

## Anatomical Traits Associated With Pod Rot Resistance In Peanut<sup>1</sup>

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### ABSTRACT

Peanut (*Arachis hypogaea* L.) cultivars resistant to *Pythium myriotylum* Drechs. and *Rhizoctonia solani* Kuhn, are needed for effective management of pod rotting diseases. The low efficiency of field screening for disease reaction in breeding for resistance has emphasized the need for improved evaluation methods. Anatomical examinations of roots, stems, leaves, pegs, and shells were made of six genotypes (TxAG-3, PI 341885, Toalson, Starr, Florunner, and Goldin I) with various degrees of resistance to pod rots in search of traits that might be used effectively in screening for disease reaction. The palisade mesophyll cells of 50-day old plants were arranged more compactly in pod rot resistant than in susceptible genotypes. An index representing total width ( $\mu\text{m}$ ) of palisade cells/mm leaf blade was more discriminative in distinguishing among genotypes than average of either cell width or cell number alone. The distribution of lignin in peanut shells was correlated with pod rot resistance. The cell walls in the epicarp and sclerenchymatous mesocarp were thicker and more lignified in the resistant than in the susceptible genotypes. Genotypic differences in lignin distribution were readily apparent at 100x when shell sections were stained with phloroglucinol. Associations between anatomical traits of stems, pegs, roots, or juvenile plant leaflets and field pod rot reaction were not consistent among all genotypes. However, lignin-distribution in pods, and an index representing  $\mu\text{m}$  of palisade cells/mm of leaf blade individually or in combination, might be used effectively to supplement field evaluations in screening breeding lines for pod disease reaction.

Key Words: *Arachis hypogaea*, *Pythium myriotylum*, *Rhizoctonia solani*, lignin distribution, phloroglucinol test, palisade mesophyll cells.

Adult plant reactions in field tests have been the basis for evaluation of peanut (*Arachis hypogaea* L.) to pod rots, caused mainly by *Pythium myriotylum* Drechs. and *Rhizoctonia solani* Kuhn (2,6,7,14,18,19), but unpredictable disease development, non-uniform field inoculum distribution, and micro-environmental effects often affect classifications. Screening techniques are needed that can be applied easily to a great number of plants which will clearly differentiate genotypes in accordance with field responses. A screening technique that would effectively discriminate before mature pod development would have advantages as follows: 1) a large number of plants could be screened in small areas; 2) the duration of the evaluation cycle would be shortened; 3) environmental controls could be provided more economically than for adult plants; and 4) the relative effects of different pathogenic isolates could be detected more readily under controlled conditions (5).

The purpose of this study was to examine selected peanut genotypes for morphological traits that might be useful as an efficient screening technique for pod rot resistance. Examinations were made of roots, stems, leaflets, pegs, and shells. Special attention was given to the lignified tissues since an association of cell lignification and pod rot resistance has been proposed (12,13), and the importance of lignin as a mechanism of disease resistance in plants has been emphasized repeatedly (1,15,16,23).

## Materials and Methods

Anatomical characteristics of shells, leaflets, stems, pegs, and roots

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of six peanut genotypes were compared for features that could be related to pod rot resistance. Cross sections of mature shells were stained with phloroglucinol, a lignin specific stain, for examination of lignin distribution.

The genotypes included TxAG-3, a selection from PI 365553 and the most pod rot resistant line; Toalson and PI 341885 (a selection of Schwarz 21), moderately resistant; Starr, slightly resistant to *Rhizoctonia solani*; and Florunner and Goldin I, susceptible (2,6,8,11,16,18).

