Phenotypic Variation of Peanut Smut (*Thecaphora frezii*) Incidence and Severity in the U.S. Peanut Mini-Core Collection

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ABSTRACT

Peanut smut is an emergent soilborne disease of peanut in South America that has significantly impacted the commercial peanut industry in Argentina. In response, plant breeders are in need of information about potential sources of smut resistance in cultivated germplasm for the rapid development of resistant cultivars. Available U.S. peanut mini-core accessions were evaluated under naturally-infested soil conditions in 2016-2019 near General Cabrera, Córdoba, Argentina, in addition to three susceptible breeding lines and four local commercial controls. Over three years, 18 mini-core accessions and two germplasm collection accessions exhibited no smut incidence in a 100-pod sample. Of those, 12 mini-core accessions and one germplasm collection accession (PI 153323) exhibited no smut incidence when all available pods were opened and examined in the 2016-2017 and 2017-2018 crop years. These 13 accessions were collected from a variety of origins across the Americas, Africa, and Asia; only three were collected from origins in South America. These results suggest that resistance mechanisms may be well-conserved across various groups within *Arachis hypogaea* L. The 13 identified accessions appear to be sources of resistance to peanut smut in *A. hypogaea* and would likely be good parent material for the development of new, resistant commercial peanut cultivars.

Key Words: *Arachis hypogaea* L., germplasm, tetraploid, resistance

Peanut smut, caused by the fungal pathogen *Thecaphora frezii* Carranza & Lindquist, is an emergent disease of peanut (*Arachis hypogaea* L.) in South America that has significantly impacted the commercial Argentine peanut industry (Rago *et al*., 2017). Originally identified on wild peanut species in Brazil in 1962, peanut smut was first reported on commercial peanuts in the 1994/1995 crop year in Argentina and quickly spread to 100% of the Argentine production areas by the 2011/2012 season (Carranza and Lindquist, 1962; Marinelli *et al*., 1995; Rago *et al*., 2017). Nationwide, mean annual production losses of 3.15% have been reported, which equate to approximately US$14,151,800; yield losses of up to 35% from smut have been reported in the more heavily-infested regions of the central-northern area of Córdoba Province. There are also global phytosanitary implications, as Argentina is currently one of the world's largest exporters of peanuts (Cazón *et al*., 2018; Oddino *et al*., 2010; Marinelli *et al*., 2008; Rago *et al*., 2017).

The management of peanut smut has been difficult, as various fungicide mixtures, seed treatments, and biocontrol measures have performed inconsistently on direct control of the pathogen (Arias *et al*., 2019; Cazón *et al*., 2018; Figueredo *et al*., 2017; Ganuza *et al*., 2017; Rago *et al*., 2017). Host plant resistance has shown potential as an effective tool for mitigating losses from peanut smut on commercial production, including some evidence of induced systemic resistance via inoculation by a specific strain of *Bacillus* spp. (Figueredo *et al*., 2017; Rago *et al*., 2017; Tonelli *et al*., 2011). A few commercial cultivars have historically displayed moderate resistance, including up to 52% reduced smut incidence (Farías *et al*., 2011; Oddino *et al*., 2013). One new cultivar was recently released with high levels of resistance to smut infection (A. Falco, personal communication).

For the continued development of new, smut-resistant peanut cultivars, it is necessary to identify reliable sources of resistance that can serve as useful parent material for hybridization and population development. Resistance has been identified and documented in a number of wild diploid *Arachis* species and Bolivian landraces (de Blas *et al*., 2019; Oddino *et al*., 2017; Soave et al., 2014). Bressano *et al*. (2019) and de Blas *et al*. (2019) demonstrated that the smut resistance trait could also be successfully introgressed into tetraploid germplasm, with relatively high transmission; Bressano *et al*. successfully utilized resistant *A. hypogaea* subsp. *fastigiata* landraces as parents, whereas de Blas *et al*. introgressed resistance from a wild, tri-species amphidiploid [(*A. cardenasii* × *A. correntina*) × *A. batizocoi*]$^{4\times}$. Little has been published, however, on the phenotypic variation of smut incidence within cultivated peanut. Iden-

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tifying resistance in cultivated, tetraploid peanut genotypes could help provide more readily-useable sources of resistance for commercial cultivar development, without the necessary pre-breeding or chromosome doubling to prepare/convert diploid germplasm for hybridization with elite tetraploid germplasm. Therefore, the purpose of this study was to evaluate the incidence and severity of peanut smut on the core of the core (or "mini-core") of the U.S. peanut germplasm collection (Holbrook and Dong, 2005) *in situ* in Argentina.

