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ARTICLE

Identification of Germplasm Resistant to Peanut Smut

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ABSTRACT

Peanut smut, caused by *Thecaphora frezzii*, is an emerging threat to global peanut production. Found in 100% of Argentinian peanut growing regions, smut infestation can result in substantial yield reductions. Although peanut smut has not been reported outside of South America, immediate proactive measures must be taken so that global peanut production will not be threatened. The first step in preventative breeding for resistance to peanut smut is to identify sources of resistance. Therefore, the objective of this study was to identify germplasm resistant to *T. frezzii* that can be used to incorporate smut resistance into cultivars optimized for U.S. peanut production areas. In this study, peanut genotypes, including accessions from the USDA germplasm collection that were purified by single-seed descent, peanut breeding lines, and U.S. cultivars, were screened for resistance. Trials were planted in test plots highly infested with *T. frezzii* in Córdoba Province, Argentina. For screening purposes, entries were retained for future testing if they scored 10% or less disease incidence. Among the entries tested in the 2017-2020 growing seasons, potential new sources of peanut smut resistance were noted. Eight USDA peanut mini-core accessions were identified as immune, demonstrating 0% incidence for three years. These accessions are being used to incorporate smut resistance into cultivars suitable for production in all growing regions of the U.S.

INTRODUCTION

Global peanut production was valued at approximately \$47 billion in 2020, with over \$1 billion attributed to the U.S. crop. (FAOSTAT, 2020). It is the third major oilseed of the world behind soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.). Peanuts are high in protein, fiber, tocopherols, unsaturated fatty acids, and other nutritionally

advantageous compounds (Ros, 2010), and are used for oil extraction, edible food products and confectionary purposes across the globe. Sustainable and profitable peanut production in the U.S. is maintained by the release of improved disease-resistant cultivars, but resistance to all threatening pathogens is not currently available.

Peanut smut, a soilborne fungal disease caused by *Thecaphora frezzii*, was first described by Carranza and Lindquist (1962) infecting wild peanut in Brazil. The disease did not appear in commercial peanut fields until 1995, when it

was reported in Argentina (Marinelli et al., 1995). Pods of plants infected by *T. frezzii* may be hypertrophied (Cazón et al., 2018). Colonized cells in the seed pods are deformed and replaced with a smutted mass (sorus) formed by teliospores, which are the inoculum source for the disease (Rago et al., 2017). Once in the soil, spores of the pathogen have been observed to remain infective for more than four years (Cazón et al., 2016). *Thecaphora frezzii* spores are easily spread by wind and weather events and the spread of the pathogen is sporadic and unpredictable. Symptoms of peanut smut infection are not visually apparent on plant foliage above ground but are revealed upon digging and shelling of peanut pods. Furthermore, pathogen spores may be concealed in peanut seed (Cazón and Rago, 2016), making visual detection less accurate.

Peanut smut has spread throughout Argentina and is now reported to be in 100% of Argentine peanut-producing fields. Disease incidence and severity varies with location but yield reductions as high as 51% have been reported (Cazón et al., 2018; Rago et al., 2017). Integrated pest management of the peanut smut has been limited. Ganuza et al. (2017) reported that treatment with the biocontrol agent *Trichoderma harzianum* ITEM 3636 may reduce peanut smut severity by up to 25%. Fungicidal management of the pathogen has proven largely ineffective. However, a report discussing a mitogenome of *T. frezzii* identified possible fungicide target genes (Arias et al., 2019), and a recent study indicated that cyproconazole or azoxystrobin application can reduce incidence significantly (Parades et al., 2021), particularly in nanocrystal-based formulations (Camilletti et al., 2021).

Host-plant resistance to peanut smut should provide sustainable protection against the disease. Resistance has been discovered among several wild *Arachis* species and landraces (Bressano et al., 2019; de Blas et al., 2019), and attempts to introgress resistance to smut from wild to cultivated peanut have been made (Bressano et al., 2019). More recently some accessions in the USDA peanut mini-core collection (Holbrook and Dong, 2005) were screened for phenotypic reaction to smut infection (Wann et al., 2020). Peanut cultivars released in the past have shown only moderate resistance to smut (Fariás et al., 2011; Oddino et al., 2013). A recent release in Argentina demonstrates resistance (https://www.criaderoelcarmen.com.ar/es/variedades.html), and the agronomic performance of that cultivar is still being investigated.

The rapid spread of this disease and lack of control measures have caused great concern in Argentina and within peanut research and production communities worldwide. Although peanut smut has not been reported in cultivated peanut outside of Argentina, proactive measures must be taken to protect the global peanut industry. Therefore, the objective of this research was to identify sources of smut resistance by screening purified peanut germplasm from the USDA peanut mini-core collection along with selected U.S. peanut cultivars and breeding lines.

MATERIALS AND METHODS

Site Description.

A smut screening test was carried out across 3 seasons (2017-2020) in fields adjacent to a farm (32°45'03.6"S 63°45'56.3"W) in the town of General Deheza (Córdoba Province, Argentina) under rainfed conditions. Soil at the site was a General Deheza coarse-silty sandy loam type (0.3% slope, coarse-silty, mixed, thermic, Entic Haplustoll). The test was planted one kilometer downwind from a peanut processing plant where peanut smut is prevalent. The production field site was on a rotation schedule of *Zea mays* L.-*Zea mays* L.-*Glycine max* (L.) Merr-*Arachis hypogaea* L. (corn-corn-soybean-peanut), where the preceding crop of the test site was always soybean.

Plant Material.

Plant materials screened in this study included 208 peanut genotypes including accessions from the USDA peanut germplasm collection, U.S. peanut breeding lines, and released U.S. cultivars (Table 1). All entries/genotypes were purified by single-seed descent (SSD) in greenhouses and seed was increased from each purified genotype prior to shipment and testing. Seeds of each entry were packaged and shipped from the U.S. to Argentina approximately two months prior to planting. Remnant seed was kept in the U.S. and stored at 4°C as potential sources of smut resistance in breeding programs. Entries demonstrating ≤ 10% disease incidence were retained for testing the following year, but all others were discarded. Therefore, the number of years each entry was tested varied from one to three (Table 1).

