Performance of Atesta and Intact Peanut Seed in Field Plots, Field Microplots, Germination and Pathogenicity Tests[1]

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ABSTRACT

Stands from atesta (bald) seed 10 and 20 days after planting were 32.0 and 35.5% less than stands from intact seed in field plots. Thirty-two and 62 days after planting the fresh weights of plants from bald seed were 32.3 and 15.3% less than those from intact seed. Pod yield of plants from bald seed was 20.4% less than that from intact seed. Sound mature kernels (SMK) produced by plants from bald seed was 1.2% less than the SMK from intact seed. When plant populations in field plots were adjusted by covariance analysis to equal numbers, no difference in yield was predicted. Plants grown from bald and intact seed in field microplots fumigated with metam-sodium had no difference in yield or SMK. Bald seed germinated in 24 hr cycles of 20 C/16 hr + 30 C/8 hr had fewer normal rapid and more normal slow germinating seedlings than intact seed. At temperatures of 30 C/16 hr + 20 C/8 hr, there was no difference in germination percentages. In soil infested with *Aspergillus niger* van Tieghem at 21 C/12 hr + 27 C/12 hr there was no difference in seedling emergence from bald or intact seed after 22 days. At temperatures of 15 C/12 hr + 21 C/12 hr, seedling emergence from bald seed in *A. niger* infested and noninfested soil was less than that of intact seed in control soil. Emergence of seedlings from bald seed was zero and that from intact seed was less than other treatments at both temperature regimes in soil infested with *Rhizoctonia solani* Kühn anastomosis group 4.

Key Words: Atesta (bald) seed, intact seed, testa, cold stress, germination vigor, *Aspergillus niger*, *Rhizoctonia solani* AG-4.

An intact testa is important for normal germination, emergence and growth of peanut (*Arachis hypogaea* L.) seed and seedlings. Carter (3) demonstrated that atesta seed (known as bald seed or "baldheads") either treated or nontreated with fungicides had ca 10% emergence compared to 95-98% emergence with intact seed. Harrison et al. (5) determined that hand planted intact seed produced an 85% stand in field plots contrasted to a 20% stand with hand planted bald seed. Higgins and Bailey (7) showed that seedlings from bald seed were stunted in growth and frequently abnormal compared to the appearance of seedlings from intact seed. Bell (1,2), Carter (3) and Clinton (4) reported that intact testae provided a physical/physiological barrier to infection of seed by several pathogens.

The objectives of this study were to compare the performance of bald and intact peanut seed in (1) laboratory germination tests; (2) field plots planted with commercial planters; (3) field microplots planted by hand; and (4) pathogenicity tests with two common peanut seed pathogens under two soil temperature regimes.

Materials and Methods

A supply of Florunner seed was obtained with ca equal amounts of bald and intact seed. These seed were hand sorted into two groups, visibly intact and completely bald with the cotyledons intact.

The bald and intact seed were assayed prior to planting for differences in seed weight, germinability and microfloral infestation. For seed weight determinations, 10 samples of 100 randomly selected seeds were used.

Prior to germination, seed were treated with a fungicide combination of 1.13 g dicloran + 1.13 g captan per kg of seed. Twenty-five seeds were placed, radicle tip downward on pH neutral, premoistened seed germination papers (Dillard Paper Co., 50 Bestfriend Road, Doraville, GA 30340). The papers were rolled and placed in a germinator with the rolls oriented in wire mesh baskets with the radicle tips downward. Each roll of 25 seeds comprised a replicate and eight replicates were germinated for each bald and intact seed.

During germination two temperature regimes were used on a 24 hr cycle, both in the dark: 30 C for 16 hr + 20 C for 8 hr and 20 C for 16 hr + 30 C for 8 hr. After 12 days seedlings were rated for percentages of normal rapid (two cotyledons, complete epicotyl, elongation of primary root > 10 cm and extensive development of fibrous roots, with elongation > 5 cm and radial enlargement > 5 mm of the hypocotyl), normal slow (opposite of normal rapid, except cotyledons and epicotyl complete), abnormals caused by pathogens, abnormals caused by other factors (mechanical damage and concealed damage), dormant seed and dead seed.

