hu *et al.*, 2003; Livingstone *et al.*, 2005). Virginia peanut cultivars genetically transformed with barley oxidase were also highly resistant to *Sclerotinia* blight in both the lab (Livingstone *et al.*, 2005) and field (Partridge-Telenko *et al.*, 2011). Other, though less-effective, mechanisms for limiting damage from oxalic acid are naturally present in soybean (Wegulo *et al.*, 1995) and common bean (Kolkman and Kelly, 2000; Tu, 1985). Tu (1985) observed differences between susceptible and resistant cultivars of *Phaseolus vulgaris* in petiole uptake and vein delimitation of oxalic acid. Similar mechanisms may be present in runner peanuts. We found Okrun and Florunner, cultivars known to be highly susceptible to *S. minor* in the field, consistently exhibited the most severe wilt symptoms when

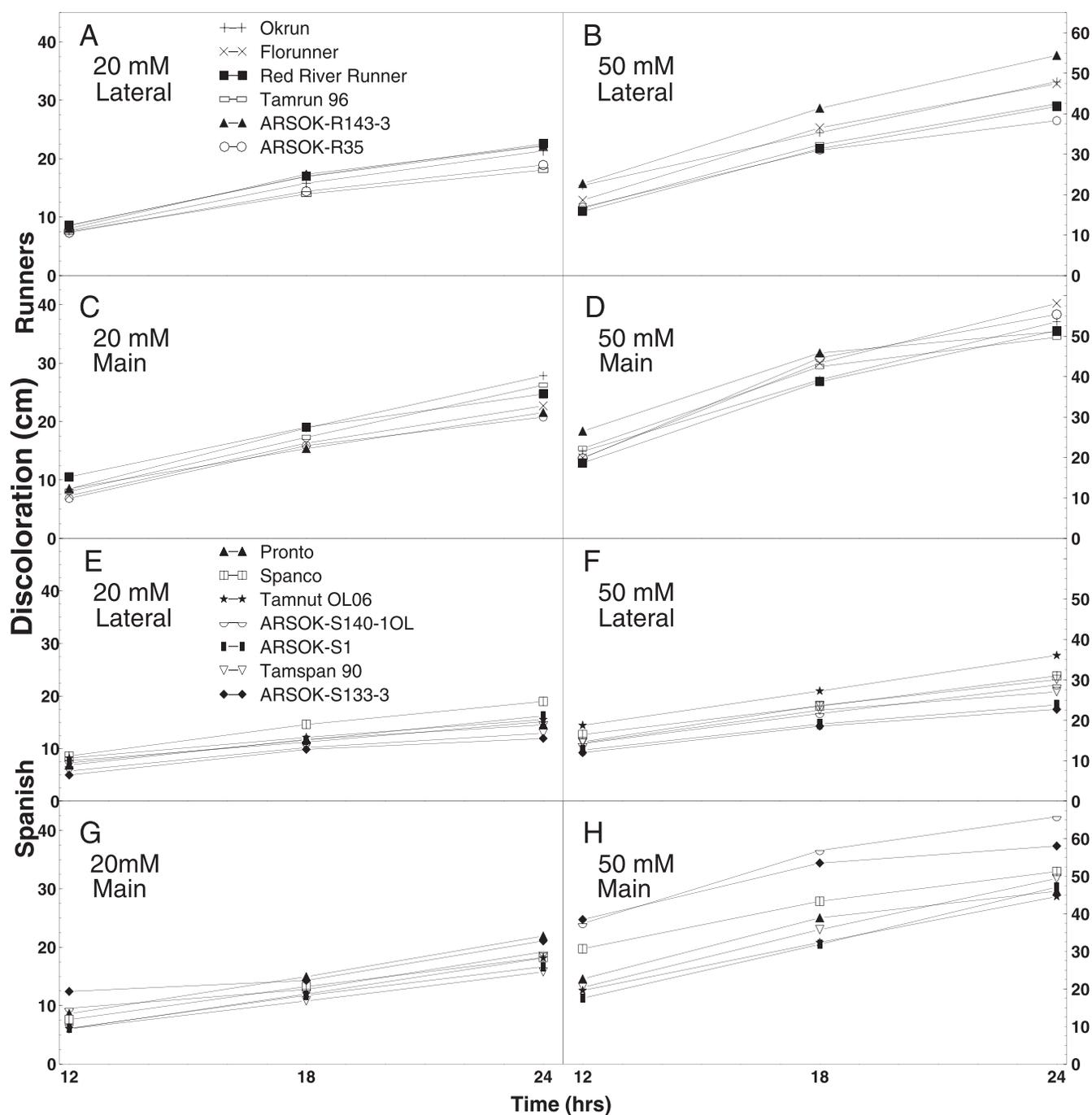


Fig. 2. Mean length of stem discoloration in runner (Okrun, Florunner, Red River Runner, Tamrun 96, ARSOK-R143-3, and ARSOK-R35; A-D) and spanish peanut genotypes (Pronto, Spanco, Tamnut OL06, ARSOK-S140-1OL, ARSOK-S1, Tamspan 90, ARSOK-S133-3; E-H). Stem discoloration in lateral and main stems of peanuts were measured after being immersed in 20 mM or 50 mM oxalic acid for 12, 18, and 24 hr.

exposed to oxalic acid. The oxalic acid concentrations and immersion times used here, which resulted in significant differences among cultivars, may be useful starting points for identifying physiologically susceptible runner genotypes.

Wilt symptoms in spanish peanuts, and stem discoloration in both runner and spanish peanuts, appeared to be less correlated to known field resistance. A small number of spanish genotypes

were used in these experiments, and we may have unknowingly chosen cultivars where canopy characteristics exert a strong influence on field resistance. Improved correlations may be obtained from a different panel of spanish entries, or by using older plants or stems (Brenneman *et al.*, 1988), but these preliminary results indicate that oxalic acid may be of limited use as a resistance assay in spanish peanuts.

Acknowledgements

The authors thank Lisa Myers and Kenneth Jackson for technical assistance. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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