Table 1. Complete listing of peanut entries tested for resistance to peanut smut during 2017-2020, along with number of years tested, total pods tested per year, and percent disease incidence per year.

Entry No. ^a	Entry ID	Country of Collection ^b	Type ^c	Seed Source ^d	Years Tested	Total Pods/Entry (#)			Average Incidence ⁴ (%) ^e		
						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
175	PI 295730	India	MC	1	2	29	273	.	10.8 j-u	.	.
176	PI 493329	Argentina	MC	1	1	51	.	.	22.8 f-t	.	.
177	PI 493356	Argentina	MC	1	2	62	268	.	4.7 n-u	26.9 b-h	.
178	PI 493547	Argentina	MC	1	1	.	200	.	.	26.5 b-i	.

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						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
179	PI 493581	Argentina	MC	1	1	59	.	.	13.7 i-u	.	.
180	PI 493631	Argentina	MC	1	1	49	.	.	14.3 i-u	.	.
181	PI 493693	Argentina	MC	1	1	59	.	.	14.9 i-u	.	.
182	PI 493717	Argentina	MC	1	1	94	.	.	13.0 i-u	.	.
183	PI 493729	Argentina	MC	1	1	74	.	.	30.6 b-l	.	.
184	PI 493880	Argentina	MC	1	1	49	.	.	12.2 l-u	.	.
185	PI 493938	Argentina	MC	1	2	.	199	65	.	1.4 (2) a-d	0.0 kl
186	PI 494018	Argentina	MC	1	2	.	349	139	.	0.2 (2) cd	0.0 kl
187	PI 494034	Argentina	MC	1	2	.	264	131	.	0.0 (2) cd	0.0 kl
188	PI 475863	Bolivia	MC	1	1	.	571	.	.	12.9 n-(2)a	.
189	PI 497318	Bolivia	MC	1	1	.	193	.	.	27.1 a-k	.
190	PI 497395	Bolivia	MC	1	1	27	.	.	53.3 ab	.	.
191	PI 497517	Brazil	MC	1	1	.	162	.	.	19.0 f-t	.
192	PI 496401	Burkina Fasco	MC	1	1	137	.	.	25.6 f-q	.	.
193	PI 496448	Burkina Fasco	MC	1	1	.	125	.	.	24.5 c-n	.
194	PI 504614	Columbia	MC	1	2	.	383	167	.	1.1 (2)cd	0.4 kl
195	PI 501272	Peru	MC	1	1	.	211	.	.	13.3 n-z	.
196	PI 502040	Peru	MC	1	3	107	210	104	3.4 p-u	8.0 w-(2)d	4.5 f-l
197	PI 502111	Peru	MC	1	1	46	.	.	21.7 g-t	.	.
198	PI 502120	Peru	MC	1	1	81
199	PI 331314	Argentina	MC	1	2	.	290	130	.	0.0 (2)cd	0.0 kl
200	PI 339960	Argentina	MC	1	1	41	.	.	49.1 a-e	.	.
201	PI 274193	Bolivia	MC	1	2	87	311	.	0.0 tu	22.0 e-p	.
202	PI 290560	India	MC	1	2	.	488	111	.	0.0 (2)cd	0.0 kl
203	PI 290566	India	MC	1	1	.	525	.	.	26.2 b-j	.

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205	PI 343398	Israel	MC	1	1	.	307	.	.	16.4 h-w	.
206	PI 343384	Israel	MC	1	1	.	176	.	.	19.7 e-w	.
208	PI 200441	Japan	MC	1	3	158	270	154	0.0 tu	0.0 (2)cd	0.0 kl
209	PI 196635	Madagascar	MC	1	1	.	462	.	.	16.2 i-w	.
210	PI 259851	Malawi	MC	1	1	150	.	.	16.9 h-u	.	.
211	PI 355271	Mexico	MC	1	1	.	272	.	.	18.0 g-w	.
212	PI 372271	Nigeria	MC	1	1	.	373	.	.	29.2 a-f	.
213	PI 399581	Nigeria	MC	1	1	.	181	.	.	35.9 a-c	.
214	PI 337406	Paraguay	MC	1	1	.	.	123	.	.	4.1 f-l
215	PI 268696	South Africa	MC	1	2	.	296	84	.	0.5 (2)cd	0.0 kl
216	PI 298854	South Africa	MC	1	1	79	.	.	49.2 a-d	.	.
217	PI 268868	Sudan	MC	1	1	.	235	.	.	14.2 m-z	.
218	PI 162655	Uruguay	MC	1	3	310	366	201	0.0 tu	0.0 (2)cd	0.0 kl
219	PI 152146	Uruguay	MC	1	3	104	401	146	0.0 tu	0.0 (2)cd	0.0 kl
220	PI 262038	Brazil	MC	1	1	55	.	.	14.5 i-u	.	.
221	PI 337293	Brazil	MC	1	3	105	546	134	0.0 tu	0.0 (2)cd	0.0 kl
222	PI 270907	Zambia	MC	1	3	26	543	27	4.2 o-u	8.9 t-(2)c	8.5 d-l
223	PI 270905	Zambia	MC	1	1	91	.	.	12.9 i-u	.	.
224	PI 268996	Zambia	MC	1	1	.	521	.	.	15.4 k-y	.
225	PI 270998	Zambia	MC	1	1	.	182	.	.	19.5 e-s	.
226	PI 268806	Zambia	MC	1	3	257	375	223	0.0 tu	0.0 (2)cd	0.0 kl
227	PI 268755	Zambia	MC	1	3	153	323	195	0.5 tu	1.2 (2) cd	0.4 kl
228	PI 270786	Zambia	MC	1	3	182	378	195	0.0 tu	0.0 (2) cd	0.0 kl
229	PI 356004	Argentina	MC	1	1	110	.	.	10.8 j-u	.	.
230	PI 259617	Cuba	MC	1	1	64	.	.	19.1 h-t	.	.

