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Evaluation of Six Peanut Genotypes for Pod Rot Resistance[1]

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ABSTRACT

Six genotypes (TxAG-3, PI 341885, Toalson, Starr, Florunner, and Goldin I) were evaluated for reaction to *Pythium myriotylum* Drechs, and *Rhizoctonia solani* Kuhn in the greenhouse to: 1) confirm field results as to the relative disease reactions of the entries; 2) ascertain whether peg versus pod exposure to pathogens confounds selection for pod rot resistance; 3) examine the utility of greenhouse inoculation in screening for pod rot resistance; and 4) evaluate simultaneous screening of plants for reaction to these fungi. Adult plants were exposed at two stages of fruit development. TxAG-3 had significantly less pod decay from each organism singly and in combination at both times of exposure to the pathogens than did the other entries. Exposure to the pathogens at the pegging and pod-filling stages of development produced similar results.

Key Words: Groundnut, Disease Screening, *Rhizoctonia solani, Pythium myriotylum,* Soil-borne diseases.

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Pod rots caused by *Pythium myriotylum* Drechs. and *Rhizoctonia solani* Kuhn are important diseases of peanuts (1,4,5,6,7,10). Losses are incurred in both yield and grade, and chemical control measures are often inadequate.

Screening for pod rot resistance in the Texas breeding program has relied on adult plant reaction in field tests, but the efficiency of the evaluations has been low because of inadequate disease pressure, non-uniform pathogen distribution, and micro-environmental effects. Frank (3) reported that erroneous conclusions can be drawn from field experiments because susceptible genotypes with short pegs form pods in the upper layer of the soil where conditions are less favorable for the pathogen. Maintenance of optimal moisture and disease pressure in the pod forming zone are major problems in screening for soil-borne disease reactions by artificial inoculation.

Differences among genotypes in field reaction to pod rot have been reported (2,3,10,11,12). The extent of variation in pod decay among genotypes commonly used as checks in Texas pod rot yield tests is exemplified in Table 1. The objectives of this study were to: 1) compare genotypes with different field reactions to pod rot for disease reactions under greenhouse conditions; 2) ascertain if the stage of fruit development at first exposure to the fungi is a major factor confounding selection for re-

sistance; 3) evaluate greenhouse inoculations in screening for pod rot resistance; and 4) evaluate the simultaneous screening for resistance to *P. myriotylum* and *R. solani* on individual plants.

Table 1. Percentage of pod decay in four genotypes at two pathogen infested sites in South Texas.

Genotype	Site 1	Site 2
Florunner	50.8 a	38.2 a
Starr	50.0 a	31.7 a
Toalson	31.2 b	9.0 b
TxAG-3	22.2 b	6.1 b

Values are means of two tests of three replications in each of two years. Principal pathogens: Site 1 - Pythium myriotylum; Site 2 - Rhizoctonia solani.

Means in columns bordered by the same letter are not significantly different at the 0.05 probability level.

Materials and Methods

Intact pegs and pods of six peanut genotypes were challenged in the greenhouse with *P. myriotylum* and *R. solani* (AG 4 type) isolated from peanut in Texas. The genotypes included: TxAG-3, the most pod rot resistant line evaluated in Texas field tests; Toalson and PI 341885, moderately resistant; Starr, slightly resistant to *R. solani* (1); and Florunner and Goldin I, susceptible (2). TxAG-3, a selection from PI 365553; Florunner; and Goldin I are virginia in botanical type, and the remaining three entries are spanish.

Single pathogen (SP) per plant

Seeds were planted in polyvinyl chloride (PVC) tubes (15.5 cm x 8 cm in dia.) filled with a medium constituted of 4 parts of twice autoclaved sandy loam soil, and two parts no. 2 and one part no. 3 vermiculite (Bell Wholesale, Inc., P. O. Box 27126, Houston, Texas, 77027). Tubes were placed in the center of 17.6 liter wooden baskets lined with polyethylene bags that were perforated for drainage. The baskets were filled outside of the tubes to 13 cm from the rim with the sterile soil mix, and the tubes were positioned to extend 3 cm above the rim of the baskets. Plants were watered daily, but soil moisture was not monitored. Hoagland's modified solution (8) and ammonium nitrate were added at nonspecified intervals beginning during early flowering to achieve and maintain growth and development.

Pythium myriotylum and *R. solani* were grown 15 days in an autoclaved 9:1 mixture of washed river sand and corn meal moistened with sterile water. The sand-corn meal inoculum was mixed at a 1/200 w/w basis (0.5%) with the sterile soil mixture. A dilution of 0.5% of each culture was used when the two fungi were mixed into the soil media.

The soil mixtures were placed in the baskets when plants were at two stages of fruit development; peg and pod-filling. Early developing pegs were removed from all plants until initiation of the experiment to facilitate uniformity of fruit age among genotypes at the time of exposure to the pathogens. Four treatments were used at each growth stage: *P. myriotylum, R. solani, P. myriotylum + R. solani,* and a non-infested sterile soil mixture control. Soil mixtures were transferred to the baskets as a surface layer approximately 10 cm deep outside the central tube during peg elongation so that the pegs grew into infested soil. Vermiculite (No. 2) was used for the surface layer during pegging in baskets containing plants to be exposed to the fungi at pod-filling. The vermiculite was removed by vacuum 60 days after peg initiation and replaced with soil mixtures identical to those described previously. Soil temperatures varied from 25 to 41 C during the study with average daily maximums and minimums of 36 and 26 C, respectively.

