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Effect of host genotype on indigenous bacterial endophytes of cotton (*Gossypium hirsutum* L.)

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Abstract

The purpose of this study was to determine if populations of indigenous bacterial endophytes in seed, stem and root tissue of cotton seedlings are influenced by host genotype. Growth chamber and field experiments were conducted to test the hypothesis that host genotype has an effect on endophytic bacterial populations in seed tissues and the developing cotton seedling. Initially, population densities of bacteria within seed of nine cotton cultivars were very low (i.e., $<\log^{10}$ 2.0 colony forming units seed^{-1}). However, after 4 days growth on water agar, population densities within developing radicles increased significantly ($\log_{10}$ 2–5 colony forming units) and significant cultivar differences were found. Significant cultivar differences occurred for populations of endophytic bacteria and the composition of bacterial functional groups differed among cultivars in field-grown seedlings at 5, 8, and 15 days after planting. Differences in the ranking of cultivars occurred for endophytic populations recovered from seed and aseptically and field-grown radicle and seedling tissues. These results suggest that whether originating from seed or from soil, cotton plants are capable of immediately establishing a carrying capacity for communities of endophytic bacteria following seed germination. During germination and development of the seedling, there are genetic and possible morphological/physiological effects that contribute to significant differences in colonization of bacterial endophytes among cotton cultivars.

Abbreviations: cfu – colony forming units; CVA – crystal violet agar; DAP – days after planting; RASS – reduced arginine starch salts; TSA – tryptic soy agar; TEB – total endophytic bacteria; TSB – tryptic soy broth; WA – water agar

Introduction

Healthy plants are internally colonized by non-pathogenic bacteria, termed endophytes (reviewed in Hallmann et al., 1997). Research has shown that there are differences in the populations of indigenous endophytic bacteria found between plant species and plant parts within plant species (Gagné et al., 1987; Hallmann et al., 1997; McInroy and Kloepper, 1994; Musson et al., 1995; Shishido et al., 1995). McInroy and Kloepper (1993, 1994) reported populations in corn ranging from $\log_{10}$ 3 cfu g^{-1} in young corn to $\log_{10}$ 10 cfu g^{-1} after 10 weeks. They also found that populations of bacterial endophytes ranged from $\log_{10}$ 3 to $\log_{10}$ 6 cfu g^{-1} in germinated surface-disinfested cotton seed, from $\log_{10}$ 3 to $\log_{10}$ 7 cfu g^{-1} fresh weight in roots of cotton, and in cotton petioles from $\log_{10}$ to $\log_{10}$ 4 cfu g^{-1}. Population densities within cotton plants decreased acropetically to the point where they could not be detected in cotton bolls (McInroy and Kloepper, 1994). Xiao et al. (1990), however, reported endophytic bacterial populations of $\log_{10}$ 4 cfu g^{-1} in cotton seedling leaves.

Little is known about how plant host genetics influences the population dynamics of bacterial endophytes. Siciliano et al. (1998) studied the microbial communities associated with roots of new cultivars of

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Table 1. Cotton cultivars used in this study

Cultivars	*Fusarium* resistance
Rowden	Susceptible
Suregrow	501 Susceptible
GA88-88	Susceptible
DES 119	Moderately resistant
Auburn 56	Moderately resistant
Deltapine 50	Moderately resistant
Stoneville LA887	Resistant
Terra 207	Resistant
GA88-15-19	Resistant

canola and wheat and found that endophytic and rhizosphere communities of the transgenic canola variety differed from those of traditional breeding systems while no such differences were found among the three wheat cultivars tested. Bird et al. (1981) and others (Bush, 1979; Tsai et al., 1975) indicated that one component of multi-adversity resistant cultivars is their ability to influence rhizosphere–rhizoplane bacteria and actinomycetes. It was shown that root exudates both influence resistance to root pathogens and earliness of the crop by encouraging the development of growth-promoting and disease suppressive microorganisms in the rhizosphere.

In a comparative evaluation of endophytic bacteria from Chinese and U.S. cotton cultivars, McInroy et al. (1997) found that, while no significant cultivar differences were observed for total bacterial populations, there were differences in the types of bacteria isolated. However, this study only involved samples from lower stem sections without surveying populations in the roots, an important initial point of entry into the plant. The purpose of the present study was to determine if host genotype influences indigenous bacterial endophyte populations in seed, stem and root tissue of cotton at germination and during subsequent seedling development.