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231	PI 288146	India	MC	1	1	.	.	41	.	.	22.3 b-d
232	PI 296550	Israel	MC	1	1	184	.	.	21.6 g-t	.	.
233	PI 370331	Israel	MC	1	1	54	.	.	14.8 l-u	.	.
234	PI 259836	Malawi	MC	1	3	102	365	83	8.4 l-u	8.1 w-(2)d	5.9 e-l
235	PI 325943	Venezuela	MC	1	2	101	214	.	6.6 m-u	20.8 e-p	.
236	PI 157542	China	MC	1	1	104	.	.	26.9 d-p	.	.
237	PI 271019	Zambia	MC	1	3	71	469	123	0.0 tu	0.0 (2)cd	0.0 kl
238	PI 268586	Zambia	MC	1	3	108	392	115	3.6 p-u	0.2 (2) cd	0.0 kl
239	PI 403813	Argentina	MC	1	2	90	174	.	4.4 o-u	22.7 d-p	.
240	PI 475918	Bolivia	MC	1	1	128	.	.	34.1 b-j	.	.
242	PI 468250	Bolivia	MC	1	1	122	.	.	22.3 g-t	.	.
244	PI 433347	China	MC	1	3	152	588	147	0.0 tu	0.0 (2) cd	3.5 g-l
245	PI 461427	China	MC	1	1	116	.	.	18.9 h-t	.	.
246	PI 478819	India	MC	1	2	.	376	70	.	0.0 (2)cd	0.0 kl
247	PI 481795	Mozambique	MC	1	3	282	514	154	0.0 tu	0.0 (2)cd	0.0 kl
248	PI 476636	Nigeria	MC	1	1	53	.	.	22.0 g-t	.	.
249	PI 476432	Nigeria	MC	1	3	179	335	101	0.8 s-u	0.0 (2)cd	0.0 kl
250	PI 476025	Peru	MC	1	3	64	303	49	2.2 r-u	8.3 v-(2)d	4.2 f-j
251	PI 259748	Peru	MC	1	3	46	166	24	8.1 l-u	9.1 w-(2)c	0.0 kl
252	PI 240560	South Africa	MC	1	3	250	461	65	0.4 tu	0.0 (2)cd	0.0 kl
253	PI 162857	Sudan	MC	1	1	78	.	.	18.3 h-u	.	.
254	PI 407667	Thailand	MC	1	2	.	281	108		0.0 (2)cd	0.0 kl
255	PI 478850	Uganda	MC	1	1	27	.	.	51.5 a-c	.	.
256	PI 482189	Zimbabwe	MC	1	3	154	284	79	2.3 q-u	1.8 (2)b-d	0.0 kl
257	PI 471952	Zimbabwe	MC	1	2	.	232	50	.	4.5 z-(2)d	3.4 g-l

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						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
258	PI 442768	Zimbabwe	MC	1	1	.	226	.	.	32.9 a-d	.
259	PI 482120	Zimbabwe	MC	1	3	132	412	137	0.0 tu	0.6 (2)cd	0.0 kl
260	PI 471954	Zimbabwe	MC	1	3	127	431	114	4.1 q-u	5.2 y-(2)d	2.3 h-l
261	PI 429420	Zimbabwe	MC	1	1	105	.	.	16.3 i-u	.	.
262	PI 461434	China	MC	1	2	.	245	142	.	0.0 (2)cd	0.0 kl
263	PI 196622	Côte D'Ivoire	MC	1	1	97	.	.	27.9 d-n	.	.
264	PI 355268	Mexico	MC	1	1	57	.	.	43.4 b-g	.	.
265	PI 337399	Morocco	MC	1	3	76	271	112	0.0 tu	0.0 (2)cd	2.3 h-l
266	Georganic	United States	C	6	1	.	449	.	.	18.7 f-v	.
267	TifGP-2	United States	BL	6	1	99	.	.	18.4 j-u	.	.
268	Tifguard	United States	C	6	1	147	.	.	17.9 h-u	.	.
269	PI 688294	Ecuador	G	1	3	89	344	120	0.0 tu	0.0 (2)cd	0.0 kl
270	PI 690056	Guatemala	G	1	1	49	.	.	12.0 j-u	.	.
271	PI 576614	Mexico	G	1	1	78	.	.	18.1 h-u	.	.
272	PI 568166	India	G	1	1	97	.	.	20.6 g-t	.	.
273	PI 648241	Ecuador	G	1	3	120	303	277	8.9 l-u	9.9 r-(2)c	10.4 c-l
274	PI 648242	Ecuador	G	1	1	70	.	.	21.0 g-t	.	.
275	PI 648245	Ecuador	G	1	3	47	331	58	9.8 k-u	9.0 s-(2)c	1.5 i-l
276	PI 648249	Ecuador	G	1	3	34	257	35	5.3 n-u	9.6 r-(2)c	13.7 b-k
277	Tifrunner	United States	C	6	1	108	.	.	26.5 d-p	.	.
278	GT-C20	United States	G	6	1	40	.	.	5.3 n-u	.	.
279	Sun Oleic 97R	United States	C	6	2	120	684	.	6.1 m-u	36.6 ab	.
280	NC94022	United States	G	6	1	9	.	.	25.6 f-r	.	.
281	Georgia 13M	United States	C	6	1	114	.	.	26.6 d-p	.	.
282	TIFNV-HIGH O/L	United States	C	6	3	55	370	142	3.1 m-u	8.8 s-(2)c	2.3 h-l

