

# Genetic and Seed Treatment Effects in Organic Peanut

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## ABSTRACT

Stand establishment has been a challenge for organic peanut production in the Southeastern United States. Field experiments were conducted in 2007 and 2009 in research plots certified for organic production to evaluate the potential of genotype selection, shelling procedure, and seed treatment with *Bacillus subtilis* to improve stand establishment and seedling emergence rates, reduce incidence of *Aspergillus* crown rot, and increase seedling biomass. Seed of 15 peanut genotypes were mechanically shelled or hand-shelled, and treated with *B. subtilis* or untreated prior to planting in early June. Percent stand was estimated for each plot 17–21 days after planting (DAP), and percent seedlings affected by *Aspergillus* crown rot and plant biomass were estimated 21–24 DAP. Seed treatment significantly affected stand establishment for three genotypes each year, Georgia-01R both years, C-99R and Tif-guard in 2007, and C34-24-69 and C724-19-25 in 2009. In all of these cases, the hand-shelled plots had significantly greater stands than the mechanically shelled plots for untreated and/or *B. subtilis* treated seed. *B. subtilis* treatment improved stands for two of the mechanically shelled treatments that significantly responded to hand shelling. Despite low incidence, there was a significant reduction of crown rot in the hand-shelled plots compared to the mechanically shelled plots across years and genotypes. The cultivar Georganic, which has been planted to organic peanut systems in Georgia in recent years, was among the genotypes with the lowest seedling establishment rates and biomass. Based on these results, it is recommended that sorting thresholds for damaged seed be higher for seed destined for organic production, and that breeding efforts that include assessments of factors involved in seedling establishment and growth be prioritized.

Key Words: *Aspergillus* crown rot, hand-shelled, shelling, seedling emergence, organic peanut.

Organic production has been the fastest growing agricultural sector in the U.S. for many years (Dimitri and Greene, 2002). Although organic peanut production in the U.S. is currently concentrated in the Southwest (Ruark and Shew, 2010), significant efforts to develop organic peanut production practices that are viable in the Southeast have recently been employed (Lamb *et al.*, 2007; Cantonwine *et al.*, 2008b; Johnson and Mullinix, 2008; Ruark and Shew, 2010). Through these efforts, stand establishment has been identified as one of the most important challenges to organic peanut production in the Southeast because the synthetic seed treatments that are ubiquitous in conventional peanut production to reduce seed and seedling diseases are not allowed in organic production. Untreated peanut seed can result in 50% fewer established plants than seed treated with synthetic seed treatments even when seed quality is high (Melouk and Backman, 1995). Poor seedling emergence can not only directly limit yield potential, but can also have an indirect impact on yield due to increased incidence of *Tomato spotted wilt tospovirus* (TSWV) (Branch *et al.*, 2003), and increased weed competition (Place *et al.*, 2010).

There are a number of biological, botanical, and mineral seed treatments that are allowable in organic production. One of the most studied is a microbial-based treatment of *Bacillus subtilis*, a gram-positive, endospore-forming bacterium. Strain GB03 is marketed as the seed treatment Kodiak (Bayer CropScience, Research Triangle Park, NC). The effect of *B. subtilis* GB03 and related strains on improving stand establishment has been variable (Elliott *et al.*, 2001; Turner and Backman, 1991; Ruark and Shew, 2010). In a recent study, Ruark and Shew (2010) found that stands were nearly identical between seed treated with a high rate of Kodiak (2.5 g/kg seed) and untreated seed in one year, yet two times greater than untreated seed in another year. Turner and Backman (1991) found that peanut seed treated with a synthetic seed treatment and *B. subtilis* AF 1 improved emergence when seed were planted early and soil temperatures were low (21°C), but did not affect emergence when planting conditions were optimal.

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Factors that affect peanut seed quality, such as maturity, drying and storage conditions, and soundness of seed after shelling, can have a great impact on germination rates (McLean and Sullivan, 1981; Sullivan, 1995). Bell (1969) showed that seed damaged by mechanical shelling can significantly reduce seedling emergence compared to seed that were shelled by hand. However, not all seed become damaged during the mechanical shelling process. Using a standard laboratory assay, Dey *et al.* (1999), demonstrated that undamaged mechanically shelled peanut seed had comparable germination as hand-shelled seed, while the damaged mechanically shelled seed where the testa had slipped or been removed had lower germination. More recently, Tarekegan *et al.* (2007) compared performance of cleaned mechanically shelled seed, where discolored, shriveled, and split seed were removed, to uncleaned seed under field conditions in Ethiopia. They found that in most cases, the plots planted with cleaned seed had greater seedling emergence rates and reduced post-emergence plant mortality than those with uncleaned seed.