All pods were harvested 150 days after planting and examined individually. Data recorded included pod stage, as described by Pattee et al. (9), pod damage on a 0 to 10 scale (0 = no decay; 10 = total pod decay), and a visual determination for internal pericarp necrosis resulting from fungal penetration through the shell. The pod damage ratings, multiplied by a factor of 10, approximates the total percentage of pod decay.

Pod disease ratings for all pods in development stage 4 (soft pericarp, small, flattened, and white kernel), or older, were averaged on a per plant basis. Statistical analyses were performed on arcsin transformed data as a 6 x 4 x 2 factorial, with a completely randomized design and 3 replications.

Multiple pathogens (MP) per plant

Individual plants of six genotypes were exposed simultaneously to both pathogens in a second effort to evaluate pod disease reactions in the greenhouse. Baskets, liners, and tubes were used as previously described. A non-sterilized soil mix of the composition listed previously was used to fill the baskets to approximately 10 cm from the rim and tubes to 2 cm from the rim. One seed per basket was planted inside the tube. Modified Hoagland's solution was applied at 14 day intervals. Flowers and pegs were removed until the first soil-mix transfer. Fungal inoculum was produced as before and mixed with the sterile soil mix on a 1/100 w/w (1.0%) basis.

Peg stage treatments were applied 60 days after planting. Infested and sterile soil mixtures were put into perforated polyethylene bags (16 cm x 22 cm) containing 2 cm of pea gravel for improved drainage. Four bags, one each with *P. myriotylum, R. solani, P. myriotylum + R. solani,* and a non-infested check, were positioned in each basket around the entral tube so that pegs could grow into the open bags. Vermiculite (No. 3) was used in place of the soil mixtures during pegging for plants that were exposed to the fungi during pod-filling and replaced 60 days later as described previously. Soil temperature varied from 20 to 35 C, with average low and high of 26 and 30 C, respectively.

Plants were harvested 44 days after pod exposure to the fungi. Data were transformed as described previously and analyzed as a 6 x 4 x 2 factorial with 3 replications and a split-plot design. Whole plots consisted of genotypes and fruit development stage at time of treatment application, sub-plots were the fungal treatments.

Results and Discussion

The reproductive development of plants was delayed in the SP test and final pod numbers varied from 3 to 31 per plant. Disease severity was low relative to that observed in field tests on pathogen infested sites. No disease was identified in the control. Tissue samples from pods with relatively high disease ratings were surface sterilized and plated on potato dextrose agar and the pathogens used to infest the soil mixture were reisolated. *Rhizoctonia solani* was reisolated more frequently than *P. myriotylum* from pods in soil infested with both fungi.

No differences in pod disease severity were found between peg and pod inoculations in the SP test. However, differences in disease ratings between infested and non-infested treatments, and between TxAG-3 and the other genotypes were significant (Fig. 1). Within

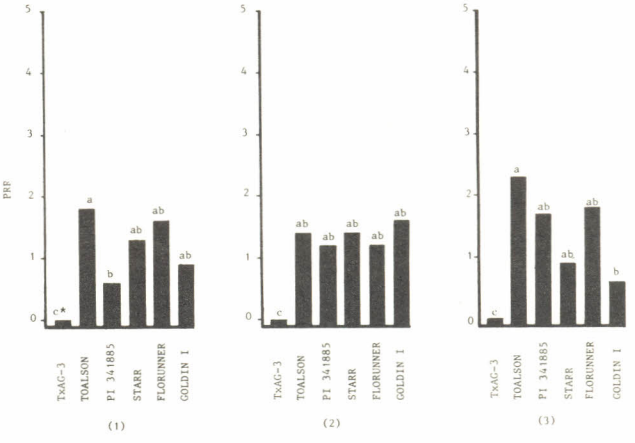

Fig. 1. Average pod rot ratings (PRR) in the adult plant-single inoculation study for 3 replications each of pod and peg inoculation. (1) *Pythium myriotylum*, (2) *Rhizoctonia solani*, (3) *P. myriotylum* and *R. solani*.

Bars topped by the same letter are not significantly different at the 0.05 probability level using Duncan's Multiple Range Test.

Within genotypes, no differences were found among the three fungal treatments. *Pythium myriotylum* alone produced more pod decay in Toalson than PI 341885, and *P. myriotylum* + *R. solani* caused more damage to Toalson than to Goldin I (Fig. 1). The entries, excluding TxAG-3, did not differ in damage from *R. solani.*

The number of pods per plant in the MP test ranged from 10 to 34, and per treatment on a plant from 2 to 12. Disease development was low compared to field tests. Less pod disease developed on TxAG-3 than on other genotypes in all treatments at both inoculations (Fig. 2). Starr, infected by *R. solani,* and PI 341885 and Toalson, infected by *P. myriotylum,* sustained more pod damage than the other entries when pegs were exposed to the fungi. Except for TxAG-3 in all treatments, and PI 341885 in Pythium infested medium, the entries were equally affected by the pod stage inoculation treatments, (Fig. 3).