Segments from various tissue of five random plants of each genotype, grown in a greenhouse, were collected and immediately placed in formalin-acetic acid-alcohol solution (FAA). The size and position of the excised segments were as follows:

a. **Shells** Segments 1 cm in width that included the peg attachment of healthy, two-seeded, dry, and mature fruits were collected and embedded in paraffin for mechanical support. Transverse sections 1 to 2 mm thick and  $\frac{1}{4}$  to  $\frac{1}{2}$  of the shell diameter in length were cut with a sharp razor blade, dehydrated, and embedded (9,17). Also, slides prepared from the same paraffin blocks were examined for lignin deposition by adding a saturated aqueous solution of phloroglucinol in 20% HCl (9). Shell section preparation was as described except that the blocks were placed in a freezer for approximately 2 h prior to cutting to ease sectioning. Two 5 min. baths in distilled water were added to the dehydration series. The slides were immediately examined with the light microscope after staining for lignin. Lignin distribution was examined in six pods of TxAG-3 and PI 341885, five of Toalson, Goldin I and Florunner, and four of Starr.

b. **Leaflets** A basal leaflet of the first leaf on the lower branch of five different 15 and 50-day old plants was collected and a segment, approximately 3 mm wide, was excised perpendicular to the main vein in the middle of the leaflets. Fifteen and 50-day old plants were chosen as representative of juvenile (vegetative) and adult (reproductive) plant stages, respectively.

c. **Stems** 5 mm segments of the main stems from 15 and 50-day old plants were excised immediately above the cotyledonary node.

d. **Pegs** The terminal 5 mm of five aerial pegs from different plants were excised.

e. **Roots** Segments 5 mm in length were excised from primary roots of 50-day old plants near the attachment of the first secondary roots.

Tissues were evacuated and fixed in FAA, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin (10,17). Sections 10-15  $\mu$ m thick were cut with a rotary microtome, mounted on slides, and stained according to a fast green and safranin schedule (10,17). Specimens were examined at 100 and 400x under a Leitz Ortholux microscope and photographed.

Measurements taken on mature shells of each genotype included the width of a vascular bundle fiber cap, the distance between adjacent caps, and the epicarp thickness. The number of cell layers in the epicarp was recorded. Six shell specimens of Goldin I, Toalson, and PI 341885, and five of the other genotypes were examined. The number of caps per specimen were counted in five randomly chosen vascular bundles of each root, stem, and peg specimen. The measurements were averaged on a per plant basis.

The number of rows of palisade mesophyll cells per mm was counted in both 15 and 50-day old plant leaflets, starting at a point approximately 1 mm from the central vein, and the width of three cells were measured. An index representing  $\mu$ m of palisade cells/mm leaf blade was calculated by multiplying the average number or rows of cells by the average cell width.

## Results and Discussion

### Shells

The morphological observations of the safranin/fast green stained mature peanut shells were in general agreement with those of other workers (3,13,20,21), although the arrangement of the epicarp cell layers and of the sclerenchymatous mesocarp was variable among genotypes. TxAG-3 shells were thick, and the cell walls in the epicarp and the fiber caps stained bright red with safranin. In all specimens, the sclerenchymatous mesocarp was very regular between the vascular bundles. In Toalson we found wide vascular bundle fiber caps and

a thick and irregular sclerenchymatous mesocarp with several outgrowths between the vascular bundles. Half of the Toalson shells examined had thin walled epicarp cells and retained little safranin stain. In general, the overall shell structure of PI 341885 was similar in appearance to Toalson, but sometimes the outgrowths of the sclerenchymatous mesocarp were more profuse than in Toalson.

The vascular bundle fiber caps were small in Starr and the sclerenchymatous mesocarp was thinner than TxAG-3. Rarely, an outgrowth was observed in Starr. The shells were thin and sporadic thick-walled epicarp cells were observed. All Florunner shells were relatively thin, and little cell wall thickening in the epicarp was observed. The sclerenchymatous mesocarp of Florunner was thin and reasonably regular. The shells of Goldin I were mostly thin and had an irregular and relatively thin sclerenchymatous mesocarp. The epicarp cell walls of some shells were thickened, although not throughout the specimens, while others were totally thin-walled.

A group of characteristics in the epicarp of peanut shells such as thickness and cell wall thickening, and the structure and arrangement of the sclerenchymatous mesocarp were associated with pod rot resistance, and should be considered collectively. TxAG-3 had a thicker epicarp and a higher number of cell layers than all other genotypes. The cell walls stained deep red with safranin, suggesting the presence of lignin. The epicarp of the susceptible cultivar Florunner was thicker than PI 341885, but more cell wall deposition was apparent in the latter.