Our objectives were to evaluate how hand-shelled or mechanically shelled seed, with or without *B. subtilis* seed treatment affected stand establishment, seedling emergence rates, and incidence of *Aspergillus* crown rot, caused by *Aspergillus niger*, for 15 peanut genotypes. Genotypic differences of these variables were evaluated when possible. The effect of genotype on seedling biomass, which is relevant to early season weed suppression in organic systems, was also evaluated.

## Materials and Methods

Field experiments were conducted in 2007 and 2009 on organically managed research plots certified for organic production in 2007 at the Horticulture Hill Farm, part of the University of Georgia, Coastal Plain Experiment Station in Tifton, GA. The 2007 field was planted to peanut the previous year, and the 2009 field was fallow cultivated the previous year to control weeds. The fields were prepared by harrowing, and plots were prepared with a bedder in 2007 and tractor tire indentions to differentiate beds in 2009. No pesticides or irrigation were applied during the experiments, which lasted 21 days in 2007 and 24 days in 2009. Weeds were removed within the plant row by hand. The field received 11.6 cm of rain across 10 rain events in 2007 and 4.9 cm of rain from 8 rain events in 2009.

The experimental design was a three factor factorial arranged in a randomized complete block

with 5 replications. There were 60 experimental treatments consisting of 15 peanut genotypes, two shelling techniques (mechanically shelled or hand-shelled), and two seed treatments [untreated or treated with *B. subtilis* ( $2.5 \times 10^{-6}$  kg Kodiak per g of seed)]. The 15 runner-type genotypes included seven cultivars: Georgia Green (Branch, 1996), Georganic (Holbrook and Culbreath, 2008), AP-3 (Gorbet, 2007), C-99R (Gorbet and Shokes, 2002), Georgia-01R (Branch, 2002), Tifguard (Holbrook *et al.*, 2008), and Tifrunner (Holbrook and Culbreath, 2007); and eight breeding lines: C11-186, C11-154-61, C12-3-114-58, C34-24-38, C34-24-69, C34-24-124, C689-6-6, and C724-19-25. Each seed lot came from seed that had been harvested from plants grown for seed production under identical conditions the previous year, and harvested at maturity. Seed were stored in the shell through the winter and shelled (mechanically or by hand) in the spring. Mechanically shelled seed were shelled using standard presizer and box shellers that are commonly used in grading procedures. Seed from both shelling treatments were cleaned to remove discolored, shriveled, or split seed. Hand shelled seed with entire or partial testa losses were also discarded, and mechanically shelled seed with entire testa losses or significant damage to cotyledons were also discarded. Treatment plots were single rows, 3.0 m long, separated by 1.0 m alleys. Thirty seed were planted by hand, 3.8 cm deep and 10 cm apart on 29 May 2007 and 2 June 2009. Wide seed spacing was used to minimize the chance of pathogen movement from seed-to-seed and plant-to-plant.

Seedling emergence was estimated as the percentage of seed resulting in an established seedling 21 days after planting (DAP) in 2007 and 17 DAP in 2009. Incidence of *Aspergillus* crown rot, expressed as the percentage of established seedlings exhibiting symptoms or signs, was recorded 21 DAP in 2007 and 24 DAP in 2009. Establishment rate was estimated as the percentage of the stand at the final evaluation that was emerged at 10 DAP. Seedling biomass was estimated 21 DAP in 2007 and 24 DAP in 2009 by harvesting the aerial parts of ten consecutive plants, avoiding plants with obvious *Aspergillus* crown rot. If there were less than ten plants in a plot, all healthy plants were harvested. Harvested plants were dried for 48 hr at 100°C, allowed to cool to room temperature in plastic bags, and weighed.

General linear model univariate analyses were performed using the statistical package SPSS version 17. When interactions were not significant, genotype, shelling treatment, and seed treatment were classified as fixed factors, and year and replication were classified as random factors. In