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						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
283	Florida 07	United States	C	6	1	141	.	.	21.6 g-t	.	.
284	SPT06-06	United States	G	6	1	48	.	.	19.6 h-t	.	.
285	N08082oIJCT	United States	G	6	1	19	.	.	10.5 j-u	.	.
286	C76-16	United States	G	6	1	117	.	.	12.8 i-u	.	.
287	NC 3033	United States	G	6	1	65	.	.	27.4 d-o	.	.
288	NM Valencia A	United States	C	6	2	62	182	.	13.7 i-u	9.5 r-(2)c	.
289	OLin	United States	C	6	2	.	362	39	.	5.5 x-(2)d	4.4 f-l
290	Florunner	United States	C	6	1	.	699	.	.	27.7 a-g	.
291	Lariat	United States	C	4	1	207	.	.	21.9 g-t	.	.
292	NC-V11	United States	C	7	1	104	.	.	14.8 h-u	.	.
293	Bailey	United States	C	7	2	93	350	.	9.7 l-u	12.3 p-(2)b	.
294	Sugg	United States	C	7	1	23	.	.	36.3 b-i	.	.
295	Sullivan	United States	C	7	1	54	.	.	48.9 a-f	.	.
296	Wynne	United States	C	7	1	61	.	.	24.5 g-r	.	.
297	Emery	United States	C	7	1	79	.	.	35.9 b-i	.	.
298	Bailey II	United States	C	7	1	133	.	.	19.2 h-t	.	.
299	FloRun 107	United States	C	11	1	106	.	.	13.2 i-u	.	.
300	TUFRrunner 511	United States	C	11	1	127	.	.	40.0 b-h	.	.
301	TUFRrunner 291	United States	C	11	1	39	.	.	19.0 h-t	.	.
302	Florida EP-113	United States	C	11	1	72	.	.	25.2 g-r	.	.
303	FloRun 157	United States	C	11	1	77	.	.	25.7 f-q	.	.
304	OLé	United States	C	4	1	143	.	.	19.5 h-t	.	.
305	COAN	United States	C	9	1	107	.	.	25.8 e-p	.	.
306	NemTAM	United States	C	9	1	8	.	.	75.0 a	.	.
307	Webb	United States	C	3	1	79	.	.	13.4 i-u	.	.

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						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
309	PI 483240	India	G	1	1	26	.	.	26.7 c-s	.	.
310	PI 483241	India	G	1	1	76	.	.	11.1 i-u	.	.
311	PI 483242	India	G	1	2	.	201	59	.	0.0 (2)cd	0.0 kl
312	PI 483243	India	G	1	1	66	.	.	12.9 i-u	.	.
313	PI 591815	India	G	1	1	69	.	.	16.6 h-u	.	.
315	PI 483245	India	G	1	1	.	.	108	.	.	0.0 kl
316	PI 483247	India	G	1	2	51	239	.	4.6 n-u	37.9 a	.
317	PI 483248	India	G	1	1	21	.	.	18.1 g-u	.	.
318	PI 483249	India	G	1	1	34	.	.	22.3 g-t	.	.
319	PI 483250	India	G	1	1	106	.	.	20.9 g-t	.	.
320	PI 598133	India	G	1	1	126	.	.	33.1 b-k	.	.
322	NM Val C	United States	C	8	1	.	255	.	.	22.7 d-p	.
323	NuMex-01	United States	C	8	1	.	195	.	.	16.0 j-x	.
324	NuMex-02	United States	C	8	1	.	148	.	.	17.4 g-w	.
325	GenTex101	United States	C	8	1	.	280	.	.	21.7 d-q	.
326	GenTex102	United States	C	8	1	.	182	.	.	14.8 m-z	.
327	GenTex-118	United States	BL	8	1	.	258	.	.	21.7 e-p	.
328	GenTex-136	United States	BL	8	1	.	183	.	.	29.8 a-e	.
329	Sunland-1	United States	C	8	1	.	183	.	.	29.2 a-f	.
330	Sunland-2	United States	C	8	1	.	130	.	.	20.9 e-p	.
331	GP-NC WS 1	United States	BL	10	1	.	415	.	.	20.0 e-r	.
332	GP-NC WS 2	United States	BL	10	1	.	336	.	.	16.0 i-w	.
333	GP-NC WS 3	United States	BL	10	1	.	216	.	.	24.6 d-m	.
334	GP-NC WS 4	United States	BL	10	1	.	254	.	.	9.1 s-(2)c	.
335	GP-NC WS 5	United States	BL	10	1	.	520	.	.	16.8 h-w	.

Table 1. Complete listing of peanut entries tested for resistance to peanut smut during 2017-2020, along with number of years tested, total pods tested per year, and percent disease incidence per year.

Entry No. ^a	Entry ID	Country of Collection ^b	Type ^c	Seed Source ^d	Years Tested	Total Pods/Entry (#)			Average Incidence ⁴ (%) ^e		
						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
336	GP-NC WS 6	United States	BL	10	1	.	319	.	.	18.7 f-v	.
337	GP-NC WS 7	United States	BL	10	1	.	447	.	.	18.0 g-s	.
338	GP-NC WS 8	United States	BL	10	1	.	421	.	.	15.3 h-z	.
339	GP-NC WS 9	United States	BL	10	1	.	398	.	.	15.3 l-y	.
340	GP-NC WS 10	United States	BL	10	1	.	325	.	.	19.3 f-t	.
341	GP-NC WS 11	United States	BL	10	1	.	257	.	.	25.8 c-l	.
342	GP-NC WS 12	United States	BL	10	1	.	469	.	.	15.9 j-x	.
343	GP-NC WS 13	United States	BL	10	1	.	349	.	.	21.4 e-p	.
344	GP-NC WS 14	United States	BL	10	1	.	513	.	.	18.9 f-u	.
345	GP-NC WS 15	United States	BL	10	1	.	266	.	.	15.8 j-x	.
346	GP-NC WS 16	United States	BL	10	2	.	545	103	.	8.4 u-(2)c	9.5 c-l
347	GP-NC WS 17	United States	BL	10	1	.	387	.	.	15.1 h-z	.
348	PI 502037	Peru	G	1	1	.	.	95	.	.	0.9 j-l
354	PI 290620	Argentina	G	1	1	.	397	.	.	21.2 e-p	.
358	PI 259658	Cuba	G	1	1	.	.	175	.	.	13.3 b-k
359	PI 288146	India	G	1	2	.	297	83	.	0.4 (2)cd	0.0 kl
363	PI 295309	Israel	G	1	1	.	.	62	.	.	12.7 b-l
368	PI 319770	Israel	G	1	2	.	313	78	.	9.6 q-(2)c	0.0 kl
371	PI 36939	Pakistan	G	1	1	.	254	.	.	36.6 ab	.
373	PI 337377	Spain	G	1	1	.	.	41	.	.	17.2 b-g
375	PI 381332	Spain	G	1	2	.	251	76	.	0.0 (2)cd	0.0 kl
378	PI 483093	Spain	G	1	1	.	190	.	.	19.9 e-r	.
379	Tamrun OL12	United States	C	3	1	.	.	104	.	.	15.1 b-j
380	Schubert	United States	C	3	1	.	301	.	.	23.0 d-o	.
381	TamVal OL14	United States	C	3	1	.	297	.	.	23.1 e-o	.