For microfloral assays, 200 randomly selected fungicide treated seeds were plated on an agar medium and arranged in 20 replicates, with a 9 x 1.5 cm petri dish of medium containing 10 seeds comprising each replicate. Plated seed were incubated at 23-24 C in 8 hr of diffuse fluorescent light + 16 hr dark for 9-10 days and the microflora enumerated. Rapidly growing colonies of *Rhizopus* sp. and *Trichoderma* sp. were enumerated 3-4 days after plating seed. The microfloral medium contained the following ingredients per liter: KH_2PO_4, 0.5 g; K_2HOP_4, 0.5 g; $MgSO_4.7\ H_20$, 0.5 g; NH_4NO_3, 0.5 g; bactopeptone, 1 g; yeast extract, 1 g; phythone, 1 g; malt extract, 1 g; sucrose, 10 g; glucose, 10 g; agar, 17 g; rosebengal, 25 mg, demineralized water, 955 ml. The medium was heated to dissolve organic components, dispensed into screw-cap bottles and autoclaved at 121 C for 20 min. Each petri dish used for plating peanut seed contained ca 20 mL of the medium.

In the field one row of bald and one of intact fungicide treated seed were planted on the same bed in Fuquay loamy sand (ca 86, 10 and 4% sand, silt and clay, respectively). Plots were 0.9 x 7.6 m. Planting depth was ca 5.1 cm with seed ca 14 cm apart in the row (ca 36% normal Florunner seeding rate for Georgia). The reduced seeding rate was an attempt to promote maximum growth and yield of individual plants from bald seed. Twenty-four plots were planted May 12. A visual cracking rating was made 6 days after planting using a scale of 0 = none to 4 = most extensive. Emergence counts were made 10 and 20 days after planting. Plants in four plots each from bald and intact seed were dug with a spade fork 32 and 62 days after planting, brushed free of soil and the fesh weight of each plant determined. Sixteen plots each from bald and intact seed were dug 149 days after planting. Pods were dried to 10% moisture, removed from the vines by hand and yields recorded. The percentages of sound mature kernels (SMK) were obtained from a 300 g pod sample from each replicate using standard Federal-State grading procedures. Pod yields were estimated with the plant populations from bald and intact seed adjusted to the same numbers by covariance analysis. Cultural and pest control practices were those recommended by the Cooperative Extension Service for commercial peanut production in Georgia (6). Sprinkler irrigation was used as needed to supplement rainfall.

Field microplots were established in concrete cylinders (surface area = 0.3 m^2, length = 1.2 m, volume = 0.1 m^3) filled with Alapaha loamy sand (ca 77, 20, and 3% sand, silt and clay, respectively). Approximately 0.4 m of each cylinder extended above ground level. Soil in each microplot was fumigated in February with 448 kg of metam-sodium applied in 253.8 kL per ha of water. Fungicide treated Florunner seed were planted on June 1 and three plants of either

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bald or intact seed were established in each of 24 microplots. The plants were arranged in a triangular pattern so that the pegging zone would occupy most of the surface area in each microplot. Cultural and pest control practices were the same as those used in field plots, except no herbicides were used.

Plants were dug 129 days after planting and dried until the pods contained 10% moisture. Weights were determined separately for total biomass and pods. The percentage of SMK was determined using all seed produced in each replicate of the bald and intact categories.

The susceptibility of bald and intact peanut seed to *Aspergillus niger* van Tieghem and *Rhizoctonia solani* Kühn anastomosis group (AG) 4 was tested under two temperature regimes in growth chambers. Both fungi were originally isolated from visibly sound peanut seed. Heat treated (70 C, 30 min) Tifton loamy sand (ca 85, 10 and 5%, sand silt and clay, respectively) was infested with 10-day old cultures of the fungi grown on 3% (w/w) cornmeal/sand inoculum. The concentration of *A. niger* was 1 part inoculum to 100 parts soil (v/v) and that of *R. solani* was 1 to 250. Infested and noninfested soils were placed in 2 L pans 19.6 x 19.6 x 5.3 cm. Duplicate sets of six treatments each replicated five times in a randomized complete block design with 10 seeds per replicate included bald or intact seed planted in noninfested or soil infested with *A. niger* or *R. solani*. One set of treatments was maintained in a chamber with 21 ± 1 C for 12 hr + 27 ± 1 C for 12 hr, and the other set was maintained in a chamber with 15 ± 1 C for 12 hr + 21 ± 1 C for 12 hr. Both temperature regimes were on 12 hr light + 12 hr dark cycles, having simulated sunlight and incandescent lamps with energy at the plant level of ca 700 $E_{s-1, m-2}$. Emergence counts were made 22 days after planting. Data in these experiments were analyzed by ANOVA and Duncan's Multiple Range test or the Waller-Duncan K-Ratio T test.

