Identification of Resistance to Rhizoctonia Limb Rot in a Core Collection of Peanut Germ Plasm

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ABSTRACT

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Diseases caused by *Rhizoctonia solani* lead to significant reductions in peanut yields and quality throughout the world. A subset of accessions from the peanut germ plasm core collection plus the commercial cultivars Florunner, Southern Runner, Georgia Browne, and Georgia Green were evaluated for resistance to limb and seedling hypocotyl infections caused by *R. solani*. Georgia Green and core accessions 95 (PI 497351), 197 (PI 331326), 208 (PI 274193), 244 (PI 343361), 246 (PI 343398), and 524 (PI 288178) had levels of resistance comparable to Georgia Browne, the only commercial cultivar reported to have partial resistance to Rhizoctonia limb rot. Eleven core accessions, representing the full range of disease expression, and the commercial cultivars were evaluated in growth chambers to quantify their susceptibility to seedling hypocotyl infections and to determine if evaluating seedlings could serve as a primary screening method to identify potential sources of limb rot resistance. The most resistant core accessions to seedling hypocotyl infections were 234 (PI 159664) and 366 (PI 268968), and the most resistant commercial cultivar was Georgia Green. There was not a significant correlation between resistance to limb rot in the field and the severity of hypocotyl infections in growth chambers, indicating that resistance to hypocotyl infections is not a good indicator of resistance to Rhizoctonia limb rot.

Peanut (*Arachis hypogaea*) growers must protect their crop from foliar and soilborne plant pathogens to optimize profits and maintain high yields. One of the most common soilborne pathogens is *Rhizoctonia solani* AG-4 (24). The diseases induced by *Rhizoctonia* in peanut include seed decay, preemergence and postemergence damping-off, hypocotyl and root necrosis, peg rot, pod rot, limb rot, and foliar blight of mature plants (4). Rhizoctonia limb rot in peanut did not become a problem for Georgia peanut growers until the early to mid 1980s (26). Since it was first reported, it is estimated that growers in Georgia lose an average of \$12.9 million each year due to Rhizoctonia limb rot infections (University of Georgia Cooperative Extension Service estimates, 1990 to 1997). Control of Rhizoctonia limb rot in peanut is based on an integrated management approach using crop rotations, proper fertilization, irrigation management, and chemical controls.

R. solani has an extensive host range, which reduces the effectiveness of some rotational crops, but rotations with grass

crops are beneficial in reducing Rhizoctonia limb rot infections (9). Until recently, chemical control of *R. solani* was limited primarily to protection of seeds and seedlings from seed decay and damping-off, because there were no fungicides available that protected the plant from limb rot infections. However, the recent registrations of tebuconazole, flutolanil, and azoxystrobin have provided growers with effective chemical control, although at a significant increase in production costs (8,12).

Evaluation of germ plasm and identification of resistance to other anastomosis groups of *R. solani* has been successful in other crops (10,16,27,30). However, host resistance has been one of the missing components of an integrated management approach in peanut production. Southern Runner has multiple disease resistance but is very susceptible to limb rot (6). Currently, Georgia Browne is the only runner cultivar reported to have partial resistance to limb rot. Unfortunately, its small seed size made it an unpopular cultivar among shellers, and it is not currently grown. Georgia Green is currently the most widely grown peanut in the southeastern United States, due in large part to its resistance to tomato spotted wilt virus (TSWV). However, it was reported to be as susceptible to limb rot as cultivars GK-7 and Florunner (6).

Although a cultivar with limb rot resistance is needed, it is more difficult to screen cultivars for resistance to limb rot

infections than for resistance to seed decay or hypocotyl infections, because plants have to be grown to maturity in the field and disease severity measurements taken at harvest. In addition, many potential fields for limb rot evaluations have high stem rot incidence caused by *Sclerotium rolfsii*, which can confound Rhizoctonia ratings. Due to these difficulties, very few studies have been conducted to identify sources of resistance to limb rot in peanut (2,6).