**Table 1. Effects of seed treatments on percent stand across years for each genotype.**

Year/genotype	Percent Stand <sup>a</sup>									
2007	MU <sup>bc</sup>		MT <sup>bd</sup>		HU <sup>ec</sup>		HT <sup>ed</sup>	SE <sup>f</sup>	<i>P</i> -value	
Georgia Green	71.4		74.6		79.2		82.2	3.97	0.07	
Georganic	68.6		68.8		70.8		74.0	4.90	0.67	
AP-3	84.0		85.2		80.8		83.2	2.82	0.48	
C-99R	61.4	ab	53.8	b	71.4	a	69.4	a	5.42	0.02
Georgia-01R	45.6	b	66.0	a	67.8	a	77.4	a	4.93	0.00
Tifguard	54.0	b	61.4	ab	73.0	a	70.6	a	5.14	0.01
Tifrunner	80.2		77.2		81.8		81.6		4.89	0.77
C11-154-61	70.6		65.4		64.6		69.4		5.63	0.66
C11-186	64.6		69.2		71.4		64.0		5.07	0.42
C12-3-114-58	70.2		65.2		66.6		68.0		7.09	0.91
C34-24-38	78.4		79.4		68.2		77.4		3.98	0.05
C34-24-69	83.8		80.2		77.4		85.4		6.19	0.58
C34-24-124	79.4		84.6		82.0		85.4		4.96	0.62
C689-6-6	72.2		62.0		63.2		62.2		8.26	0.56
C724-19-25	72.6		78.0		81.2		84.0		5.99	0.30
2009										
Georgia Green	59.0		66.0		63.6		69.2		6.55	0.45
Georganic	70.2		66.2		60.4		64.3		7.15	0.60
AP-3	69.9		70.7		70.4		72.5		7.52	0.99
C-99R	52.7		52.5		51.3		53.3		7.69	0.99
Georgia-01R	24.0	b	35.3	ab	50.4	a	51.7	a	5.82	0.00
Tifguard	66.7		62.2		64.0		63.6		6.13	0.90
Tifrunner	46.0		56.4		59.3		61.3		5.70	0.07
C11-154-61	48.0		46.6		55.3		49.2		7.01	0.62
C11-186	39.0		44.0		50.8		54.7		10.10	0.44
C12-3-114-58	48.7		46.8		53.3		58.3		6.00	0.24
C34-24-38	64.0		54.7		60.4		54.0		5.13	0.20
C34-24-69	54.7	ab	50.0	b	64.6	ab	70.0	a	6.45	0.03
C34-24-124	60.4		55.3		66.7		72.5		8.68	0.26
C689-6-6	40.0		34.7		39.3		35.0		8.46	0.88
C724-19-25	47.8	b	67.6	a	75.8	a	81.7	a	5.30	0.00

<sup>a</sup>Mean percentage of seed producing seedlings. Letters within rows that differ are statistically different.

<sup>b</sup>Mechanically shelled and cleaned to remove discolored, shriveled, or split seed, and seed with significant damage to cotyledons or entire loss of testa.

<sup>c</sup>Untreated.

<sup>d</sup>Treated with *B. subtilis* ( $2.5 \times 10^{-6}$  kg Kodiak per g of seed).

<sup>e</sup>Hand-shelled and cleaned to remove discolored, shriveled, or split seed, and seed with minimal damage to cotyledons or partial loss of testa.

<sup>f</sup>Standard error of means.

cases where shelling treatment  $\times$  seed treatment interactions were significant, all four seed treatment combinations [mechanically shelled untreated (MU), mechanically shelled *B. subtilis* treated (MT), hand shelled untreated (HU), and hand shelled *B. subtilis* treated (HT)] were classified as a fixed factor (combined seed treatment). Factor effects on percent stand were evaluated by genotype because of the inability to standardize seed lots for germination variability. Stand count was evaluated as a covariate of biomass using linear regression by year. Studentized residuals were computed to analyze treatment effects on biomass minus influences of stand differences. When the effect of genotype could be evaluated, Tukey's

honestly significant difference tests ( $P < 0.05$ ) were conducted to distinguish level differences within the factor.

## Results and Discussion

Due to significant 3- and 2-way interactions ( $P < 0.01$ ), percent stand was evaluated using year, genotype, and combined seed treatment as fixed factors. Combined seed treatment significantly affected three genotypes each year, Georgia-01R both years, C-99R and Tifguard in 2007, and C34-24-69 and C724-19-25 in 2009 (Table 1). In all of these cases, the hand-shelled plots had significantly

**Table 2. Effect of genotype on establishment rate in 2007 and 2009.**

Genotype	Percent of Final Plant Stand 10 DAP <sup>a</sup>			
	2007 <sup>b</sup>		2009 <sup>b</sup>	
Georgia Green	77.9	A	85.9	AB
Georganic	43.6	E	72.2	B
AP-3	62.1	BC	87.9	A
C-99R	63.8	ABC	87.5	A
Georgia-01R	56.1	CDE	82.4	AB
Tifguard	62.4	BC	85.0	AB
Tifrunner	66.4	ABC	77.3	AB
C11-154-61	76.5	AB	88.0	A
C11-186	47.1	DE	77.0	AB
C12-3-114-58	51.9	CDE	81.8	AB
C34-24-38	56.3	CDE	78.9	AB
C34-24-69	59.7	CD	75.4	AB
C34-24-124	57.3	CDE	84.0	AB
C689-6-6	47.2	DE	75.9	AB
C724-19-25	61.3	CD	86.5	AB

<sup>a</sup>Days After Planting.