Results and Discussion

There were no significant differences in the weights of bald (65.9 g) and intact (64.5 g) seed. Seed weight therefore, would not have affected the results of the field planting. There were no significant differences among any of the germination categories when bald and intact seed were germinated at 30 C/16 hr + 20 C/8 hr (Table 1). However, normal rapid germination of bald seed was significantly reduced (P = 0.05) and normal slow germination was significantly increased (P = 0.05), when the seed were maintained at 20 C/16 hr + 30 C/8 hr. There were no differences between bald and intact seed for abnormal, dormant and dead seed.

The germination tests showed that under favorable moisture and temperature conditions there were no differences in the germinability of bald and intact seed. However, bald seed exposed to prolonged cold stress produced fewer normal rapid and more normal slow germinating seedlings than did intact seed. The primary implications are that, with bald seed in cold field soil (ca 20 C or <), germination and emergence would be slowed and normal growth of seedlings reduced.

The occurrence of peanut seed pathogens in bald and intact seed was low. Among the microflora isolated, *Rhizopus arrhizus* Fischer, *A. flavus* Link, *A. niger*, and *R. solani* AG-4 are potent peanut seed pathogens, and these fungi were infrequently isolated from fungicide treated seed. With the exception of significantly more (P = 0.05) *R. arrhizus* in bald (0.5%) than intact (0.2%) seed, the isolation frequencies of potent peanut seed pathogens was the same for both bald and intact seed. The role of these fungi in pathogenesis to bald seed cannot be discounted, however, since tests (3,4) have shown that bald seed are much more susceptible to pathogens than intact seed.

The visual rating of seedlings cracking the ground six days after planting was significantly less (P = 0.05) for bald than for intact seed (Table 2). The emergence counts at 10 and 20 days after planting were significantly higher (P = 0.05) for intact than for bald seed (Table 2). There was 32.0% fewer plants from bald than intact seed 10 days after planting and 35.5% fewer after 20 days, suggesting that bald seed were more susceptible

Table 1. Germination of bald and intact peanut seed under two daily temperature regimes.

Germination, % [a]				
30 C for 16 hr + 20 C for 8 hr				
	Normal [b]		Abnormal [c]	
Seed	Rapid	Slow	Diseased	Other
Bald	62.0 a	12.5 a	20.0 a	2.0 a
Intact	67.0 a	8.5 a	20.5 a	2.0 a
	Dormant		Dead	
Bald	0.0 a		3.5 a	
Intact	0.5 a		1.5 a	
20 C for 16 hr + 30 C for 8 hr				
	Normal [b]		Abnormal [c]	
Seed	Rapid	Slow	Diseased	Other
Bald	60.0 b	15.5 a	17.0 a	3.5 a
Intact	69.0 a	6.0 b	19.5 a	4.0 a
	Dormant		Dead	
Bald	0.0 a		4.0 a	
Intact	0.5 a		1.0 a	

[a] Means within columns followed by the same letter are not significantly different at P = 0.05 by Duncan's Multiple Range test.

[b] Normal rapid: two cotyledons, complete epicotyl, elongation of primary root> 10 cm, extensive development of fibrous root system, elongation >5 cm and radial enlargement> 5 mm of hypocotyl. Normal slow: opposite of normal rapid, except cotyledons and epicotyl complete.

[c] Diseased: seedborne pathogens. Other: mechanical damage and/or concealed damage.

Table 2. Visual ground cracking rating, emergence counts and fresh weight of plants from bald and intact peanut seed, with days after planting indicated in each category.

	Cracking rating	Emergence counts [b]	
Seed	after 6 days [a,b]	10 days	20 days
Bald	2.3 b	42.6 b	41.5 b
Intact	2.8 a	62.6 a	64.3 a
	Fresh weight of plants, g		
	32 days [b,c]	62 days [b,d]	
Bald	6.7 b	100.9 b	
Intact	9.9 a	119.1 a	

[a] Visual cracking rating : 0 = none - 4 = most.

[b] Means within columns followed by the same letter are not significantly different at P = 0.05 by Duncan's Multiple Range test.

[c] n = 195 for bald and 200 for intact.

[d] n = 254 for bald and 279 for intact.

to preemergence stresses (e.g., pathogens).

For 20 days after planting the average minimum and maximum soil temperatures at 5.1 cm depth were 22.4 and 36.4 C and the overall average was 29.4 C. Therefore, cold and heat stresses probably were not limiting factors in reducing emergence of bald seed. The seed treatment fungicide was a dust formulation which adhered poorly to bald seed. The reduced amount of fungicide adhered to bald seed coupled with increased vulnerability to decay by seed and soilborne pathogens probably accounted for a major part of the reduced emergence.