Table 1. Complete listing of peanut entries tested for resistance to peanut smut during 2017-2020, along with number of years tested, total pods tested per year, and percent disease incidence per year.

Entry No. ^a	Entry ID	Country of Collection ^b	Type ^c	Seed Source ^d	Years Tested	Total Pods/Entry (#)			Average Incidence ⁴ (%) ^e		
						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
382	PI 381331	Spain	G	1	1	.	358	.	.	20.1 e-o	.
387	Toalson	United States	C	9	1	.	.	97	.	.	0.0 kl
388	Tamspan 90	United States	C	9	1	.	.	46	.	.	6.3 d-l
389	Comet	United States	C	4	1	.	.	45	.	.	0.0 kl
390	Southwest Runner	United States	C	4	1	.	.	55	.	.	0.0 kl
391	Contender	United States	C	4	1	.	.	143	.	.	22.4 b-d
392	13-3532	United States	BL	11	1	.	.	155	.	.	9.1 c-l
393	13-1125	United States	BL	11	1	.	.	127	.	.	18.6 b-h
394	M3	United States	BL	8	1	.	.	60	.	.	13.8 b-k
395	M4	United States	BL	8	1	.	.	64	.	.	39.7 a
396	M7	United States	BL	8	1	.	.	124	.	.	5.3 f-l
397	NM 308-2	United States	BL	8	1	.	.	119	.	.	26.3 ab
398	AU-NPL17	United States	C	5	1	.	.	151	.	.	5.4 e-l
401	TSIL5	United States	BL	10	1	.	.	67	.	.	6.0 d-l
402	TSIL6	United States	BL	10	1	.	.	40	.	.	4.7 e-l
403	TSIL7	United States	BL	10	1	.	.	163	.	.	13.9 b-k
404	TSIL15	United States	BL	10	1	.	.	222	.	.	10.1 c-l
405	TSIL26	United States	BL	10	1	.	.	129	.	.	23.5 bc
406	TSIL29	United States	BL	10	1	.	.	137	.	.	17.7 b-g
407	TSIL42	United States	BL	10	1	.	.	217	.	.	13.4 b-k
408	TSIL45	United States	BL	10	1	.	.	144	.	.	6.3 e-l
409	TSIL49	United States	BL	10	1	.	.	116	.	.	12.4 b-k
410	TSIL51	United States	BL	10	1	.	.	155	.	.	7.5 e-l
411	TSIL54	United States	BL	10	1	.	.	69	.	.	6.0 e-l
412	TSIL58	United States	BL	10	1	.	.	113	.	.	17.3 b-g

Table 1. Complete listing of peanut entries tested for resistance to peanut smut during 2017-2020, along with number of years tested, total pods tested per year, and percent disease incidence per year.

Entry No. ^a	Entry ID	Country of Collection ^b	Type ^c	Seed Source ^d	Years Tested	Total Pods/Entry (#)			Average Incidence ^e (%)		
						2017-2018	2018-2019	2019-2020	2017-2018	2018-2019	2019-2020
413	TSIL62	United States	BL	10	1	.	.	121	.	.	7.6 e-l
414	TSIL67	United States	BL	10	1	.	.	168	.	.	10.8 c-l
415	TSIL75	United States	BL	10	1	.	.	154	.	.	10.5 b-l
416	TSIL78	United States	BL	10	1	.	.	62	.	.	8.3 c-l
417	TSIL79	United States	BL	10	1	.	.	104	.	.	11.5 c-k
418	TSIL80	United States	BL	10	1	.	.	226	.	.	9.4 c-l
419	TSIL81	United States	BL	10	1	.	.	185	.	.	16.9 b-h
420	Florun 331	United States	C	11	1	.	.	198	.	.	16.1 b-h
155 (R)	Ascasubi Hispano	Argentina	C	2	3	348	1,721	495	0.3 tu	0.4 (2)d	0.0 l
160 (S)	Colorado Irradiado INTA	Argentina	C	2	3	452	1,992	431	24.1h-m	34.9a	16.3b-e
Mean									16	14	6.9

^a Entry 155 = resistant control; entry 160 = susceptible control
^b From www.grin-global.org
^c BL=Breeding line; C = Cultivar; G = U.S. Peanut germplasm accession; MC = U.S. Peanut mini-core germplasm accession
^d 1= USDA ARS National Plant Germplasm System (single seed descent purified); 2 = J. Baldessari, INTA, Argentina; 3 = M. Burow, Texas AgriLife Research; 4 = K. Chamberlin, USDA ARS; 5 = C. Chen, Auburn University; 6 = C. Holbrook, USDA ARS; 7 = T. Isleib, North Carolina State University; 8 = N. Puppala, New Mexico State University; 9 = C. Simpson, Texas AgriLife Research; 10 = T. Stalker, North Carolina State University; 11 = B. Tillman, University of Florida
^e Values followed by the same letter are not significantly different at P < 0.05

Field Experimental Design.