Materials and Methods

This study was modeled after similar germplasm screenings published in cotton (*Gossypium hirsutum* L.) by Basal *et al.* (2003) and in the U.S. peanut mini-core by Dean *et al.* (2009); it was also arranged in the field similarly to the peanut mini-core evaluation reported by Upadhyaya (2005). Field trials were conducted with the Maniagro S.A. peanut company, at the La Riojana research farm near General Cabrera, in the Córdoba Province of Argentina, in the 2016-2017, 2017-2018, and 2018-2019 crop years. All plots were planted on 31 Oct. 2016, 27 Oct. 2017, and 24 Oct. 2018, by hand-dribbling seed through a 2-row Monosem precision air planter (Monosem, Inc., Edwardsville, KS) at an approximate seeding rate of 3.2 seed/m and depth of 6 cm. Fresh seed for each planted mini-core accession was obtained from the USDA National Plant Germplasm System (NPGS) peanut collection in Griffin, GA for planting in 2016, therefore quantities were limited to only 25 seed per plot in the 2016-2017 season. As a result, individual plot dimensions in 2016-2017 were 1.8 m (2 rows) x 2 m. Seed harvested from each genotype in 2016-2017 was kept and replanted for evaluation in 2017-2018; therefore, plot dimensions were larger in 2017-2018 and 2018-2019, measuring 1.8 m x 6 m. Row spacing was 91 cm each year. All plots were planted such that planting began and ended in the alleys between plots, then alleys were hand-cleared of additional peanut seedlings after emergence. Additionally, all plots were planted in minimal tillage (disc-harrowed soil only) into wheat (*Triticum aestivum* L.) stubble every year, in a dryland production scenario with consistently heavy natural peanut smut pressure each year. Overall management throughout the growing season was conducted according to local guidelines for commercial production.

At the beginning of this study, only the 104 U.S. peanut mini-core accessions registered by Chen *et al.* (2014) were available for public distribution in the NPGS collection. Thus, these 104 accessions were obtained and planted in 2016-2017, along with five additional accessions from the NPGS germplasm collection. Three advanced, uniform breeding lines (14-1-0066, 16-1-0033, and 16-1-0089), selected from a breeding program operated by International Peanut Group and Maniagro S.A., were used as susceptible controls. Four local Argentine cultivars were also included as commercial controls (though not resistant), including cultivars Granoleico (INASE Reg. No. 7907), MA-88 (INASE Reg. No. 17235), MA-757 (INASE Reg. No. 17240), and MA-767 (INASE Reg. No. 17234). Five of the planted mini-core accessions in 2016-2017 did not germinate and were therefore excluded from the final overall analyses. However, new seed for four of those accessions was reobtained from the NPGS collection and planted in 2017-2018. One accession (PI 429420) was planted only in 2018-2019, due to limited seed availability and poor emergence the previous years. All plots were arranged randomly each year for three subsequent years. Therefore, experimental design was a randomized complete block, blocked by year (three total blocks), with genotype as treatment.

Plots were naturally infested with peanut smut each year. The location of the field trials was in an area with historically-elevated levels of smut incidence, near two large commercial peanut shellers in General Cabrera and nearby Carnerillo. Thus, ambient soil teliospore density at the field location was approximately 4500 teliospores/g soil during the course of these evaluations, which would be classified as "highly-infested" (Oddino *et al.*, 2010). All plots were dug on 10 April 2017, 13 April 2018, and 8 April 2019, which were 161 days after planting (DAP), 168 DAP, and 166 DAP, respectively. A 100-pod sample of each plot was randomly collected immediately after digging for determination of smut incidence and severity. All pods from each sample were individually hand-opened and visually rated for smut infection; affected pods were rated using a 0-4 visual scale developed by Astiz Gassó *et al.* (2008) and photographically documented by Cazón *et al.* (2018), de Blas *et al.* (2019), and Rago *et al.* (2017). Any pod with a scored value equal to or greater than "1" was considered affected. Smut incidence was subsequently expressed a percentage of the whole sample. Mean severity values of affected pods for each genotype were calculated using the following equation:

$$\text{Severity} = (1x_1 + 2x_2 + 3x_3 + 4x_4)/(\text{total pods})$$

where x_n is the number of pods with n severity

grade and "total pods" is the number of total affected pods identified in a sample.

In 2016-2017, given the small plot sizes, some plots did not produce greater than 100 total pods; in these cases, all available pods were opened and rated for smut incidence and resultant incidence expressed as a percentage of the whole. There was sufficient production from plots in 2017-2018 and 2018-2019 to provide at least 100 pods per plot for analysis, including for all of the missing/non-emergent genotypes in 2016-2017 (except for PI 429420). There was sufficient production of PI 429420 in 2018-2019 to evaluate at least 100 pods. In 2016-2017 and 2017-2018, in plots with 0.0% smut incidence from the 100-pod samples, all of the remaining pods from each respective plot were opened and analyzed, to determine disease incidence in a larger sample size.

Statistical analyses were conducted with R software (R Core Team, 2016), using the Agricolae package. All data were analyzed by analysis of variance and means were separated via Tukey's HSD Test ($P \leq 0.05$) for the main genotype comparison and Fisher's Least Significant Difference Test ($P \leq 0.05$) for the comparison of the smaller group of genotypes in 2017-2018 and 2018-2019. Pearson and Spearman correlation analyses between smut incidence and severity values were also conducted in R, using the "cor.test" function.

Results and Discussion

Significant genetic variation for both smut incidence and severity was found among the evaluated genotypes, although genotype and block were both significant in these evaluations ($P \leq 0.05$; data not shown). As mentioned above, this experiment was blocked by year, so the significance in block was likely due to the natural year-to-year environmental variation inherent in utilizing naturally-infested soils. However, blocking the experiment in time minimized confounding year-to-year effects on genotype. Additionally, a significant genotype effect across varying levels of natural infestation indicates stable phenotypic expression of both resistance and susceptibility.

Smut Incidence and Severity

Mean smut incidence ranged 0.0 - 23.0% in the evaluated mini-core and germplasm collection accessions, whereas the susceptible controls ranged 16.6 - 18.6% and the local evaluated cultivars ranged 11.0 - 16.3% (Table 1). Approximately 20 germplasm accessions exhibited no observable incidence of smut over all three years; of those 20, two accessions (PI 119204 and PI 153323) were germplasm accessions that are not designated as part of the official mini-core collection. Additionally, all of those accessions had less smut incidence than the three susceptible controls and four commercial cultivars ($P \leq 0.05$). One accession (PI 497517) exhibited the greatest level of smut incidence (23.0%) which made it greater than all other evaluated mini-core and germplasm accessions ($P \leq 0.05$). Thirty-three germplasm accessions had lesser smut incidence than the susceptible controls and the evaluated cultivars, the least of which (0.0%) constituting more than a 10-fold reduction in comparative incidence than the commercial controls.

Mean severity of smut infection ranged 0.00 - 3.06 among the mini-core and germplasm collection accessions (Table 1); susceptible controls were 2.60 - 2.66 and evaluated cultivars ranged 2.54 - 2.63, but were not different ($P > 0.05$). The 20 mini-core and germplasm accessions with no smut incidence also had smut severity ratings of "0.00"; therefore, all of those accessions had lesser severity than the susceptible controls and the four commercial cultivars ($P \leq 0.05$). Correlation analyses between smut incidence and severity were conducted only on the affected pods rated during the course of these evaluations. Severity was negatively correlated with percent incidence ($P \leq 0.05$), with a Pearson correlation coefficient of - 0.148; the Spearman correlation coefficient was - 0.214. These correlation results suggest that smut severity decreases as overall incidence increases, indicating that there is varied progression of the disease throughout infected pods on a given plant. Since smut infection does not occur until peanut pegs reach the soil, this differential incidence/severity rate is likely linked to the variable pegging timing and subsequent maturity of different pods (Marraro Acuña *et al.*, 2013). However, given the relatively low correlation coefficients for both analyses it is also likely that different genotypes have differential rates of smut severity, despite large or small levels of infestation—this would indicate that some genotypes could have a substantial incidence of smut, but at a more commercially-tolerable level.