Materials and methods

Cultivars

Nine cotton (*Gossypium hirsutum* L.) cultivars were used in this study: 'Rowden', 'Suregrow 501', 'GA88-88', 'DES 119', 'Auburn 56', 'Deltapine 50', 'Stoneville LA887', 'Terra 207', and 'GA88-15-19'. Cultivars were chosen on the basis of their relative susceptibility to Fusarium wilt (Table 1) as indicated in the 1994 Alabama Cotton Variety Report (Glass and Bransby, 1995).

Media

In growth chamber experiments, 5% Difco Tryptic Soy Agar (TSA) was used to determine total populations of endophytic bacteria. However, for the field experiments, several selective media were used to determine populations of different functional groups of endophytic bacteria. Ten percent TSA was used for isolating total endophytic bacteria (TEB) and heat-tolerant bacteria, reduced arginine starch salts agar-RASS (Herron and Wellington, 1990) for actinomycetes, crystal violet agar (CVA) for Gram-negative bacteria (3.0 g l^{-1} of Difco Tryptic Soy Broth, 15.0 g l^{-1} of Bacto agar, and 5.0 mg l^{-1} of crystal violet), S1 media for fluorescent pseudomonads (Gould et al., 1985), water agar (WA) for oligotrophic bacteria (20 g l^{-1} of Bacto agar), and colloidal chitin agar (4.25% chitin) for chitinolytic bacteria (Lingappa and Lockwood, 1962). In order to mimic the low-nutrient micro-environment inhabited by bacterial endophytes, 5% TSA was used as an isolation medium for bacterial endophytes in seed and ten percent TSA and WA were used as isolation media for bacterial endophytes in seedlings, respectively. To isolate heat-tolerant bacteria, sterile 10-ml polyethylene test tubes containing 1 ml of the triturated homogenate of radicles or seedlings was heat-treated at 80°C for 20 min prior to making dilutions. All media were amended with 2 ml l^{-1} of 100 ppm cycloheximide and nystatin to inhibit fungal growth.

Growth chamber experiment

Growth chamber experiments (conducted under aseptic conditions) were used to determine populations of bacterial endophytes in the seed and developing radicles of cotton. The experiment was set up as a completely randomized design with six replications per cultivar. Five seed of each cultivar for both seed and radicle isolations were surface-disinfested in a sodium hypochlorite solution (1.05% available chloride) for 3 min and rinsed five times with sterile distilled water. One-tenth ml of the final two washes was plated on TSA and incubated at 28 °C for 72 h (Musson et al., 1995). No fungal or bacterial contaminants were detected. To determine internal bacterial population densities within seed, the five seed were triturated in two milliliters of 0.2 M phosphate buffer in a Kleco Tissue Pulverizer (Kinetic Laboratory

Equipment Company, Visalia, CA). To isolate bacteria from within radicles, five seeds were placed on a water agar plate which was parafilmed, placed in a growth chamber, and allowed to germinate and grow for 4 days at 27 °C. Radicles were removed aseptically and triturated as described previously. Two-tenth ml of homogenate from both sources (seed and radicles) was spread plated on 5% TSA and dilutions were made to 10^{-4} for plating with a spiral plater (Spiral Systems, Bethesda, MD). Plates were incubated at 28 °C for 72 h and the $\log_{10}$ cfu seed^{-1} or radicle was calculated.

Field experiment

A field experiment was conducted at the Plant Breeding Unit, E.V. Smith Experiment Station in Shorter, AL from April to July, 1998 to determine total populations and functional groups of bacterial endophytes at different stages of cotton seedling development. The term 'functional group', for the purposes of this study, is an ecological reference to mean those groups of bacteria which represent a collection of all taxa which have the capacity to perform a specific function (e.g., iron chelation by siderophore producing bacteria). The soil was a Cahaba fine sandy loam (Fine-Loamy, Siliceous, Thermic Typic Hapludults) with a latitude of 32°42′ N. After soil was disced to a depth of 11–15 cm, seed-beds were prepared and cotton seed of each cultivar were planted with a manual planter.