The cumulative test (2017-2020) included 208 experimental entries and two control entries (one resistant and one susceptible). Control entries were planted each year while the evaluated entries varied in number, as mentioned previously (Table 1). The control entries were replicated a different number of times depending on the season. In 2017, the replications (four blocks) were split, and the first block contained the resistant control five times and the susceptible four times, along the diagonal of the block. In the second block, the resistant control was present five times and the susceptible five times. Finally, the third block contained the resistant control five times and the susceptible four times. In 2018, the control entries were planted along three diagonal transects of the plot, with 23 replications of the resistant and 22 replications of the susceptible entries. In 2019 the design was similar to the previous season, with 3 diagonals containing alternating resistant and susceptible controls. The resistant control was

present 26 times and susceptible was present 25 times. As the previous season, both were also included as entries with 3 reps for each.

Prior to planting, soil samples were taken, and quantitation of disease pressure was performed as described by Marinelli *et al.* (2008). The tests were planted on 18 Dec. 2017, 7 Nov. 2018, and 23 Dec. 2019, using an augmented grid design with three replications, using single plants as experimental units (one plant per plot). Plots were spaced one m apart on all sides. Plots were managed for weeds and diseases following the extension guidelines of the Instituto Nacional de Tecnología Agropecuaria (INTA).

Harvest and Evaluation of Smut Resistance.

Plants were dug 120, 147, and 140 days after planting in 2018, 2019, and 2020, respectively. Each pod was opened by hand and the presence or absence of *T. frezzii* sori or spore masses on the kernels of each pod was recorded. The smut

incidence for each plot (as described below) was considered as the evaluation for smut reaction. Each replication was evaluated by a different person and the same evaluators were employed over the three seasons.

Statistical Analysis.

To determine the inter- evaluator reliability among smut evaluations performed by different persons, all the pods from 3 plots (replications) belonging to 10 genotypes of unknown smut reaction were hand-opened and subsequently displayed over a board in an ordered manner so that each pod corresponded to an ordered number. Five persons took turns individually evaluating each pod for the presence or absence of smut. In this way, it was possible to assign 5 evaluations for each numbered pod. Based on these 5 ratings, it was possible to do an inter-evaluator reliability assessment. Interrater reliability was calculated from 5 evaluators and 2,803 individual pods using Fleiss' Kappa in R (RStudio Team, 2020). Percentage of Smut Incidence (%SI) in a plot was calculated as $\%SI = [(number\ of\ infected\ pods / total\ number\ of\ pods) * 100]$.

Analysis of variance (ANOVA) was used to ascertain differences among entries for percent smut incidence using PROC MIXED in SAS Version 9.4 (SAS Institute, 2013). To determine difference among all entries tested, each year was analyzed separately because the entries evaluated differed each year due to removal of susceptible genotypes. Since standard errors differed, generation of a single LSD value was not possible, and treatment mean letter grouping was accomplished by using the SAS macro PDMIX800 (Saxton, 2000). Analysis was performed across years for those entries tested (28 plus two controls) for three consecutive years (Table 3), where year was included as a random effect. Protected post-hoc pairwise comparisons were conducted on the least square means (DIFF option in an LSMEANS statement). A significance level of $P < 0.05$ was used for the pairwise comparisons. To seek association between collection origin and/or growth habit with disease incidence, analysis of variance was performed to assess disease differences for the four continents of collection origin.

RESULTS AND DISCUSSION

The peanut growing season in Argentina roughly spans the months of November through May. Field plots were not irrigated, and the yearly rainfall total varied over the 2017-2020 period of testing. In 2017-2018, the total yearly rainfall at the testing site was 255 mm, as the region experienced a severe drought. Rainfall during the 2018-2019 and the 2019-2020 seasons was much closer to the average (605 mm) for the region at 706 mm and 614 mm, respectively. Soilborne inoculum at the testing site was consistent over the testing period at 5790 (\pm 1006), 6100 (\pm 1169), and 5650 (\pm 1307) teliospores/g soil, in 2017-2018, 2018-2019, and 2019-2020, respectively (Table 2).

There was strong agreement among scores given by the five evaluators, evidenced by a Kappa value of 0.868 ($P < 0.001$). A value of 0.454 was obtained when Kappa was calculated using the 554 pods that were evaluated as infected by at least 1 evaluator, indicating that the 5 evaluators were in reasonable agreement as to when a pod was infected. The average number of pods per entry varied by year (Table 1). A total of 106 entries were tested in 2017-2018, along with accepted resistant and

susceptible controls. The observed incidence of smut ranged from 0-75%, with a mean incidence of 16.4%. The resistant control, Ascasubi Hispano (<https://inta.gob.ar/variedades/ascasubi-hispano>) averaged 0.3% incidence while the susceptible control, PI 386349 (Colorado Irradiado INTA) averaged 24.1%. Significant differences in disease incidence were noted among entries. Thirty-five (33%) of the entries averaged less than 10% incidence and were retained for further testing the following year. The range of smut incidence for most entries fell between those of the susceptible and resistant controls. Thirteen entries were statistically similar in resistance to the accepted resistant control Ascasubi Hispano, and 7 entries exhibited extreme susceptibility with incidences statistically different and above that of the accepted susceptible control Colorado Irradiado INTA.

Table 2. Thecaphora frezzii spore count (disease pressure) in testing sites near General Deheza, Argentina, 2017-2020.

Growing Season	Field Location	Spore Count
2017-18		
	1	4900
	2	7500
	3	5300
	4	3800
	5	6500
	6	6000
	7	5500
	8	5900
	9	6500
	10	6000
	Mean	5970 \pm 1006
2018-19		
	1	6700
	2	5900
	3	4100
	4	7000
	5	6600
	6	7400
	7	6500
	8	4600
	Mean	6100 \pm 1169
2019-20		
	1	5800
	2	5900
	3	7700
	4	4200
	5	6500
	6	5500
	7	6100
	8	3500
	Mean	5650 \pm 1307

In the 2018-2019 growing season, 103 entries (including 35 retained from the prior year) were tested and smut incidence ranged from 0-37.9%, with a mean incidence of 13.5%. The resistant and susceptible controls averaged 0.4 and 34.9% smut incidence, respectively. Significant differences in disease incidence were again noted among entries. Forty-four (43%) of

the entries averaged less than 10% incidence and were retained for further testing the following year. Resistance was confirmed for a second year for 27 of the 35 entries retained from testing in 2017-2018, and these were retained for a third year of testing. The range of smut incidence for most entries fell between the range of the susceptible and resistant controls.