Currently, the U.S. germ plasm collection for peanut consists of 7,432 accessions representing a wide range of genetic diversity (14). To screen each accession for resistance to soilborne and foliar diseases would take an exorbitant amount of time and resources. To increase the efficiency of germ plasm screening, a core collection was developed (14). Utilizing a core collection enables a subset of accessions to be screened more efficiently for disease resistance. The core collection can be used as a starting point to screen accessions for resistance to a particular disease, or it can be used with existing data to identify other accessions within the collection that could contain certain desirable traits. If a core selection does have resistance to a particular disease, then it is likely that other accessions within the same cluster possess the same trait, since theoretically they are genetically similar to the core selection. Studies conducted from 1986 to 1992 to screen the entire germ plasm collection for resistance to late leaf spot have been used to test the efficiency of the core collection in identifying resistance (13). From those studies, this approach would have identified the overall best four, and eight of the 10 best sources of resistance in the entire collection.

Studies with other host plant species have attempted, with limited success, to determine relationships between resistance expressed in seedlings and resistance expressed in adult plants (11,15,19,28,29,31). One objective of these studies was to develop more efficient methods of screening genotypes for disease resistance. This type of screening technique would work well in breeding programs looking for qualitative resistance and as a method of eliminating susceptible genotypes in large numbers of early-generation lines or germ plasm.

The objectives of this study were to (i) screen 66 core accessions from the peanut core collection along with Florunner, Georgia Browne, Georgia Green, and

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Southern Runner for limb rot resistance, (ii) screen 11 core accessions and four commercial cultivars for hypocotyl resistance, and (iii) determine if resistance to limb infections and to seedling hypocotyl infections were correlated, which could be used to develop a faster method of screening genotypes for limb rot resistance.

MATERIALS AND METHODS

Inoculum production. Whole oat seeds were soaked in water for 24 h and autoclaved in 3.78-l plastic Nalgene containers at 250°C for 90 min on 2 consecutive days. Sterile oat seeds were inoculated with *R. solani* AG-4 isolated from infected peanut plants in 1996 and 1997. Several isolates were used in order to utilize a broader genetic base of the pathogen and maximize disease development under field conditions. Each container of sterile oat seeds was inoculated with one isolate using 1-cm-diameter hyphal plugs. After 14 days of incubation at 27°C, the infested oat seeds were placed in shallow plastic pans and dried in a Blue M oven (Blue M Electric Co., Blue Island, IL) at 40°C for 48 h. Infested oat seeds were stored in large Fibre Drums (Sonoco Co., Marietta, GA) until used.

Field screening. Sixty-six core accessions possessing one or more desirable agronomic characteristics for breeding and four commercial cultivars (Florunner, Georgia Browne, Georgia Green, and Southern Runner) were planted in a Pelham coarse sand (20) at the Coastal Plain Experiment Station in two-row plots 2.94 m long. Plots were separated by fallow alleys 1.52 m long. Entries were arranged in a randomized complete block design with five replicates. The field had been planted to peanut 3 years prior to our test and was chosen because of the low incidence of stem rot in the previous years. Selections were planted on 23 May 1996 and 20 May 1997, and conventional cultural practices were used to maintain the plots (3). To ensure uniform disease development, all plots were infested with inoculum produced as described previously. In 1996, plants were inoculated on 11 September with 88.5 kg of oat seeds infested with *Rhizoctonia solani* per ha. In 1997, plants were inoculated on 8 September with 80.6 kg of infested oat seeds per ha, and another 28.0 kg/ha was applied on 8 October. An average of 18.8 g of inoculum per m was manually applied to the outside half of each row within the plots in both years. Previous research has shown artificial inoculations produce very similar disease reactions under suitable environmental conditions (7,23,25). In 1997, to increase the level of disease, an all-terrain vehicle was driven down the rows to press the limbs into contact with the inoculum and to slightly injure the limbs. To create favorable conditions for disease development, approximately 1.27 cm of water was

applied daily with an overhead sprinkler for 4 to 5 days after inoculation.