The experiment was set out as a completely randomized design with a 9 × 3 factorial and five replications per treatment. Treatments consisted of nine cultivars sampled at three times (5, 8, and 15 days after planting, DAP). Due to the time involved in processing plant material, seeding (by replicate) was staggered over 16 days (April 22, April 23, April 24, May 6, and May 7, respectively). At each sample time, whole-plant samples were taken and the hypocotyls removed. Samples were weighed, placed into sterile 50-ml centrifuge tubes, and 25 ml of sterile distilled water added before vortexing for 30 s to remove adhering soil particles. Samples were removed, placed in a second sterile 50-ml centrifuge tube for surface-disinfestation with 25 ml of sodium hypochlorite solution (1.05% available chloride), and shaken for 2–3 mins. The hypochlorite solution was decanted, and the samples rinsed three times with 25 ml of sterile distilled water. As a sterility check, 0.1 ml of the final rinse solution was placed in a test tube containing 10 ml of Difco Tryptic Soy Broth (TSB) and shaken for 72 h at 25 °C (Musson et al., 1995). Samples that were contaminated were discarded. Following surface-disinfestation, samples were removed from the tube, triturated in 3–5 ml of 0.2 M phosphate buffer in a Kleco Tissue Pulverizer (Kinetic Laboratory Equipment Company, Visalia, CA), serially diluted and spiral plated on selective media as described above. Plates were incubated at 28 °C for 48–72 h for 10% TSA, CVA, and S1 media, and up to 6 days for WA, RASS, and colloidal chitin media.

Table 2. Mean populations of bacterial endophytes isolated from aseptic seed and 4-day-old radicles of nine cotton cultivars

Cultivar	Seed ($\log_{10}$ cfu/seed)	4-Day-old radicles ($\log_{10}$ cfu/radicle)
Auburn 56	1.25 ab[a]	4.83a
DES 119	0.91b	3.23ab
Deltapine 50	1.17ab	1.77b
GA88-15-19	1.50a	2.70b
GA88-88	0.78b	3.24ab
Rowden	0.95b	4.72a
Stoneville LA887	1.22ab	2.10b
Suregrow 501	1.04ab	2.49b
Terra 207	0.95b	3.64ab
Mean	1.08	3.19
LSD(0.05)	0.52	1.93

[a]Means followed by the same letter are not significantly different at the 0.05 probability level, according to LSD test procedure using GLM in PC-SAS.

Data analysis

Population data for seed and radicles were converted to $\log_{10}$ cfu per seed or radicle and log cfu per gram of tissue for field-grown seedlings, with populations below detection thresholds being scored as 0 for calculation of means (Kloepper and Beauchamp, 1992). Analysis of variance for both growth chamber and field experiments was performed using the General Linear Model (GLM) procedure in PC-SAS (SAS Institute, Cary, NC). Significant differences between cultivars and cultivar × time interactions were determined using the least significant difference (LSD) test at $P = 0.05$.

Results

Growth chamber experiment

Mean populations of indigenous bacterial endophytes in seed and 4-day-old radicles (across all cultivars)

were $\log_{10}$ 1.08 cfu seed^{-1} and $\log_{10}$ 3.19 cfu radicle^{-1} (Table 2). Although not statistically significant at $P = 0.05$, analysis suggested a trend toward Auburn 56, Stoneville LA887, GA88-15-19, Suregrow 501, and Deltapine 50 harboring the highest populations of TEB at $\log_{10}$ 1.25, 1.22, 1.50, 1.04, and 1.17 cfu seed^{-1}, respectively (Table 2). However, there were significant cultivar differences for TEB in radicle tissue. Auburn 56 and Rowden had the highest population of TEB while Stoneville LA887, GA88-15-19, Suregrow 501, and Deltapine 50 had the lowest populations recovered (Table 2).

Field experiment

In general, average populations of endophytic heat-tolerant and chitinolytic bacteria were $\log_{10}$ 3.58 and $\log_{10}$ 2.25 cfu g^{-1} tissue^{-1}, respectively. Similar population densities were also found among cultivars for heat-tolerant and most cultivars for chitinolytic bacterial populations. However, Deltapine 50 tended to have the highest numbers of chitinolytic bacteria at 15 DAP and the lowest populations at 8 DAP with populations at 5 DAP not significantly different from populations at 8 and 15 DAP (Table 5). Although not statistically significant, Auburn 56 had the highest population densities of heat-tolerant bacteria at 5 DAP and this decreased by 15 DAP.