Although the safranin/fast green staining method provided interesting information, it would not be practical for use as a screening technique. The procedure was time consuming, required experience, and the differences among genotypes were not readily discerned. However, the shell study supported the report of Pettit and co-workers (12) that lignin is involved in pod rot resistance, probably as a 'pre-existing' mechanism, since only healthy appearing pods were used.

Lignins have been demonstrated to be important mechanisms of resistance to fungal penetration (1,4,12,15). Russell (15) stated that there is very little published information concerning 'pre-existing' resistance mechanisms, however, Pettit and co-workers (12) observed that as pods of PI 365553 mature lignified tissue is deposited near the outer surface, while in Florunner, lignified tissue occurs only near the inner surface. This difference was associated with pod rot resistance. In our studies of the shell, the safranin stain revealed that the arrangement of lignified tissue was variable among genotypes, and apparently was related in these genotypes to pod rot resistance.

Lignified areas were readily visible through the microscope when stained with phloroglucinol, and provided a sharp contrast between the most pod rot resistant and susceptible genotypes, TxAG-3 and Goldin I, respectively. Within TxAG-3 the shells were very similar and appeared to vary in thickness only. These shells had a thick, uniform sclerenchymatous mesocarp which stained deep red violet. Vascular bundle fiber caps also stained intensely, were thin and uniform, and fully covered the vascular bundles. Fibers were observed between the vascular

bundles, underneath the epicarp; a contrast to the observations on all other genotypes. The epicarp consisted of cells with thick lignified walls and a uniform layer of highly lignified material close to the mesocarp. Using a magnification of 100x it was easy to observe that TxAG-3 shells possess two layers of lignified material, near the outer and inner surfaces (Fig. 1).

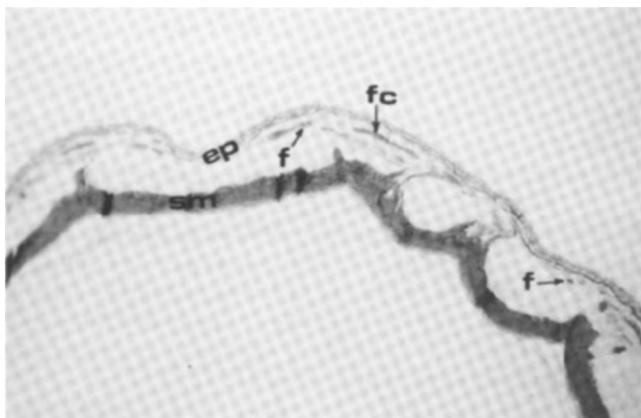


Fig. 1. Cross section of the shell of a mature TxAG-3 pod stained with phloroglucinol. (ep) epicarp, (f) fibers, (fc) fiber cap, and (sm) sclerenchymatous mesocarp. Magnification 200X.

The sclerenchymatous mesocarp of Goldin I shells were irregular, often thin, and stained lighter than the more pod rot resistant genotypes (Fig. 2). Some cell wall thickening was observed in the epicarp of two specimens and the cell walls stained lightly.

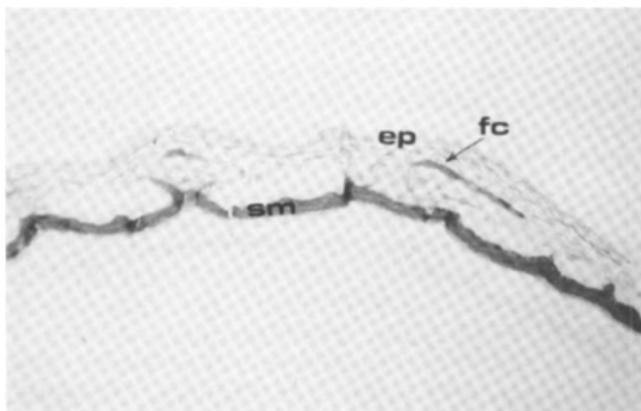


Fig. 2. Cross section of the shell of a mature Goldin I pod stained with phloroglucinol. (ep) epicarp, (fc) fiber cap, and (sm) sclerenchymatous mesocarp. Magnification 200X.