The average fresh weight of peanut plants from bald seed was significantly less (P = 0.05) at 32 and 62 days after planting than that from intact seed (Table 2). Higgins et al. (8) determined that raw peanut testae contained an average of 36 μg per g of thiamin chloride and 4.8 mg per 100 g of nicotinic acid. These and possibly other growth factors in the testae may have been responsible for the more rapid growth of plants from intact seed than from bald seed.

The pod yield of plants from bald seed (1562.2 kg/ha) was significantly less (P = 0.05) than the yield from intact seed (1963.0 kg/ha). However, when plant populations from bald and intact seed were statistically adjusted to the same number, no significant differences in yield were predicted. To test the statistical prediction, plants were grown from bald and intact seed in field microplots containing metam-sodium fumigated soil. There were no differences in average yields of total biomass from bald (385.0 g) or intact seed (411.3 g) or pods from bald (213.4 g) or intact seed (224.4 g).

With plants grown in the field, those from bald seed had a small but significantly lower (P = 0.05) percentage of SMK (72.4%) than did plants from intact seed (73.6%). There was no difference in SMK, where plants from bald seed (71.8%) or intact seed (72.9%) were grown in field microplots. Small differences in SMK, however, can be important because each point increase in SMK increases the value of pods ca $6.35/metric ton.

Bald and intact fungicide treated seed were planted in noninfested soil and soil infested with a high inoculum concentration (1:100 v/v) of a moderately virulent strain of *A. niger* or a high inoculum concentration (1:250 v/v) of a highly virulent strain of *R. solani* AG-4. When these seed were incubated at 21 C/12 hr + 27 C/12 hr, there were no differences in percentage emergence after 22 days between bald and intact seed planted in noninfested soil or soil infested with *A. niger* (Table 3). Emergence of bald and intact seed in soil infested with *R. solani* was significantly lower (P = 0.05) than with *A. niger* infested or noninfested soil (Table 3). There was significantly higher (P = 0.05) emergence of intact than bald seed in *R. solani* infested soil (Table 3). With a temperature regime of 15 C/12 hr + 21 C/12 hr, emergence of intact seed in noninfested soil was significantly greater (P = 0.05) than treatments other than intact seed in *A. niger* infested soil (Table 3). Emergence of bald seed in *R. solani* infested soil was zero at both temperature regimes.

The retarding effect of cold stress on germination of seed and emergence of seedlings is illustrated by an 84% emergence of bald seed in noninfested soil at 21 C/12 hr + 27 C/12 hr vs 70% emergence with the same treatment at 15 C/12 hr + 21 C/12 hr (Table 3).

Table 3. Emergence percentages after 22 days under two daily temperature regimes, with bald and intact peanut seed planted in noninfested soil or soil infested with *Aspergillus niger* or *Rhizoctonia solani*.

Seed	Soil treatment	Emergence, % [a] 21 C/12 hr + 27 C/12 hr	15 C/12 hr + 21 C/12 hr
Intact	Noninfested	94 a	92 a
Intact	*A. niger*	94 a	84 ab
Bald	*A. niger*	86 a	56 c
Bald	Noninfested	84 a	70 bc
Intact	*R. solani*	16 b	10 d
Bald	*R. solani*	0 c	0 d

[a] Means within columns followed by the same letter are not significantly different at P = 0.05 by the Waller-Duncan K-Ratio T test.

There is no doubt that the physical integrity and chemical composition of peanut testae are important in the health of germinating peanut seeds and normal growth of peanut seedlings (1,2,3,4,8). LaPrade et al. (9) also demonstrated that the thickness and integrity of wax on the outer surface of testae affected seed infection by *A. flavus*. Carter (3) found that with seed planted in soil infested with *A. flavus* there was 48% more emergence of seed with colored testae than with white testae. Sanders (10) determined that low concentrations of tannins occurred in colored testae early in development of pods (0.92%, fresh weight), but these increased with maturity (6.95%, f. wt.). and then dropped slightly before final curing (6.04%, f. wt.). Tannins are high molecular weight polyphenolic compounds that are inhibitory to some pathogenic fungi of peanut seed (3,11).

This study has shown that, although plants from bald seed are slower germinating and slower growing than those from intact seed, there was no difference in the potential yield of plants from the two types of seed. This was predicted by covariance analysis of yield data from a field plot test and corroborated by yield data from a field microplot study. There was 1.2% less SMK produced by plants from bald than intact seed in field plots and 1.1% less in field microplots. These differences in percentages of SMK possibly were caused by differences in maturity distribution caused by slower germination and slower growth of plants from bald seed.

In conclusion this study has highlighted two major problems with bald peanut seed relative to intact seed: germination and emergence were hindered more by cold stress and susceptibility to peanut seed pathogens was greater.

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