Plants were inverted on 10 October 1996 and on 5 November 1997. A single disease assessment was made after inversion. The process of inverting the vines in paired rows placed the inoculated half of each row on the inside of the windrow. Five primary reproductive limbs were selected arbitrarily from the inside half of each row in each plot. Plants with obvious symptoms of TSWV were not sampled. The mean numbers of lesions, girdling lesions, and lesions >2.54 cm were calculated per stem. Yield data were collected in 1996 but not in 1997 due to heavy damage by TSWV. TSWV ratings were taken approximately 1 month before harvest in both years. The percentage of infected 0.30-m row segments was calculated by taking the number of 0.30-m segments that exhibited viral symptoms divided by the total number of 0.30-m segments in each plot. To obtain clearly defined, nonoverlapping groups within the large number of entries and reduce the chance of overlooking resistant genotypes, the FASTCLUS procedure of PC-SAS (22) was used to identify groups of genotypes with different levels of susceptibility to limb rot.

Seedling experiment. The core accessions used in this study were selected using data from the field screening study to represent the full range of resistance to limb rot infections. Eleven core accessions and the four commercial cultivars were evaluated for resistance to hypocotyl infections. Since the seeds were not treated with fungicide, they were immersed in a hot (50°C) calcium hydroxide solution (1.58 g/liter) for 20 min to reduce the level of fungal contamination and improve germination (1). After treatment, 100 seeds were germinated in plastic containers lined with moist paper towels in an incubator at 30°C for 36 to 48 h.

Seeds were planted in plastic Cone-Tainers (4 cm diameter × 20.5 cm; Stuewe & Sons, Inc., Corvallis, OR) half filled with a pasteurized soil:promix (1:2 vol/vol)

mixture. One germinated seed was placed in each Cone-Tainer and covered with approximately 1.27 cm of sterile soil mixture. Ten Cone-Tainers were used for each genotype and replicate. The experiment was a randomized complete block design with five replicates.

One isolate (JY-1) of *R. solani* from a severely diseased peanut seedling was used as inoculum for the soil drench. This isolate was highly virulent to peanut seedlings in preliminary studies. The JY-1 isolate was grown on potato dextrose agar (PDA) at room temperature (24.7 to 26.6°C) until the mycelium covered the entire petri dish (36 to 48 h). Contents of five petri dishes were macerated with 500 ml of sterile deionized water in a Waring blender for 15 s on low speed and 15 s on high speed. Seven Cone-Tainers were inoculated with 20 ml each (275 CFU/ml) of the blended inoculum. Three Cone-Tainers were non-inoculated controls for every entry in each replicate. Controls were treated with a soil drench prepared according to the method described above, except five petri dishes of sterile PDA were used. Inoculum was applied when the young peanut seedlings had one to two tetrafoliate leaves unfolded and leaflets were flat (V-1 to V-2 growth stage) (5). Since there was some variability in time of emergence, additional plates of inoculum were started daily for 3 days so that all plants were inoculated with mycelium of similar age.

After inoculation, seedlings were placed in a growth chamber at 23°C for 2 weeks. This temperature was selected to maximize fungal growth and hypocotyl infections. After 2 weeks, seedlings were removed from the Cone-Tainers and roots were removed from the hypocotyls. Seedlings were washed in tap water, and the hypocotyls were rated on a 1 to 6 scale used by the cottonseed treatment committee of the National Cotton Council (21), but modified as follows: 1 = no symptoms, 2 = discoloration and/or small pinpoint lesions, 3 = small, distinct necrotic lesions, 4 = large necrotic lesions, 5 = girdling lesion, and 6

Table 1. Field reactions of 66 selected genotypes representing a subset of the peanut core collection and four commercial peanut cultivars to *Rhizoctonia* limb rot from 1996 and 1997

Cluster ^a	No. of entries ^b	Cluster means			
		Lesions per stem ^c	Girdling lesions per stem ^c	Lesions >2.54 cm ^c	TSWV intensity ^d
1	1	1.45	0.01	0.08	29.01
2	4	1.17	0.09	0.18	26.53
3	12 (FR)	1.03	0.09	0.18	22.66
4	15 (SR)	0.86	0.05	0.09	21.99
5	30	0.71	0.03	0.06	23.53
6	8 (GG + GB)	0.50	0.01	0.02	21.58

^a Data for lesions per stem, girdling lesions per stem, and lesions >2.54 cm were clustered to determine which entries belong in each cluster. Clustering was done using the FASTCLUS procedure of PC-SAS.