Endophytic populations of all other bacterial groups tested increased with time, except for populations of fluorescent pseudomonads (Tables 4 and 5). Mean endophytic populations of fluorescent pseudomonads increased after 5 DAP from $\log_{10}$ 0.39 cfu g tissue^{-1} to $\log_{10}$ 1.72 cfu g tissue^{-1} at 8 DAP and then decreased to $\log_{10}$ 0.74 cfu g tissue^{-1} at 15 DAP. This trend was also observed for populations of endophytic fluorescent pseudomonads in DES 119 (Table 5). However, fluorescent pseudomonad populations in Auburn 56 and Suregrow 501 were highest at 8 DAP and not detectable at 15 DAP.

There were some significantly large cultivar differences in populations of endophytic Gram-negative and oligotrophic bacteria, while small differences were observed among cultivars for total bacteria (Table 4). Deltapine 50 was the only cultivar where differences in populations of actinomycetes were observed between sample times where endophytic populations were highest at 15 DAP (Table 5).

When averaged across all sample times, there were significant differences in population densities among cultivars for endophytic bacterial functional groups, with the exception of heat-tolerant bacteria and actinomycete groups (Table 3). This finding was also observed among cultivars at particular sample times, with the exception of those at 8 DAP (Table 4). At 8 DAP, the highest populations of actinomycetes were observed in Auburn 56 while Deltapine 50 and Terra 207 had no detectable populations. There was a trend toward Stoneville LA887, DES 119, and Suregrow 501 exhibiting the highest populations of heat tolerant bacteria while Terra 207 had the lowest (Table 4). Small differences were also observed among cultivars for total bacteria (Table 3). These differences, as well as those for oligotrophic bacteria, occurred between 8 and 15 DAP (Table 4). There were no differences among cultivars observed for TEB or oligotrophic bacteria at 5 DAP. Significant differences among cultivars for populations of Gram-negative bacteria occurred at all sample times with rankings for cultivars also changing at each sample time (Table 4). Very low to no populations of fluorescent pseudomonads among cultivars were observed at 5 DAP (Table 4). Auburn 56 had the highest populations of fluorescent pseudomonads while DES 119, Deltapine 50, GA88-15-19, GA88-88, Stoneville LA887, and Terra 207 had no detectable populations. Populations increased at 8 DAP where, again, Auburn 56 and the highest populations and Deltapine 50, GA88-88, and Stoneville LA887 continued to have the lowest populations (Table 4). There were no differences among cultivars for fluorescent pseudomonads at 15 DAP. No differences among cultivars for chitinolytic endophytic bacteria occurred at 5 or 8 DAP (Table 5). However, at 15 DAP, Deltapine 50 had the highest populations of chitinolytic bacteria and GA88-88, Suregrow 501, and Terra 207 had the least (Table 5).

Of all the cultivars for which differences or significant trends were found for functional endophytic bacterial groups, Deltapine 50 ranked the highest in population density in three of the functional groups (TEB, oligotrophic, and chitinolytic bacteria) while GA88-88 ranked the lowest in four the functional groups (TEB, Gram-negatives, fluorescent pseudomonads, and oligotrophic bacteria) (Table 3).

Discussion

Populations of TEB were very low in seed of all nine cotton cultivars. However, after germination, population densities increased significantly for 4-day-old aseptic and field grown cotton seedlings and ranged

Table 3. Overall mean population (Log_{10} cfu/g of tissue) of bacterial endophyte groups isolated from nine cotton cultivars under field conditions