The Toalson shells examined varied in thickness. The sclerenchymatous mesocarp was not uniform, was often thick, and stained intensely for lignin. In one specimen, this tissue was very close to the outer surface of the shell. Cells in the epicarp with lignified walls were observed, but they were not as lignified as those of TxAG-3.

An irregular and thick, apparently highly lignified, sclerenchymatous mesocarp, was characteristic for PI 341885 shells. Outgrowths were observed in four of the six shells examined. The degree of lignification in the

epicarp was intermediate between Toalson and TxAG-3. The two shells with a more uniform sclerenchymatous mesocarp had more lignified material in the epicarp, but still not as much as TxAG-3.

The sclerenchymatous mesocarp in one of the Starr shells contained some small outgrowths, but thin regions that did not stain as intensely as TxAG-3 were observed in all four pods. Cell wall lignification was visible in the epicarp, although not nearly as much as TxAG-3. Some lightly stained lignified material was observed in the epicarp of one Starr pod close to the mesocarp.

The Florunner specimens examined were thin and seemed to have less lignified sclerenchymatous mesocarp than the more pod rot resistant genotypes. The sclerenchymatous mesocarps were uniformly thin in four shells while the fifth specimen was a little thicker and more irregular. Little or no cell wall lignification was observed in the epicarp. Some lignified material was observed in the epicarp near the mesocarp, but did not form a continuous band and stained lightly.

The main features observed on the phloroglucinol stained sections were in agreement with observations on shells stained with safranin and fast green, but the phloroglucinol stain provided a much better contrast. This stain would facilitate the study of the specimens if used as a screening technique. The staining procedure is very simple, and although the preparation is not permanent, microscope slides can be examined quickly, and a decision regarding resistance to pod rot should be reached easily.

The sclerenchymatous mesocarp was reported by Pettit and co-workers (12,20) to be lignified and act as a barrier to fungi. Observations in the present study suggest that both thickness and uniformity of this tissue are related to resistance to the disease. The sclerenchymatous mesocarp of TxAG-3 was thick and uniform; Toalson and PI341885 were thick and irregular with outgrowths, and the sclerenchyma was thin in the other genotypes. A combination of all the previously described characteristics could account for the pod rot resistance of TxAG-3, the development of surface rots on PI 341885 with little damage to the kernels (6,12), and the lack of resistance in Florunner and Goldin I.

#### Leaflets

Leaflet specimens from 50-day old plants were collected, and easily sectioned and stained with safranin/fast green. The description by Brennan (3) of the palisade mesophyll in peanut leaflets were confirmed in this study. The arrangement of the cells varied among entries and a strong relationship between this characteristic and pod rot reaction was apparent. The palisade mesophyll cells in TxAG-3 were more compactly arranged than in the other genotypes, and a difference in the arrangement of these cells was observed, from the most pod rot resistant genotypes, with the most compact arrangement, to the most susceptible, with more intercellular space, as shown in Figs. 3 and 4.

The index representing  $\mu\text{m}$  of palisade cells/mm of leaflet blade made quantification of the observations easier and provided a better numerical characterization than either measurement separately. Analysis of the index produced the same statistical results as the analysis of cell width and demonstrated numerically that the cell

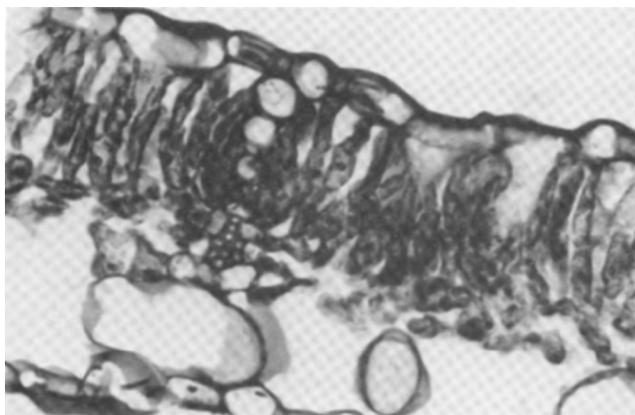


Figure 3. Cross section of a basal leaflet from the first leaf of a primary lateral branch of a 50-day old TxAG-3 plant showing compact arrangement of palisade cells. Magnification 3,000X.