The results of the mini-core accessions that had no emergence in the 2016-2017 evaluation are presented in Table 2. One accession (PI 429420) again had no emergence in 2017-2018, so it was excluded from the statistical analyses. Genotype was significant ($P \leq 0.05$) for both smut incidence and severity; block was not significant for either parameter ($P > 0.05$; data not shown). Smut incidence ranged 0.0 - 13.0% among the additional mini-core and germplasm accessions; the suscepti-

Table 1. Incidence and severity of peanut smut (100-pod samples) on 97 peanut mini-core accessions, five other germplasm collection accessions, three susceptible breeding lines, and four local commercial cultivars near General Cabrera, Argentina in 2016-2019.

		Peanut Smut				Peanut Smut	
PI	Type[a]	Incidence	Severity	PI	Type	Incidence	Severity
		%	0-4			%	0-4
119204	G	0.0 e	0.00 b	475918	MC	8.3 a-e	1.52 ab
153323	G	0.0 e	0.00 b	493693	MC	8.3 a-e	1.99 ab
240560	MC	0.0 e	0.00 b	497639	MC	8.6 a-e	2.12 ab
259617	MC	0.0 e	0.00 b	259658	MC	8.6 a-e	2.71 ab
268696	MC	0.0 e	0.00 b	259836	MC	8.6 a-e	2.30 ab
155107	MC	0.0 e	0.00 b	259851	MC	8.6 a-e	2.30 ab
268806	MC	0.0 e	0.00 b	200441	MC	9.0 a-e	1.87 ab
270905	MC	0.0 e	0.00 b	268586	MC	9.0 a-e	2.18 ab
274193	MC	0.0 e	0.00 b	331297	MC	9.0 a-e	2.40 ab
288210	MC	0.0 e	0.00 b	162655	MC	9.0 a-e	2.49 ab
290566	MC	0.0 e	0.00 b	407667	MC	9.0 a-e	1.45 ab
313129	MC	0.0 e	0.00 b	476432	MC	9.0 a-e	1.32 ab
337399	MC	0.0 e	0.00 b	290560	MC	9.3 a-e	2.13 ab
337406	MC	0.0 e	0.00 b	355271	MC	9.3 a-e	2.46 ab
478850	MC	0.0 e	0.00 b	372305	MC	9.3 a-e	2.20 ab
481795	MC	0.0 e	0.00 b	493356	MC	9.3 a-e	2.21 ab
482120	MC	0.0 e	0.00 b	493581	MC	9.3 a-e	1.17 ab
482189	MC	0.0 e	0.00 b	262038	MC	9.6 a-e	2.41 ab
494018	MC	0.0 e	0.00 b	270786	MC	9.6 a-e	2.11 ab
497395	MC	0.0 e	0.00 b	399581	MC	10.0 a-e	2.21 ab
270998	MC	0.3 de	1.33 ab	493631	MC	10.0 a-e	2.82 ab
461434	MC	0.3 de	0.89 ab	493717	MC	10.0 a-e	2.23 ab
471954	MC	0.3 de	2.12 ab	496448	MC	10.0 a-e	2.22 ab
475863	MC	0.3 de	1.41 ab	331314	MC	10.3 a-e	2.56 ab
494034	MC	0.3 de	1.33 ab	461427	MC	10.3 a-e	1.23 ab
268868	MC	0.6 de	0.89 ab	295309	MC	10.6 a-e	2.27 ab
290536	MC	0.6 de	0.89 ab	442768	MC	10.6 a-e	2.54 ab
288146	MC	1.6 c-e	2.49 ab	370331	MC	11.0 a-e	2.30 ab
471952	MC	1.6 c-e	2.10 ab	403813	MC	11.0 a-e	1.90 ab
268755	MC	2.0 c-e	0.44 ab	196622	MC	11.3 a-e	2.36 ab
504614	MC	3.0 c-e	1.62 ab	268948	G	11.6 a-e	2.20 ab
338338	MC	3.3 c-e	1.52 ab	476025	MC	11.6 a-e	2.47 ab
493329	MC	4.0 b-e	1.63 ab	343384	MC	12.0 a-e	2.47 ab
493547	MC	4.3 b-e	1.31 ab	196635	MC	12.3 a-e	2.46 ab
157542	MC	4.6 b-e	2.22 ab	336941	G	12.6 a-e	2.26 ab
496401	MC	4.6 b-e	2.54 ab	295250	MC	13.0 a-e	1.93 ab
271019	MC	5.0 b-e	2.43 ab	298854	MC	13.0 a-e	2.59 ab
319768	MC	5.0 b-e	2.59 ab	478819	MC	13.0 a-e	2.11 ab
343398	MC	5.0 b-e	3.06 a	497318	MC	13.0 a-e	2.04 ab
162857	MC	5.0 b-e	1.71 b-l	372271	MC	13.6 a-e	2.11 ab
337293	MC	5.6 b-e	2.77 a-c	152146	MC	14.0 a-e	2.17 ab
493729	MC	6.0 b-e	1.43 d-l	149636	G	14.0 a-e	2.43 ab
502120	MC	6.3 b-e	2.50 a-i	295730	MC	14.0 a-e	2.39 ab
292950	MC	6.6 b-e	2.03 a-k	296550	MC	14.0 a-e	2.02 ab
296558	MC	6.6 b-e	1.79 a-k	159786	MC	15.3 b-d	2.25 ab
355268	MC	6.6 b-e	1.61 b-l	497517	MC	23.0 a	2.57 ab
268996	MC	7.0 b-e	2.60 a-f				
290594	MC	7.0 b-e	2.16 a-k				
356004	MC	7.3 b-e	2.37 a-j	14-1-0066	S	18.6 ab	2.60 ab
493938	MC	7.3 b-e	1.82 a-k	16-1-0033	S	18.6 ab	2.66 ab
323268	MC	7.6 b-e	2.17 a-k	16-1-0089	S	16.6 a-c	2.63 ab
339960	MC	7.6 b-e	2.13 a-k				