Cultivar	Total	Heat-tolerant	Actinomycetes	Gram-negative	Fluorescent pseudomonads	Oligotrophs	Chitinolytics
Auburn 56	5.29abc[a]	3.65a	1.82a	4.24ab	1.42a	5.17ab	2.52
DES119	5.50a	3.42a	1.22a	4.69ab	0.79ab	5.48a	2.26ab
Deltapine 50	5.62a	3.56a	1.37a	4.73ab	0.61ab	5.54a	2.70a
GA88-15-19	5.42ab	3.65a	1.86a	4.10ab	1.20ab	5.20ab	2.23ab
GA88-88	4.89c	3.60a	1.66a	3.73b	0.44b	4.93b	2.27ab
Rowden	5.42ab	3.84a	1.48a	4.30ab	1.39a	5.39ab	2.55a
Stoneville LA887	5.04bc	3.48a	1.15a	3.80b	0.57ab	5.03ab	2.78a
Suregrow 501	5.26abc	3.65a	1.66a	4.66ab	1.16ab	5.02ab	1.62ab
Terra 207	5.24abc	3.34a	0.81a	4.97a	1.23ab	5.10ab	1.25b
LSD(0.05)	0.48	0.61	1.17	1.04	0.96	0.55	1.26
Overall mean	5.29	3.58	1.45	4.35	0.98	5.20	2.25

[a] Means followed by the same letter are not significantly different at the 0.05 probability level, according to LSD test procedure using GLM in PC-SAS.

between log_{10} 2–5 colony forming units. This in agreement with the finding of McInroy and Kloepper (1994). We conclude that there is a maximal carrying capacity for endophytes in cotton plant tissues. Whether originating from seed or from soil, cotton plants are capable of immediately establishing initial threshold communities of endophytic bacteria after germination. Not only can this threshold be affected by environmental factors, but apparently by cultivar type; as indicated by the significant cultivar effects for endophytic bacteria recovered from within seed and the internal tissues of field-grown seedlings. These population communities comprise a number of functional groups which fluctuate according to plant age, environment conditions, and host genotype. These results are consistent with those of McInroy et al. (1997) who found that, while no significant cultivar differences could be observed for total populations, differences in the types of endophytic bacteria isolated were found for cotton cultivars sourced from China versus United States. For example, 21-day-old stem sections of Chinese cultivars had more *Bacillus* species than U.S. cultivars.

We speculate that differences in ranking among cultivars for populations of bacterial endophytes in seed and radicles could be attributed to competition for, lack of, or decrease in the amount and quality of exudates from emerging radicles (Neal et al., 1973). The increase in population densities of bacterial endophytes after germination and densities observed in populations of actinomycetes in Deltapine 50 over sample time was probably due to an increase in the amount of exudates released from germinating seed and exudates released from roots during seedling development. Once seed begin to germinate, nutrients (exudates) are released from the emerging radicle and the subsequent developing mass of root system. These nutrients provide an adequate food source to facilitate large increases in microbial population density when compared to the low nutrient environment of the seed (Baker and Cook, 1974). Under aseptic conditions or in field-soil, these bacterial micro-communities (i.e., the developing radicle and the rhizosphere) will compete with each other and with other microorganisms for niche space and nutrients. This can cause shifts in community structure (Hallmann et al., 1997; Mahaffee, 1997) as the seedling develops.

Differences in ranking among cultivars for populations of bacterial endophytes in seed and radicles could also have been due to passive colonization of seed by some bacteria which subsequently were unable to enter the emerging radicles during germination. The seed coat morphology of cotton is very rough with deep pores called ovule fiber cells through which cotton fibers develop. Cotton seed also have a distal opening known as the chalazal end. This opening and the ovule cell fiber pits can be prime sites for escape and colonization of bacteria (i.e., under the seed coat or deep within cell pits) even after surface-disinfestation (McInroy, 1993). Furthermore, Bowman et al. (2001) found cultivar differences for the numbers of ovule cells in cotton and Bell (1995, personal communica-

Table 4. Mean populations (Log_{10} cfu/g of tissue) of bacterial endophyte groups of nine cotton cultivars grown under field conditions at 5, 8, and 15 day sample times