^b Includes the four commercial cultivars FR = Florunner, GB = Georgia Browne, GG = Georgia Green, and SR = Southern Runner.

^c Average of 10 primary reproductive limbs per plot.

^d Percentage of 0.30-m row segments exhibiting symptoms of tomato spotted wilt virus.

= dead seedling. Seedling height and fresh weight were measured. Height was measured from the cotyledonary leaf node to the tip of the last unfolded tetrafoliolate leaf. The percentage of healthy seedlings (ratings of 1 or 2) for each replicate was calculated. Percent reduction in height and weight for inoculated seedlings compared with noninoculated seedlings was calculated. The experiment was repeated.

Percentage data were analyzed using analysis of variance and means separated into nonoverlapping groups using the FASTCLUS procedure of PC-SAS. The genotypes were clustered according to percent healthy seedlings. Correlations between resistance to limb and hypocotyl infections were determined for the genotypes evaluated in the seedling experiment using Pearson correlation coefficients.

RESULTS

Field screening. There were no significant year $\times$ genotype interactions for lesions per stem, girdling lesions per stem, or lesions >2.54 cm per stem, and data from both years were combined. The clustering procedure of the combined data yielded six clusters of genotypes with means that had levels of resistance to limb rot infections ranging from 0.50 to 1.45 lesions per stem (Table 1). Mean number of lesions per stem, number of girdling lesions, and lesions >2.54 cm decreased from cluster two to cluster six. Cluster six, with the lowest means for all three variables, contained partially *R. solani*-resistant cultivars Georgia Browne, Georgia Green, and six core accessions.

Incidence of TSWV was relatively high in both years of the experiment, and genotypes demonstrated a wide range of susceptibility. Of the core accessions partially resistant to limb rot, only core accession 95 (PI 497351) and 524 (PI 288178) had a level of TSWV infection equivalent to Georgia Green, a cultivar partially resistant to TSWV. Incidence of TSWV over the 2-year period was lower for these two core accessions than for the other four accessions within that cluster (Table 2). There was no significant correlation between

TSWV and any of the other variables from the field experiment (Table 3).

Seedling experiment. There was not a significant run $\times$ genotype interaction for the seedling data, and data from the two experiments were combined. The clustering procedure yielded five clusters. The percentage ranges were 60 to 26 for healthy seedlings, 28 to 14 for reduction in height, and 25 to 20 for reduction in weight (Table 4). Accessions 234 (PI 159664) and 366 (PI 268968) were the most resistant to hypocotyl infections, while 335 (PI 153323) and Florunner were the most susceptible. Of the four commercial cultivars, Georgia Green had the least seedling hypocotyl infections with 50% healthy seedlings. There was no significant correlation between percent healthy seedlings and percent reduction in height, or between percent healthy seedlings and percent reduction in weight. However, there was a significant correlation between percent reduction in height and weight (Table 3). There were no significant correlations

between percent healthy seedlings and any of the variables from the field experiment or between percent healthy seedlings and number of lesions per stem for the 15 entries in the seedling experiment (Table 3).

DISCUSSION

Use of the core collection enhanced the search for resistance to *Rhizoctonia* limb rot in this study. The number of core accessions evaluated could be managed more easily than the several hundred accessions that these core accessions represented. The six core accessions that were the most resistant to limb infections represented 458 individual accessions within the clusters from which these six partially resistant accessions were selected. These accessions could be additional sources of resistance to limb infections.

Barnes et al. (2) identified two genotypes that had partial resistance to *Rhizoctonia* limb rot, both of which had a more erect growth habit, which may allow for more rapid drying after rain, dew, or

Table 3. Correlation coefficients and *P* values for variables from the field and greenhouse peanut experiments evaluating selected genotypes and four commercial peanut cultivars for resistance to *Rhizoctonia* limb rot and seedling hypocotyl infections