<sup>b</sup>Means in the same column followed by the same letter are not significantly different ( $P \geq 0.05$ ).

greater stand counts than the mechanically shelled plots for at least one of the *B. subtilis* seed treatment factors (untreated or treated). The application of *B. subtilis* as a seed treatment significantly improved stand counts for mechanically shelled Georgia-01R in 2007 and mechanically shelled C724-19-25 in 2009, but did not affect stand counts for any of the hand-shelled treatments. In both years, the genotypes that responded to hand shelling and/or *B. subtilis* seed treatment had relatively low stand counts in the mechanically shelled untreated plots (< 62% emergence). Of the 6 seed lots with stand counts < 62% that were not significantly affected by combined seed treatment, 4 showed a numerical increase in stand count with hand-shelling and 3 showed a numerical increase with *B. subtilis* seed treatment (Table 1). These trends are worth noting as the power of our analysis was relatively low due to multiple factor interactions.

Where significant, reduced stand counts of mechanically shelled plots may have been due to more pre-emergence seed and seedling disease since seed with minor damage to cotyledons or partial testa loss were included in seed lots of these plots. Because the fields received rain shortly after planting each year, *Rhizopus* spp. and *Pythium* spp. are the most likely pathogen candidates for seed mortality (Melouk and Backman, 1995). The quantity and quality of seed damage to cotyledons and testa occurring in the seed lots of the mechanical shelling treatment were not evaluated, but it is possible that the genotypes that did not respond to hand-shelling had less damage in their mechanically shelled seed than those genotypes

where hand-shelling improved stands. The significant response to *B. subtilis* by mechanically shelled Georgia-01R in 2007 and C724-19-25 in 2009, which was not detected in the respective hand-shelled treatment, supports the possibility that differences in seed damage correlate with response to shelling treatment. Turner and Backman (1991) suggested that *B. subtilis* seed treatments were of greatest value to improve peanut emergence under stressful field conditions, such as low temperature or moisture. Our data suggests that this may also be true for seed stress, such as that caused by mechanical shelling. The lack of response to *B. subtilis* observed for the majority of the genotypes in this study might also be a result of the optimal (warm, moist) planting conditions each year.

Although the seed in this study were grown and processed the same, we were not able to standardize seed lots for germination variability, and are therefore unable to make conclusions about the cause of differences observed for stand establishment across genotypes. We can however make conclusions about how the genotypes differed in their establishment rates, resistance to post-emergence crown rot, and seedling biomass. Establishment rates, the percentages of established seedlings at 17–21 DAP that had emerged by 10 DAP, were significantly affected by genotype ( $P < 0.01$ ), and are presented by year due to a significant genotype-year interaction ( $P < 0.01$ ) (Table 2). In 2007, Georgia Green, C11-154-61, Tifrunner, and C-99R were among the genotypes with the highest establishment rates, while Georganic was among the genotypes with the lowest establishment rates. Georganic had the lowest numerical rate again in 2009, but only C11-154-61,

**Table 3. Percentage of seedlings presenting symptoms of *Aspergillus* crown rot.**

Genotype	2007 <sup>a</sup>		2009 <sup>a</sup>	
Georgia Green	0.2	A	2.4	AB
Georganic	0.5	A	2.3	AB
AP-3	0.7	A	4.4	AB
C-99R	0.8	A	6.9	AB
Georgia-01R	0.2	A	3.6	AB
Tifguard	1.8	AB	4.5	AB
Tifrunner	0.5	A	1.4	A
C11-154-61	3.9	B	6.8	AB
C11-186	2.3	AB	3.7	AB
C12-3-114-58	1.6	AB	5.3	AB
C34-24-38	1.1	AB	3.1	AB
C34-24-69	0.0	A	3.7	AB
C34-24-124	0.0	A	2.7	AB
C689-6-6	0.5	A	6.9	AB
C724-19-25	0.2	A	7.3	B

<sup>a</sup>Means in the same column followed by the same letter are not significantly different ( $P \geq 0.05$ ).