Eighty-five entries were tested in 2019-2020, including 44 retained from the prior year. Observed smut incidence ranged from 0-39.7%, with a mean incidence of 6.9%. Resistant and susceptible controls averaged 0 and 16.3% disease, respectively. Significant differences in reaction to smut were again noted among entries. A third year of resistance (less than 10% incidence) was confirmed for 25 of the 44 entries retained from the prior year and a second year of resistance was confirmed for 12 entries. An additional 16 entries exhibiting less than 10% smut incidence were identified and those having less than 5% disease were retained for testing outside the scope of this study.

According to the Germplasm Resources Information Network (GRIN, 2021), entries used in this study were collected from or developed in four continents, (1) Asia, (2) Africa, (3) South America, and (4) North America. Analysis of variance performed to determine if an association between

disease incidence and collection origin exists generated an overall p-value of 0.09 and did not warrant a pairwise comparison. Although there did appear to be some natural separation between these continents, no significant association between collection origin and disease susceptibility was found.

Twenty-five entries tested over a three-year period averaged less than 10% disease incidence (Table 3). Among those 25 entries, eight entries appeared immune to smut, averaging 0% infection. Taxonomy information for the immune group of entries (taken from GRIN) revealed that 5 of the 8 entries belonged to *A. hypogaea* subsp. *hypogaea* var *hypogaea*. The other three immune entries were of *A. hypogaea* subsp. *fastigiata*, with one each of var *aequatoriana*, var *fastigiata*, and var *vulgaris*. Examination of growth habit information, also taken from GRIN, showed that all growth habits (erect, bunch, spreading or prostrate) were represented among entries with 10% or less disease incidence, including the immune entries. According to GRIN, many entries were reported to have 'mixed' growth habit, indicative of seed that may have been pooled upon collection. In short, no significant correlation between disease incidence and origin, growth habit, or taxonomy was found for the entries tested in this study.

Table 3. U.S. peanut germplasm evaluated for three years, 2017-2020, near General Deheza, Córdoba Province, Argentina, exhibiting ≤ 10% incidence of peanut smut, including susceptible (entry 160) and resistant (entry 155) controls.a

Entry	PI/Cultivar	Continent of Collection or Development	Country of Collection or Development	Taxonomy	Growth Habitb	Mean Infection (%)
247	PI 481795	(SE) Africa	Mozambique	hyp var hyp	M (E/B/P)	0.00 i
218	PI 162655	S. America	Uruguay	hyp var hyp	E	0.00 i
226	PI 268806	(E) Africa	Zambia	hyp var hyp	B	0.00 i
221	PI 337293	S. America	Brazil	hyp var hyp	P	0.00 i
228	PI 270786	(E) Africa	Zambia	hyp var hyp	B	0.00 i
237	PI 271019	(E) Africa	Zambia	fast var vul	B	0.00 i
208	PI 200441	Asia	Japan	fast var fast	E	0.00 i
269	PI 688294	S. America	Ecuador	fast var aequ	M (B/P)	0.00 i
219	PI 152146	S. America	Uruguay	fast var fast	E	0.01 i
252	PI 240560	(S) Africa	South Africa	hyp var hyp	E	0.15 i
155	Ascasubi Hispano	S. America	Argentina	hyp var hyp	E	0.19 i
259	PI 482120	(S) Africa	Zimbabwe	hyp var hyp	B	0.19 h-i
249	PI 476432	(W) Africa	Nigeria	hyp var hyp	B	0.25 g-i
227	PI 268755	(E) Africa	Zambia	hyp var hyp	B	0.67 f-i
265	PI 337399	(N) Africa	Morocco	hyp var hyp	M (B/P)	0.78 f-i
244	PI 433347	Asia	China	unknown	B	1.16 f-i
238	PI 268586	(E) Africa	Zambia	hyp var hyp	M (B/P)	1.29 f-i
256	PI 482189	(S) Africa	Zimbabwe	fast var fast	E	1.54 e-i
260	PI 471954	(S) Africa	Zimbabwe	fast var fast	E	3.85 d-h
250	PI 476025	S. America	Peru	fast var fast	M (B/P)	4.88 d-h
196	PI 502040	S. America	Peru	fast var fast	B	5.39 c-g
251	PI 259748	S. America	Peru	fast var per	M (E/B/P)	5.71 c-f
282	PI 680611/TiFNV-HIGH O/L	N. America	USDA-Georgia	hyp var hyp	P	6.72 b-e
275	PI 648245	S. America	Ecuador	fast var aequ	M (E/B/P)	6.76 b-e

Table 3. U.S. peanut germplasm evaluated for three years, 2017-2020, near General Deheza, Córdoba Province, Argentina, exhibiting ≤ 10% incidence of peanut smut, including susceptible (entry 160) and resistant (entry 155) controls.a

Entry	PI/Cultivar	Continent of Collection or Development	Country of Collection or Development	Taxonomy	Growth Habit ^b	Mean Infection (%)
222	PI 270907	(E) Africa	Zambia	hyp var hyp	M (B/P)	7.17 b-e
234	PI 259836	(SE) Africa	Malawi	fast var fast	M (B/P)	7.48 b-d
276	PI 648249	S. America	Ecuador	fast var aequ	M (B/P)	10.53 b-c
273	PI 648241	S. America	Ecuador	hyp var hir	P	10.89 b
160 (S)	Colorado Irradiado INTA	S. America	Argentina	fast var fast	E	24.81 a

^a Means within a column followed by the same letter are not significantly different at P < 0.05.

^b Growth habit from www.grin-global.org: B = bunch, E = erect, P= prostrate, S= spreading, M = mixed.