Cultivar	Total	Heat Tolerant	Actinomycetes	Gram-Negative	Fluorescent Pseudomonads	Oligotrophs	Chitinolytics
Auburn 56	4.86a[a]	3.90a	134a	2.55abc	144a	4.25a	2.03a
DES119	5.32a	3.16a	1.16a	2.79abc	0.00b	4.91a	2.13a
Deltapine 50	5.01a	3.54a	1.60a	3.73ab	0.00b	4.97a	2.55a
GA88-15 19	4.81a	3.55a	1.48a	0.89c	0.00b	4.45a	2.07a
GA88-88	4.91a	3.33a	1.66a	2.04abc	0.00b	4.74a	2.92a
Rowden	4.79a	3.93a	0.79a	1.26bc	0.99ab	4.59a	2.48a
Stoneville LA887	4.67a	3.23a	1.37a	3.24ab	0.00b	4.56a	3.37a
Suregrow 501	4.60a	3.71a	1.67a	3.18abc	0.93ab	4.24a	2.43a
Terra 207	4.84a	3.68a	0.87a	4.65a	0.00b	4.62a	1.82a
LSD(0.05)	0.72	1.76	2.38	2.68	1.34	0.96	2.32
Auburn 56	5.22ab	3.56ab	1.93a	4.78ab	2.83a	5.51ab	5.22a
DES119	5.46ab	3.66a	0.55ab	5.34a	2.22abc	5.72a	5.46a
Deltapine 50	5.49ab	3.61ab	0.00b	4.35ab	0.67bc	5.33ab	5.49a
GA88-15 19	4.74ab	3.53ab	1.21ab	4.50ab	1.92abc	4.81ab	4.74a
GA88-88	4.65b	3.96a	1.23ab	4.07ab	0.61c	4.65b	4.65a
Rowden	5.17ab	3.59ab	1.31ab	4.96a	2.27abc	5.23ab	5.17a
Stoneville LA887	4.72ab	3.69a	1.17ab	3.38b	0.56c	4.66b	4.72a
Suregrow 501	5.50a	3.77a	1.16ab	5.18a	2.49ab	5.33ab	5.50a
Terra 207	5.02ab	2.92b	0.00b	4.70ab	1.92abc	4.96ab	5.02a
LSD(0.05)	0.84	0.71	1.54	1.41	1.92	0.97	1.01
Auburn 56	5.79abc	3.49a	2.19a	5.38abc	0.00a	5.74ab	2.42abc
DES119	5.70abc	3.38a	1.92a	5.55abc	0.00a	5.69ab	2.22abc
Deltapine 50	6.41ab	3.51a	2.85a	6.21ab	1.14a	6.39	4.28a
GA88-15 19	6.59a	3.84a	2.81a	6.25a	1.43a	6.19ab	2.19abc
GA88-88	5.11c	3.44a	2.08a	4.75c	0.61a	5.36b	1.58bc
Rowden	6.17ab	4.04a	2.20a	6.07abc	0.85a	6.19ab	3.07ab
Stoneville LA887	5.74abc	3.52a	0.91a	4.76bc	1.16a	5.87ab	2.97abc
Suregrow 501	5.55bc	3.49a	2.14a	5.32abc	0.00a	5.33b	1.36bc
Terra 207	5.78abc	3.48a	1.56a	5.51abc	1.53a	5.63ab	0.80c
LSD(0.05)	0.96	0.69	2.28	1.45	1.64	0.99	2.23

[a] Means followed by the same letter are not significantly different at the 0.05 probability level, according to LSD test procedure using GLM in PC-SAS.

Table 5. Mean populations (Log_{10} cfu/g of tissue) of bacterial endophyte groups at sample times for nine cotton cultivars grown under field conditions