Variables	N ^a	Correlation coefficients	<i>P</i> value
Field experiment			
Lesions per stem vs. girdling lesions per stem	140	0.364	0.0020
Lesions per stem vs. lesions greater than 2.54 cm per stem	140	0.562	0.0001
Girdling lesions per stem vs. lesions greater than 2.54 cm per stem	140	0.621	0.0001
Lesions per stem vs. tomato spotted wilt virus (TSWV)	140	0.063	0.6030
Girdling lesions per stem vs. TSWV	140	0.089	0.4600
Lesions greater than 2.54 cm per stem vs. TSWV	140	0.090	0.4600
Seedling experiment			
Percent healthy seedlings vs. percent reduction in height	30	-0.127	0.6510
Percent healthy seedlings vs. percent reduction in weight	30	0.307	0.2660
Percent reduction in height vs. percent reduction in weight	30	0.705	0.0030
Field and seedling experiment			
Percent healthy seedlings vs. TSWV	30	0.107	0.7040
Percent healthy seedlings vs. lesions per stem	30	0.284	0.3060
Percent healthy seedlings vs. girdling lesions per stem	30	0.181	0.5180
Percent healthy seedlings vs. lesions greater than 2.54 cm per stem	30	0.346	0.2060

^a Combined over all trials.

Table 2. Characteristics of selected peanut genotypes exhibiting the highest level of resistance to *Rhizoctonia* limb rot in field trials

Genotype ^a	PI number	Country of origin	Growth habit	Plant size ^b	Maturity (days)	Lesions per stem ^c	TSWV intensity (%) ^d
246	343398	Israel	Spreading bunch	Medium	140	0.56	25.0
244	343361	Israel	Spreading bunch	Medium	140	0.57	20.0
524	288178	India	Spreading	Small	150	0.48	15.0
197	331326	Argentina	Spreading	Large	140	0.51	36.0
95	497351	Bolivia	Spreading	Extra large	150	0.52	9.0
Georgia Browne	...	United States	Spreading bunch	Medium	140	0.54	12.0
Georgia Green	...	United States	Spreading bunch	Medium	140	0.44	7.0
208	274193	Bolivia	Spreading bunch	Large	140	0.36	45.0

^a Numbers correspond with numbers used in the core collection (14).

^b Plant size at harvest. Varieties that describe plant size according to United States Peanut Descriptors, 1995. ARS Bull. 132. Small = "Chico," medium = "Early Bunch," large = "Floriant," and extra large = "BPZ 53."

^c Values are means of 10 stems.

^d Mean percentage of 0.30-m row segments exhibiting symptoms of tomato spotted wilt virus for 1996 and 1997.

irrigation and a less favorable microclimate for *R. solani*. Also, the erect growth habit of these genotypes reduced limb contact with *R. solani* in the soil. This was not observed among the partially resistant genotypes identified in our field study, since only genotypes with either spreading or spreading/bunch growth habits were tested. Late maturity and a large, spreading vine are characteristics usually associated with cultivars such as Southern Runner that are more susceptible to Rhizoctonia limb rot. Plant size varied among the resistant genotypes, from small to extra large. Some partially resistant genotypes matured 10 days later than Florunner. The resistance could be attributed to disease escape. However, in this study, all genotypes were harvested simultaneously and disease pressure was relatively high both years. Differences among genotypes may be the result of physiological or biochemical responses rather than differential maturity or canopy shape and size.

Of the commercially released runner peanut cultivars evaluated in the field, Georgia Green and Georgia Browne were the most resistant to limb infections. Both cultivars also have very good partial resistance to TSWV. Georgia Green and Georgia Browne were not immune to limb infections by *R. solani*. However, the lesions that formed on the limbs of these cultivars were smaller than those on Florunner and Southern Runner and did not girdle the stems as frequently. This should result in decreased loss of pods in the field. Unfortunately, the high levels of TSWV in 1997 compromised yield data and made it difficult to verify the relationship between disease intensity and yield.

Experimental evidence for the relative susceptibility of Southern Runner and Florunner to Rhizoctonia limb rot has been conflicting. One study showed that Florunner and Southern Runner were equally susceptible to Rhizoctonia limb rot (2), but a later study showed that over a 3-year period, Southern Runner was more susceptible to Rhizoctonia limb rot than Florunner (6). Our data agreed with the first study. Southern Runner is a later-maturing

cultivar than Florunner and in most situations remains in the field 2 to 3 weeks longer. In our study, all the genotypes were inverted at the same time. Had we allowed Southern Runner to remain in the field the additional time to completely mature, we might have observed higher levels of disease. Core accession 95 is the only other genotype with resistance to limb infections that has a level of resistance to TSWV comparable to Georgia Green and would be the most likely candidate for development into a commercially acceptable cultivar.