To date, all commercial cultivars planted widely in Argentina are susceptible to *T. frezzii* infection, which has contributed to the rapid spread of this pathogen through the growing region of Cordoba (Cignetti *et al.*, 2010). Without effective chemical means of control, identification, and incorporation of resistance genes to develop cultivars with host-plant resistance remains the best option for peanut smut management. Sources of resistance in wild *Arachis* species and landraces have been identified (de Blas *et al.*, 2019; Oddino *et al.*, 2017; Soave *et al.*, 2014) and some have been introgressed into a cultivated, tetraploid background (Bressano *et al.*, 2019; de Blas *et al.*, 2019) in a stable and heritable manner. However, reports of smut resistance occurring naturally in cultivated, tetraploid backgrounds are scarce.

Wann *et al.* (2020) recently evaluated peanut germplasm, including 97 peanut accessions from a purified U.S. peanut mini-core collection (Chen *et al.*, 2014), for reaction to *T. frezzii* in infested fields near General Cabrera, also in Córdoba Province, Argentina. Disease incidence ranging from 0-23% was observed, and twelve accessions tested from the U.S. peanut mini-core collection had no incidence of *T. frezzii* infection. The results obtained in our study support those findings in part. The genotypes evaluated in this study include 81 of the 97 mini-core plant introductions (PIs) examined by Wann *et al.* Of the accessions present in both studies, only two (PI 268806 and PI 481795) exhibiting zero disease by Wann and colleagues appeared immune in our study. However, four accessions reported with no smut incidence by Wann *et al.* (PIs 268696, 337399, 482120, and 494018) were highly resistant (< 1% disease), but not immune in the trials conducted in this study.

The germplasm evaluated in this study were not from the collection of Chen *et al.* (2014) which was purified using phenotypic characteristics. Instead, each mini-core accession used in our study was purified from seed provided by the USDA National Plant Germplasm Repository System in Griffin, Georgia using SSD. This difference in germplasm source may explain some differences between these results with those reported by Wann and colleagues (2020). A genetic comparison of our purified accessions with those developed by Chen *et al.* (2014) would test this hypothesis. Other possible explanations for the differences between the studies include differing test locations, disease pressures, environmental conditions, and experimental design.

Of the 34 breeding lines and cultivars examined in this study, TifNV-High O/L (Holbrook *et al.*, 2017) demonstrated moderate resistance to *T. frezzii* infection, at 6.7% over 3 years.

Within the pedigree of TifNV-High O/L, the background of ancestor C99-R (Gorbet and Shokes, 2002) contains two PIs (203396 and 259785) that may have contributed to smut resistance. Neither of these PIs were evaluated for smut resistance here nor by Wann *et al.* (2020). The ancestry of TifNV-High O/L also includes TxAG-6, an amphidiploid resulting from a [(*A. cardenasii* x *A. diogo*) x *A. batizoco*] cross and subsequent colchicine treatment. The resistance seen in TifNV-High O/L may also come from TxAG-6 since smut resistance has been reported previously in a [(*A. correntina* x *A. cardenasii*) x *A. batizoco*]^{sd} amphiploid (de Blas *et al.*, 2016). Several spanish cultivars no longer in production demonstrated moderate or high resistance to peanut smut. Toalson (Simpson *et al.*, 1979) and Comet (released in 1971 by J.S. Kirby, Oklahoma Agricultural Experiment Station) were resistant to *T. frezzii*, but both have only been evaluated for one growing season in our trials thus far. Toalson has previously been evaluated by others in Argentina and demonstrated smut resistance (Baldessari, personal communication). Toalson has an unusually thick hull, which may contribute to its resistance to the pathogen. OLin (Simpson *et al.*, 2003) and Tamspan 90 (Smith *et al.*, 1991) were both derived from crosses involving Toalson, and demonstrated moderate resistance at 5.5% (2 years) and 6.5% (1 year) disease incidence, respectively. Southwest Runner (Kirby *et al.*, 1998), which resulted from a cross between Comet and Florunner (Norden *et al.*, 1969), was also resistant to *T. frezzii*, exhibiting no disease during the one growing season it was evaluated. Toalson, Comet, and Southwest Runner also have a common ancestor in the cultivar Starr (Simpson, 1972b), which may be the potential source of their resistance. Starr was developed from a cross between Spantex (Simpson, 1972a) and PI 161317, a spanish-type line collected from Uruguay in 1947, that also appears in the pedigrees of six other spanish-type cultivars- Comet, Tamnut 74 (Simpson and Smith, 1975), Toalson, Pronto (Banks and Kirby, 1983), Spanco (Kirby *et al.*, 1989), and Tamspan 90, according to Isleib and colleagues (2001).

To date, we have evaluated 208 peanut genotypes, comprised of germplasm accessions, breeding lines, and cultivars, for resistance to *T. frezzii*. Among those evaluated for three years, we identified eight entries that appear immune to smut, averaging 0% incidence. An additional eight entries have exhibited no infection by *T. frezzii* over a 2-year period. No association among collection origin, taxonomy, or growth habit was found among those accessions exhibiting complete resistance to smut. These data indicate that resistance to peanut

smut exists in the U.S. peanut germplasm collection and can be incorporated into cultivars suitable for commercial production. While completely resistant PI accessions were found, none of the cultivars or breeding lines evaluated for three years in this study exhibited complete resistance. However, several did exhibit moderate resistance and could serve as potential parents for the development of resistant cultivars if crossed with immune PIs. Because the germplasm used in this study was purified by SSD and increased before evaluation, the entries identified as immune will serve as a valuable resource for peanut breeding programs across the globe.

Highly resistant and susceptible materials identified in these studies have been used to develop recombinant inbred line (RIL) populations for mapping molecular markers associated with peanut smut resistance. Traditional and experimentally aggressive approaches will be used in tandem to develop these mapping populations. The identified molecular markers will be deployed for use by peanut breeding programs and will allow rapid screening of breeding and other populations for peanut smut resistant materials, thus increasing the breeding efficiency and reducing the number of materials that must be phenotyped in the field.

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