Sample time	Total	Heat-tolerant	Actinomycetes	Gram-negative	Fluorescent pseudomonads	Oligotrophs	Chitinolytics
Auburn 56							
5	486a[a]	3.90a	1.34a	2.55b	1.44ab	4.25b	2.03a
8	5.22ab	3.56ab	1.93a	4.78a	2.82a	5.51a	3.51a
15	5.79a	3.49b	2.19a	5.38a	0.00b	5.74a	2.42a
LSD(0.05)	0.85	0.39	2.13	1.76	1.70	0.95	2.36
DES119							
5	5.32a	3.16a	1.65a	2.79b	0.00b	4.91a	2.13a
8	5.46a	3.66a	0.56a	5.34a	2.22a	5.72a	2.44a
15	5.70a	3.78a	1.92a	5.55a	0.00b	5.69a	2.22a
LSD(0.05)	1.12	1.43	2.10	2.36	1.45	1.35	2.58
Deltapine 50							
5	5.01a	3.54a	1.60ab	3.73a	0.00a	4.97a	2.55ab
8	5.49a	3.61a	0.00b	4.35a	0.67a	5.33a	1.87b
15	6.41a	3.51a	2.85a	6.21a	1.14a	6.39a	4.28a
LSD(0.05)	1.42	0.65	1.91	2.92	1.94	1.75	2.17
GA88-15-19							
5	4.81b	3.55a	1.48a	0.89c	0.00b	4.45b	2.07a
8	4.74b	3.53a	1.21a	4.50b	1.92a	4.81b	2.44a
15	6.59a	3.84a	2.81a	6.25a	1.43ab	6.19a	2.19a
LSD(0.05)	0.82	1.61	2.94	1.31	1.90	0.69	2.63
GA88-88							
5	4.91ab	3.33a	1.66a	2.03b	0.00a	4.74b	2.92a
8	4.65b	3.96a	1.23a	4.07a	0.61a	4.65b	2.50a
15	5.11a	3.44a	2.08a	4.75a	0.61a	5.36a	1.58a
LSD(0.05)	0.31	1.42	2.18	1.56	1.38	0.50	2.45
Rowden							
5	4.79b	3.93a	0.79a	1.261,	0.99a	4.59b	2.48a
8	5.17b	3.59a	1.31a	4.96a	2.27a	5.23ab	2.09a
15	6.17a	4.04a	2.20a	6.07a	0.85a	6.19a	3.07a
LSD(0.05)	0.82	0.89	2.16	1.78	2.36	1.01	2.80
Stoneville LA887							
5	4.66b	3.23a	1.37a	3.24a	0.00a	4.56b	3.67a
8	4.72b	3.69a	1.17a	3.39a	0.56a	4.66b	2.01a
15	5.74a	3.52a	0.91a	4.77a	1.16a	5.87a	2.97a
LSD(0.05)	0.96	1.29	2.08	2.62	1.32	1.10	1.70

tion) indicated that populations of bacteria can be as high as $\log_{10}$ 1–6 cfu g^{-1} of dry cotton fiber alone. This suggests that, in addition to seed size (differences in seed size were observed between varieties), cultivar differences in endophytic populations of bacteria could also have been due to differences in the number and percentage of ovule fiber cells occupied by bacteria or the amount of bacteria residing underneath

Table 5. contd.

Suregrow 501							
5	4.60b	3.71a	1.67a	3.18b	0.93ab	4.24b	2.43a
8	5.50a	3.77a	1.16a	5.18a	2.49a	5.33a	1.23a
15	5.55a	3.49a	2.14a	5.32a	0.00b	5.33a	1.36a
LSD(0.05)	0.47	0.66	2.18	1.41	2.04	0.55	2.85
Terra 207							
5	4.84a	3.68a	0.87a	4.65a	0.00a	4.62b	1.82a
8	5.02a	2.92a	0.00a	4.70a	1.92a	4.96ab	1.25a
15	5.78a	3.48a	1.56a	5.51a	1.53a	5.63a	0.80a
LSD(0.05)	1.08	1.31	1.87	1.05	1.96	1.00	2.20

[a] Means followed by the same letter are not significantly different at the 0.05 probability level, according to LSD test procedure using GLM in PC-SAS.

the seed coat after surface-disinfestation. Once these bacteria initially establish themselves on or within the seed coat, gradual exclusion may occur through some form of competition or antagonism with other seed endophytes (Baker and Cook, 1974; Clark, in Baker and Snyder, 1965) and this may change population density and community structure under field conditions.

Ranking cultivars based on population densities of bacterial endophytes in seed did not match the cultivar ranking of population densities in aseptic or field-grown seedlings. For instance, Deltapine 50 was one of the three cultivars with the highest TEB populations in seed but was one of the three lowest ranked in populations of endophytic bacteria in aseptic radicles. However, under field conditions, seedling populations of TEB, oligotrophic, and chitinolytic bacteria for this cultivar were among the highest populations. This change could have been due to abrasion of roots by soil particles which could facilitate entry of numerous bacteria; whereas, in aseptically grown radicles, source bacteria was confined to those populations originating from the seed, some of which may not have had the capacity to enter the emerging radicle. Overall results suggest that, during germination and development of the seedling, there are genetic and possible morphological or physiological affects that contribute to significant differences in the colonization of cotton cultivars by bacterial endophytes.

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