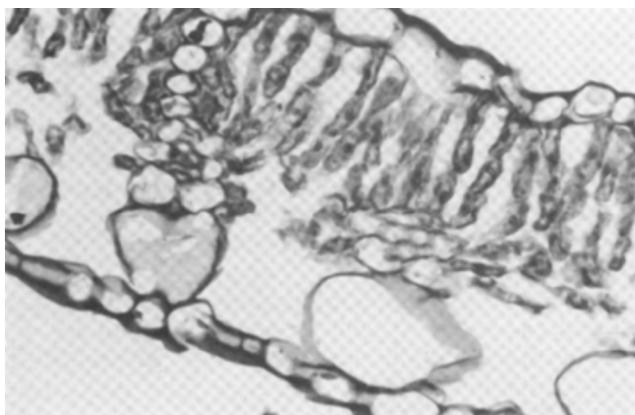


Figure 4. Cross section of a basal leaflet from the first leaf of a primary lateral branch of a 50-day old Goldin I plant showing wide interspaces among palisade cells. Magnification 3,000X.

arrangement of pod rot resistant genotypes were more compact than those of susceptible genotypes (Table 1).

Table 1. Average number of cells per mm, average cell width, and index of the palisade mesophyll cells of peanut leaflets collected from 50-day old plants.

	Genotype						C.V. (%)
	TxAG-3	Toalson	PI 341885	Starr	Florunner	Goldin I	
Cells/mm	59.8a*	54.8ab	52.8bc	47.4c	46.8c	46.6c	9.57
Cell width (μm)	11.0a	10.8a	11.0a	9.18b	8.5bc	7.5c	9.05
Index** (μm/mm)	607.3a	592.4a	579.3a	436.4b	398.4bc	349.7c	8.08

\* Means within rows followed by the same letter are not significantly different at the 0.05 probability level using Duncan's Multiple Range Test.

\*\* Index (μm/mm) = cells/mm x cell width (μm). Statistical analyses were made using arcsine transformed data.

In 15 day-old leaflets the relationship with disease resistance was not so apparent. Some differences among genotypes in palisade cell number/mm of leaflet and palisade cell width were found but the rankings among genotypes were not consistent with field pod rot disease reactions. More research is needed to determine the consistency of the association of mesophyll cell arrangement and pod rot resistance over a large number of

genotypes, and to ascertain the genetic control.

#### Stems

Association of stem anatomy and pod disease reaction of 50-day old plants was not as good as the association of disease reaction with leaves of 50-day old plants. The genotypes PI 341885 and TxAG-3 had more fiber caps per stem than Starr, Florunner, and Goldin I. The fiber caps of Florunner were located deeper in the stem than those of Toalson. Goldin I had wider fiber caps than all other entries and were located closer together than those of PI 341885. The main difference of apparent importance was that the more pod rot resistant genotypes had more fiber caps that were closer to the outer surface of the stem. The major difference found among stems of 15-day old plants was in number of fiber caps/stem. An average of 30.0 fiber caps/stem was counted in TxAG-3 compared to 24.6 and 26.4 for Florunner and Goldin I, respectively. However, the ranking of the genotypes was different from that of 50-day old stems. It appeared that the relative number and depth of fiber caps changed with age of peanut stems, and association with pod rot resistance might exist only in mature plants.

#### Pegs

In immature aerial pegs it was difficult to determine the exact delimitation of the vascular bundle fiber caps because the fibers were still immature. The fiber caps of all genotypes were approximately the same in depth and width, but they were closer together in TxAG-3. The fiber caps were more numerous in PI 341885 than Starr, Goldin I, and Florunner. The more pod rot resistant entries had a higher number of fiber caps in aerial pegs than the more susceptible genotypes, although the latter did not differ statistically from Toalson and TxAG-3. Although the number of fiber caps in the pegs was lower than in the older stems, the ranking of the genotypes was similar in both plant parts. The study of the pegs confirmed the similarities of stems and pegs related by Thomas and co-workers (21,22,28). As in the young stems, no differences were found in fiber cap depth and width in the pegs.