Recent studies (28,29) with the barley leaf scald and strawberry leaf scorch pathosystems showed a strong correlation between resistance expressed in seedlings and resistance expressed in mature plants. Xue et al. (28) evaluated 66 barley genotypes for resistance to leaf scald in seedlings and mature plants and identified 32 genotypes that had a moderate level of resistance in both. Similar results were obtained in later work identifying potential sources of resistance in strawberries to leaf scorch. Nine progeny populations derived from four different strawberry varieties were identified that had resistance to leaf scorch in the seedling and adult-plant stages. In both studies, these findings could have been useful in evaluating potential sources of qualitative resistance or eliminating susceptible germ plasm in large numbers of genotypes. Our hypothesis was that resistance to Rhizoctonia limb rot would be positively correlated with resistance to peanut seedling infection. Had this hypothesis been true, a seedling screen could be used as a primary method of identifying potential sources of resistance to limb infections. Unfortunately, analysis of the data did not reveal any significant relationship that could be exploited in such a manner.

For both field and seedling data, there was almost a threefold difference between entries in the most resistant and most susceptible clusters for number of lesions per stem and percent healthy seedlings. In both cases, Georgia Green was the most resistant to limb and seedling hypocotyl infec-

tions compared with three other commercial cultivars. Data from the other genotypes were consistent with those in which resistance in broccoli to downy mildew (11), wheat to leaf blotch (15), and rapeseed to blackleg (19) was only present in either seedlings or mature plants, but not both. All of this work supports earlier evidence in the wheat-rust pathosystem in which expression of some resistance genes is based on plant growth stage (17,18).

Georgia Green is currently the predominant commercial peanut cultivar planted in the southeastern United States. It is grown primarily because of its high yield potential and partial resistance to TSWV. The resistance of Georgia Green to hypocotyl infections has not been reported previously. The significant resistance to limb rot infections differs from a previous report where it was less susceptible than Southern Runner but as susceptible as Florunner, Sunrunner, and GK-7 (6). However, results of that study may have been confounded by high levels of stem rot (*Sclerotium rolfsii*). Although Georgia Green is being grown for other reasons, its resistance to Rhizoctonia limb rot and seedling disease represent added value to the grower. This is particularly true since cotton is now the crop most commonly rotated with peanut in the southeastern states, thus increasing the risk of *Rhizoctonia*-induced diseases in both crops.

Although a more efficient screening method for Rhizoctonia limb rot was not found, one commercial cultivar and six core accessions with levels of resistance to limb infections equal to that of the partially resistant cultivar Georgia Browne were identified. In addition, Georgia Green and two core accessions (234 and 366) have more than a twofold increase in the level of resistance to seedling hypocotyl infections compared with Florunner. This information will be of great value to plant breeders in their effort to identify sources of resistance to soilborne disease caused by *R. solani*. The use of host resistance to manage pest problems is crucial to growers to reduce input costs and increase profitability.

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Table 4. Combined results of an evaluation of 11 selected genotypes and four commercial peanut cultivars for resistance to seedling hypocotyl infections caused by *R. solani*

Cluster ^a	Genotypes	Core accessions and standard cultivars ^b	Cluster means		
			% healthy seedlings ^c	% reduction in height ^d	% reduction in weight ^d
1	2	335, FR	26.1	20.7	23.9
2	3	197, 302, 287	36.6	28.1	21.9
3	3	283, SR, GB	42.1	20.4	25.6
4	5	512, 513, 208, 458, GG	50.4	21.4	21.0
5	2	234, 366	60.0	14.4	20.3

^a Data for percent healthy seedlings were clustered to determine which entries belong in each cluster. Clustering was done using the FASTCLUS procedure of PC-SAS.

^b FR = Florunner, GB = Georgia Browne, GG = Georgia Green, and SR = Southern Runner.

^c Percent seedlings with a rating of one or two.

^d Percent reduction in height and weight of inoculated seedlings compared with noninoculated controls.

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