Table 1. Continued.

PI	Type[a]	Peanut Smut		PI	Type	Peanut Smut	
		Incidence	Severity			Incidence	Severity
158854	MC	8.0 a-e	2.37 a-j	Granoleico	CC	16.3 a-c	2.58 ab
325943	MC	8.0 a-e	2.48 a-i	MA-88	CC	13.6 a-e	2.57 ab
502111	MC	8.3 a-e	1.33 f-l	MA-757	CC	11.0 a-e	2.54 ab
290620	MC	8.3 a-e	1.91 a-k	MA-767	CC	13.6 a-e	2.63 ab

Means within a column followed by the same letter are not different according to Tukey's HSD Test at $P = 0.05$.

[a]Type of evaluated material (G, other germplasm collection accession; CC, local commercial cultivar; MC, mini-core accession; S, susceptible control).

ble controls ranged 20.5 - 22.5% and the commercial cultivars were 11.0 - 18.0%. Of the evaluated germplasm accessions, PI 494795 was the only one to exhibit no smut incidence both years. Smut severity ranged 0.00 - 2.64; PI 494795 had a lower severity (0.00) than all of the evaluated genotypes in this group ($P \leq 0.05$).

Resistant Genotypes and Geographic Origins

Genotypes found to have 0.0% smut incidence from both sampling methods are presented in Table 3. Again, all of the pods from the harvested plots in 2016-2017 and 2017-2018 for these genotypes were opened and found to have no smut incidence. In total, there were 13 genotypes that had 0.0% incidence in all evaluated pods: 12 mini-core accessions and a germplasm accession (PI 372335). The total number of evaluated pods per genotype ranged 15 - 1064. It is likely that these genotypes are resistant to infection by *T. frezii* and would be potential sources of resistance in *A. hypogaea* to use for introgression into breeding populations and eventually commercial cultivars.