#### Roots

No discriminatory feature that could be associated with pod rot resistance was apparent in neither the external nor internal morphology of 50-day old plant tap roots. PI 341885 roots had more fiber strands than Toalson, TxAG-3, and Florunner. The fiber strands of Goldin I, TxAG-3, and PI 341885 were positioned deeper in the roots than those of Toalson and Florunner. The strands were wider in TxAG-3 than in Starr and PI 341885, and were positioned closer together in Toalson than in Florunner, Goldin I, and TxAG-3. Fiber strands in Starr and PI 341885 roots were intermediate in interspace length and did not differ from any other genotype.

Our measurements on the root fiber strands indicated that the caps of TxAG-3 were wide and deep inside the root, but the interspace between them was comparatively wide because of a low number of fiber strands. The interspace was wide in Florunner and Goldin I and the fiber strands were moderately wide, but the bundles of Florunner were close to the surface and in relatively low numbers. Goldin I had more fiber strands placed deeper in the root than did the other genotypes.

Some measures other than those on shells and 50-day

old plant leaflets showed some relation to the usual comparative field pod rot reactions of the genotypes. A comparison of measurements on the most resistant and susceptible genotypes for anatomical traits that differed at the 5% probability level are presented in Table 2. Repetitious among the observations was the indication that pod rot resistant and susceptible genotypes differ in the proportion of sclerotized tissue in various plant parts. The differences among genotypes and for the varied levels of resistance were more distinct in the 50-day old plant leaflets and in the shells. Thus, examinations at these stages is considered to be the most reliable for screening.

Table 2. Anatomical trait measurements of a pod rot resistant and susceptible cultivar that differ at the 5% probability level.

Plant Part/Anatomical Trait	Cultivars	
	TxAG-3 (Resistant)	Goldin I (Susceptible)
Shell (mature)		
cell layers/epicarp (no.)	4.8	2.7
epicarp thickness ( $\mu\text{m}$ )	78.3	42.5
Leaflet		
15-day old plant		
palisade width/leaflet ( $\mu\text{m}/\text{mm}$ )	777.6	626.0
50-day old plant		
palisade cells/mm (no.)	59.8	46.6
palisade cell width ( $\mu\text{m}$ )	11.0	7.5
palisade width/leaflet ( $\mu\text{m}/\text{mm}$ )	607.3	349.7
Stem		
15-day old plant		
fiber caps/stem (no.)	30.0	26.4
50-day old plant		
fiber caps/stem (no.)	29.0	21.0
Peg		
fiber cap interspace ( $\mu\text{m}$ )	24.9	46.1
Root	no difference	

The lack of differences among these genotypes in the root sections is not congruent with the result of seedling screening by Jones and Woodard (11). They subjected seedling roots to *P. myriotylum* oospore suspensions in water and reported percentages of seedling wilt to increase progressively for PI 365553, Toalson, Tamnut 74, and Florunner, respectively. If the extent and distribution of lignification alone were the determinant for disease reaction, it would seem that anatomical differences should have been found in the roots for agreement with their results.

The association of thickness of the sclerenchymatous mesocarp with resistance causes some concern for breeding. If the thickness or density of the mesocarp causes significant effects on shell weight, it could be assumed that the percentage of shell-out would be lower in pod rot resistant lines. However, if the uniformity and localization of lignin are major factors in pod rot resistance, selection of thin-shelled genotypes with the desired lignin deposition might result in minimal effects on peanut grades.

Analyses of the calculated index showed that the more resistant genotypes had less intercellular space than the susceptible ones (Table 1). Perhaps calculation of the index would be more laborious than necessary for screening large populations; a well-trained technician might rate cell arrangement adequately for preliminary decisions regarding selection. Evaluation of adult plant leaflet tissue followed by shell lignin distribution examinations of selected plants or lines might be used for the selection of elite lines for final field disease comparisons. Additional studies are needed on the applicability of these techniques to a wider range of germplasm, the timing of the deposition of lignin, the inheritance of lignin content and distribution, and the association of pod lignin and palisade cells in leaflets.

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