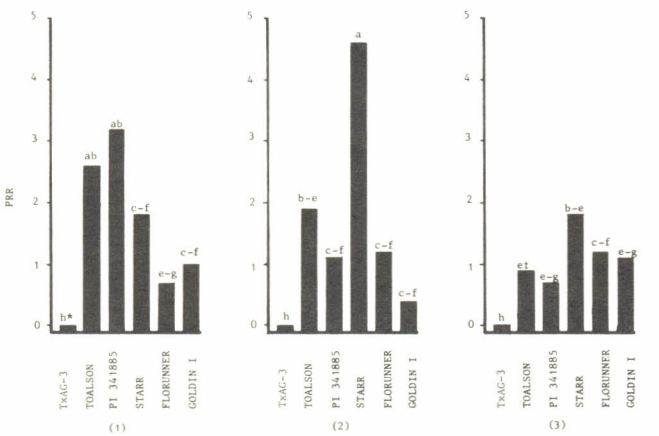

Fig. 2. Average pod rot ratings (PRR) in the adult plant-multiple inoculation study for 3 replications of peg inoculation. (1) *Pythium myriotylum,* (2) *Rhizoctonia solani,* (3) *P. myriotylum* and *R. solani.*

Bars topped by the same letter are not significantly different at the 0.05 probability level using Duncan's Multiple Range Test.

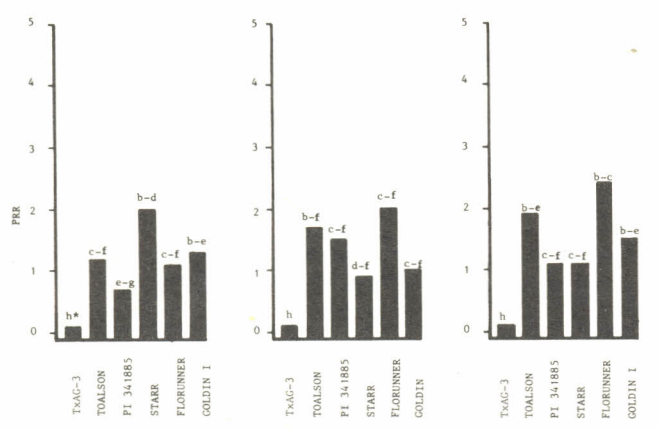

Fig. 3. Average pod rot ratings (PRR) in the adult plant-multiple inoculation study for 3 replications of pod inoculation. (1) *Pythium myriotylum,* (2) *Rhizoctonia solani,* (3) *P. myriotylum* and *R. solani.*

Bars topped by the same letter are not significantly different at the 0.05 probability level using Duncan's Multiple Range Test.

The results suggest that fruit stage, pegging or mid-podfill, at initial exposure to the fungi was not critical to the evaluation. This reduced our concern that differences in stage of development among plants in segregating populations might confound selection for pod rot resistance in field tests.

Confirmation in the greenhouse of the pod rot resistance apparent in field tests was only partially successful. The resistance of TxAG-3 was evident in both greenhouse tests, but PI 341885 and Toalson did not differ in resistance from Starr, Florunner, or Goldin I. Perhaps the level of resistance was inadequate for detection under greenhouse conditions, the disease pressure was too low, or the sample numbers were inadequate. Other organisms, possibly interacting with these pathogens or other unknown factors might have affected field reactions. Previous work has shown that the resistance of PI 341885 results in part from failure of the fungus to penetrate through the shell rather than reduced pericarp infection *per se.* (3).

Sterilized soil was used in the rooting media as a precaution against plant loss and confounding effects of other soil-borne organisms in the SP test. This, based on foliar symptoms first observed at flowering, apparently affected the nitrogen availability to the plants, and probably contributed to the slow reproductive development. Plant color was much better in the MP test, and relatively pathogen free, non-sterile soil might be preferred over twice autoclaved soil as the rooting media. Soil sterilization for use in the peg and pod zone was considered important to reduce confounding effects from other organisms in both the development and evaluation of disease.

The single inoculation per plant method required too much labor and space for use as a screening technique on large populations. Both the space and labor requirements were reduced by multiple inoculations per plant. The latter method also allows the evaluation of single plant genotypes, such as advanced generation plant selections from a single seed descent or bulk breeding program, for reactions to multiple pathogens for dual rather than tandem selection. The consistently low pod disease rating for TxAG-3 when exposed to *P. myriotylum* and *R. solani* singly and in combination supports field data regarding the resistance of this genotype to both organisms. This same consistency gives indication of the adequacy of this procedure in screening for the TxAG-3 level of resistance. The MP procedure should be useful in screening TxAG-3 x adapted cultivar progeny and other germplasm sources for useful levels of pod rot resistance. Refinements in the experimental procedure such as closer monitoring and control of moisture, and larger samples might permit classification of lesser levels of resistance.

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