It was hypothesized by the authors that these 13 accessions could have originated in regions of South America with significant ambient *T. frezii* populations, thereby suggesting possible co-development of resistant germplasm with the increased presence of the pathogen. Table 4 presents the name, geographic collection origin and date, and taxon information for the 13 resistant accessions, adapted from information published by Chen *et al.* (2014) and data currently available in the NPGS

Table 2. Additional evaluation (100-pod samples) of peanut smut incidence and severity on seven peanut mini-core accessions, one germplasm collection accession (which were either missing or did not emerge in the 2016-2017 evaluation), three susceptible breeding lines, and four local commercial cultivars near General Cabrera, Argentina in 2017-2019.

PI	Type[a]	Peanut Smut	
		Incidence	Severity
		%	0-4
494795	MC	0.0 f	0.00 c
270907	MC	1.5 ef	1.33 b
493880	MC	5.5 d-f	2.26 ab
429420[b]	MC	8.0	1.12
371521	MC	9.0 c-f	1.73 ab
502040	MC	9.5 c-f	2.64 a
372335	G	10.0 c-e	2.15 ab
476636	MC	13.0 a-d	1.87 ab
14-1-0066	S	21.0 a	2.48 ab
16-1-0033	S	22.5 a	2.53 a
16-1-0089	S	20.5 ab	2.62 a
Granoleico	CC	18.0 a-c	2.59 a
MA-88	CC	14.5 a-d	2.53 a
MA-757	CC	11.0 b-e	2.50 ab
MA-767	CC	15.0 a-d	2.53 a

Means within a column followed by the same letter are not different according to Fisher's Least Significant Difference test at $P = 0.05$.

[a]Type of evaluated material (G, other germplasm collection accession; CC, local commercial cultivar; MC, mini-core accession; S, susceptible control).

[b]Was not present in both years of evaluation and was therefore excluded from statistical analyses.

Table 3. Evaluated U.S. peanut mini-core and germplasm collection accessions with no incidence of peanut smut across three site-year locations near General Cabrera, Argentina, 2016-2019.

PI	Type[a]	No. of Sampled Pods		
		2016-2017	2017-2018	2018-2019
153323	G	339	500	100
155107	MC	110	596	100
240560	MC	100	940	100
259617	MC	405	986	100
268806	MC	410	699	100
288210	MC	350	1064	100
290566	MC	160	750	100
337399	MC	15	849	100
337406	MC	234	700	100
478850	MC	220	551	100
482120	MC	290	971	100
482189	MC	210	930	100
494018	MC	280	640	100

[a]Type of germplasm accession (MC, mini-core accession; G, other germplasm collection accession).

Table 4. Descriptions of mini-core and other germplasm accessions with no incidence of peanut smut near General Cabrera, Argentina in 2016-2019.[a]

PI	Accession Name	Collection Origin	Year Donated[b]	Taxon
153323	CC335	South Africa	1946	*A. hypogaea* L.
155107	LE 39 Aceitero Federacion	Uruguay	1946	*A. hypogaea* L. supbsp. *fastigiata* var. *vulgaris*
240560	Natal Common	South Africa	1957	*A. hypogaea* L. supbsp. *hypogaea* var. *hypogaea*
259617	No. 15233	Cuba	1959	*A. hypogaea* L. supbsp. *fastigiata* var. *vulgaris*
268806	SB152	Zambia	1960	*A. hypogaea* L. supbsp. *hypogaea* var. *hypogaea*
288210	526	India	—	*A. hypogaea* L. supbsp. *fastigiata* var. *vulgaris*
290566	SI 35	India	1963	*A. hypogaea* L. supbsp. *fastigiata* var. *fastigiata*
337399	White Spanish 32	Morocco	1968	*A. hypogaea* L. supbsp. *hypogaea* var. *hypogaea*
337406	Fav 153	Paraguay	1968	*A. hypogaea* L. supbsp. *fastigiata* var. *fastigiata*
478850	ICG 2716 (EC76446)	Uganda	—	*A. hypogaea* L. supbsp. *fastigiata* var. *fastigiata*
482120	Kaboko	Zimbabwe	1983	*A. hypogaea* L. supbsp. *hypogaea* var. *hypogaea*
482189	Kasawaira	Zimbabwe	1983	*A. hypogaea* L. supbsp. *fastigiata* var. *fastigiata*
494018	RCM 710	Argentina	1984	*A. hypogaea* L. supbsp. *fastigiata* var. *vulgaris*

[a]From information published by Chen *et al*. (2014) and GRIN (2019).
[b]Refers to the date that accession was added to the U.S. National Plant Germplasm System collection.