AP-3 and C-99R had significantly higher rates than Georganic that year. Because speed of emergence is negatively related to the length of time seed and pre-emergence seedlings are susceptible to pathogens (Melouk and Backman, 1995), emergence rates should be an important consideration for cultivar selection in organic systems. Shelling procedure and seed treatment did not significantly affect establishment rates ( $P > 0.51$ ) or interact with genotype ( $P > 0.05$ ).

Despite relatively low disease pressure, incidence of *Aspergillus* crown rot was significantly lower for seedlings from the hand shelled plots than mechanically shelled plots ( $P < 0.01$ ). Across years, plots with mechanically shelled seed lost 3.1% of established plants to crown rot compared to 2.1% of established plants in the hand shelled treatment plots. Many of the plants affected by crown rot presented signs of *A. niger* on cotyledons, and perhaps the increase in incidence in the mechanically shelled plots were due to additional infection courts of damaged cotyledons during the shelling process. Crown rot was not affected by seed treatment ( $P = 0.97$ ), and few significant differences were observed among genotypes each year (Table 3). C11-154-61 was among the genotypes with the greatest incidence of crown rot both years.

Seedling biomass was measured to better understand genotype variation in early season growth for the potential of cultivar development to help with weed management. If plants grow faster, they have the potential to shade out emerging weeds and may help reduce weed pressures. Neither *B. subtilis* seed treatment nor shelling procedure had an effect on biomass ( $P > 0.60$ ), but genotype significantly affected biomass across years ( $P < 0.01$ ). C11-154-61, C724-19-25, Tifguard, AP-3, C99R, and Georgia-01R had

significantly greater biomass than Georganic, and C689-6-6 (Table 4). The relatively low biomass observed for Georganic and C689-6-6 may be related to the slow rates of establishment observed for these cultivars (Table 2).

The cultivar Georganic has many excellent qualities suitable for organic production, including high yield potential, and enhanced disease resistance to foliar pathogens, such as *Cercospora arachidicola*, *Cercospora personatum*, and TSWV (Holbrook and Culbreath, 2008; Cantonwine *et al.*, 2006; Cantonwine *et al.*, 2008a). As such, Georganic has been planted to organic peanut

**Table 4. The mean above ground biomass of peanut genotypes across years.**

Genotype	Biomass <sup>a</sup> (g)	
Georgia Green	1.49	BCDEF <sup>b</sup>
Georganic	1.35	EF
AP-3	1.61	ABC
C-99R	1.54	ABCD
Georgia-01R	1.51	ABCD
Tifguard	1.60	ABC
Tifrunner	1.49	CDEF
C11-154-61	1.69	A
C11-186	1.49	BCDEF
C12-3-114-58	1.49	BCDE
C34-24-38	1.47	CDEF
C34-24-69	1.38	DEF
C-34-24-124	1.45	CDEF
C689-6-6	1.29	F
C724-19-25	1.68	AB

<sup>a</sup>Biomass at 21 d (2007) and 24 d (2009). Studentized residuals of biomass regressed against stand count were used to identify genotype differences.

<sup>b</sup>Means followed by the same letter are not significantly different,  $P \geq 0.05$ .

systems in Georgia in recent years. However, the slow rates of establishment and slow seedling growth, as observed in this study, have been seen in other studies (R.S. Tubbs, unpubl. data, 2009–2010) and in on-farm trials (M.A. Boudreau, unpubl. data, 2006–2008). Planting the highest quality seed, such as seed from cold storage, with a low level of damage, and high germination rate, may improve establishment rates. Cultivar evaluations for organic production must not only strive to enhance post emergence disease resistance and maximize yield potential (Branch and Culbreath, 2008), but also improve traits related to the rate and consistency of establishing a stand and resisting seedling diseases.

### Summary and Conclusions

Hand shelling did not affect the rate that seedlings emerged or above ground biomass, but did reduce incidence of *Aspergillus* crown rot compared to the mechanical shelling treatment, and increased the percentage of seed that emerged for some of the genotypes with poor emergence rates. Although hand-shelling seed for production is not practical for most organic production fields, these results show the importance of planting seed from well-sorted seed lots where damaged seed are removed. Seed lots intended for organic production should be sorted using a lower damage threshold than those for conventional production, where seed with minor damage are of little concern due to the synthetic seed treatments. There were no negative effects observed with the use of *B. subtilis*, but in most cases *B. subtilis* did not improve the variables observed in this study, and when it did, the effect was not as great as that of hand shelling. As we saw in this study, without effective seed treatment stands have the potential to be so poor that profitable production is unrealistic. Additional research is needed to evaluate alternative seed treatments and to enhance cultivar traits involved in seedling establishment and growth.

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