Genetic Resources Information Network (GRIN, 2019) for the material. The accessions were collected from a wide array of geographic origins spanning the Americas, Africa, and Asia. Only three accessions (PI 155107, PI 337406, and PI 494018) were collected from locations in South America (Uruguay, Paraguay, and Argentina, respectively). Therefore, this information does not directly suggest that these resistant accessions developed in concert with the concurrent development and proliferation of the *T. frezii* pathogen on peanut. However, it does suggest that mechanisms of resistance may be well-conserved across the various collected accessions within *A. hypogaea*. Development of resistance may have occurred in a common progenitor of these lines prior to the distribution of *A. hypogaea* to other continents. Alternatively, these resistant accessions may also be carrying different mechanisms of resistance. Therefore, genomic analysis and further phenotypic analysis would be necessary to elucidate the phylogenetic relationships among these resistant accessions and identify the subsequent mechanisms responsible for conferring resistance. Identifying different mechanisms of resistance to peanut smut would allow plant breeders to introgress multiple resistance loci into cultivars, thereby reducing the likelihood of *T. frezii* overcoming host plant resistance in the field.

Summary and Conclusions

The data presented herein suggest that the incidence and severity of peanut smut varies significantly within *A. hypogaea* and that there are potential sources of resistance within the U.S. peanut mini-core collection. While this was a screening of a limited subset of germplasm accessions, identifying resistant accessions within the mini-core will inform more targeted screening of related *A. hypogaea* groups in the larger germplasm collections worldwide. This information will be valuable for plant breeders wanting to develop smut-resistant cultivars. The identification of both resistant and susceptible germplasm will also be useful for elucidating the genetic nature of smut resistance in peanut, as well as aiding in genomic marker development.

Over three years, 18 mini-core accessions and two germplasm collection accessions (PI 153323 and PI 119204) exhibited no smut incidence in a 100-pod sample, with respective severity values of "0.00". One additional mini-core accession (PI 494795) also exhibited 0.0% smut incidence in 2017-2018 and 2018-2019. There were 12 mini-core accessions and one germplasm collection accession that had no smut incidence when all harvested pods were opened and inspected in 2016-2017 and 2017-2018. These 13 accessions would likely be good sources of resistance for research and cultivar development. Identifying such resistance in *A. hypogaea* provides tetraploid sources for introgressing smut resistance into new, elite peanut cultivars or breeding populations, without requiring pre-breeding of resistant diploid germplasm. Additionally, using *A. hypogaea* sources of resistance can help reduce potential linkage drag of undesirable alleles from resistant wild *Arachis* species, as evidenced in other crops (Wann *et al*., 2017). However, depending on the source, using a tetraploid source of resistance may make it more complicated to pyramid resistance from both

subgenomes in peanut, as described by de Blas *et al*. (2019).

The 13 resistant accessions identified in these evaluations represent both the *fastigiata* and *hypogaea* subspecies of *A. hypogaea*. Only three of these were collected in South America, which does not suggest potential co-development of resistant germplasm commensurate with the historic proliferation of *T. frezii* on peanut in South America. However, this does suggest that mechanisms of resistance to *T. frezii* incidence may be well-conserved across *A. hypogaea* germplasm—this could aid in identifying the specific mechanism(s) of smut resistance in many different groups of cultivated peanut, which would thereby increase the efficiency of selection for plant breeders in the development of resistant cultivars. Nevertheless, further research is needed to identify these specific mechanisms of resistance and to identify other groups of *A. hypogaea* with resistance. Additionally, more information is needed on the genetic nature of smut resistance in peanut, to better utilize the trait and maximize its stability in elite germplasm.

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