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Quantification of the geocarposphere and rhizosphere effect of peanut (*Arachis hypogaea* L.)

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Abstract

Roots and pods of field-grown peanut (groundnut) (*Arachis hypogaea* L.) were sampled at the R3, R5, and R7 developmental stages and examined in comparison to root- and pod-free soil for microbial populatiou densities to assess the geocarposphere and rhizosphere effects. G/S (no. geocarposphere microorganisms/no. soil microorganisms) and R/S (no. rhizosphere microorganisms/no. soil microorganisms) ratios were calculated for total fungi, *Aspergillus flavus*, spore-forming bacilli, coryneform bacteria, fluorescent pseudomonads, and total bacteria isolated on low- and high-nutrient media. A clear geocarposphere effect was evidenced by increased population densities of bacteria and fungi associated with developing pods compared to soil. G/S and R/S ratios were generally greater than 1.0 for all groups of microorganisms except bacilli. G/S ratios were greater for total bacteria than for total fungi at two of the three sample times, suggesting that bacteria were stimulated more than fungi in the zone around developing pods. In contrast, R/S ratios were higher for total fungi than for total bacteria at two of three sample times. The preferential association of fungi and bacteria with early developmental stages of the pod indicates that some microorganisms are particularly well adapted for colonization of the peanut geocarposphere. These microorganisms are logical candidates for evaluation as biological control candidates for *A. flavus*.

Introduction

Peanut, or groundnut (*Arachis hypogaea* L.) is unique among major crop plants in that the flower is pollinated aerially, and the seed matures underground. The soil immediately surrounding the pod is termed the geocarposphere (Garren, 1966). Garren (1966) pointed out that there are major anatomical differences between roots and fruits, and that intense physiological activity occurs in soil when peanut pistils develop into fruits. This suggests that the geocarposphere may, like the rhizosphere, consist of an area containing greater amounts of carbohydrates and amino acids than plant-free soil and thereby contain higher populations of diverse microorganisms. However, we are not aware of any reports examining the relative population densities of microorganisms in the geocarposphere and soil or differences between the geocarposphere and rhizosphere.

Fungal constituency of the geocarposphere has been examined in association with efforts to understand the microbial ecology of the toxin-producing fungi *Aspergillus flavus* and *A. parasiticus* (*A. flavus* group). Several research groups revealed over 70 species of fungi associated with healthy pods (Garren, 1964; Jackson,

1964; Jackson, 1965; Joffe, 1969; Joffe and Borut, 1966). In addition, *Aspergillus* spp. and several other fungi were reported in the endo-geocarposphere as colonists of seed kernels (Gilman, 1969; McDonald, 1970; Norton et al., 1956; Porter et al., 1972). Total populations of fungi from pods were not reported in these studies. Rao (1962) compared population densities of fungi in the rhizosphere to populations in soil, expressed as R/S ratios (Katznelson, 1946), for several peanut cultivars. This is the only published report of R/S ratios for peanut, and the results are probably not applicable to North America since organic amendments were used in Rao's study, and peanuts in North America are grown in sandy soils low in organic matter.

In contrast to the numerous reports cataloguing fungal species in the geocarposphere, there are no comprehensive reports of bacterial species in this region. In a study on the effect of foliar fungicides on rhizosphere and geocarposphere microflora, Hancock (1981) enumerated geocarposphere bacteria on Thornton's medium (1922). Some bacteria were noted as contaminants on fungal medium in the studies cited above, but isolations from the geocarposphere on bacterial media were not reported.

Aflatoxin production by *A. flavus*-type fungi results in rejection of shelled peanuts for human consumption when toxin levels exceed 20 ppb in the US (Davidson et al., 1982, Peanut Administrative Committee, 1978). Annual losses due to toxin contamination vary from year to year, depending on the environment (Diener et al., 1982). Many older fungicides labelled for use on peanut have not been effective in reducing toxin levels (Diener et al., 1982); however, some new fungicides have reduced the incidence of *A. flavus* in peanut kernels when applied to foliage for leafspot control (Bowen and Backman, 1989). If chemical control of *A. flavus* and aflatoxins continues not to be feasible, other means of control must be considered. Biological control is a theoretical possibility if agents can be found that occupy the same niche (i.e. infection site) as *A. flavus*-type fungi. These fungi are ubiquitous in soil, particularly in the geocarposphere, and penetrate the developing seed pod (Diener et al., 1982). Hence, microorganisms preferentially adapted to colonization of the geocarposphere would be logical candidates for screening as biological control agents.

The main objective of this study was to compare population densities of microorganisms in peanut geocarposphere to those in rhizosphere and soil. The emphasis was placed on bacteria since previous studies, cited above, concentrated on fungi. The project was designed to test the hypothesis that a geocarposphere effect, similar to the rhizosphere effect, exists with peanut such that this zone contains higher microbial population densities and/or different microbial species than soil without roots or pods. This information is necessary for designing approaches to biological control of seed-borne peanut pathogens using bacterial inoculants.

Materials and methods

Sampling procedures

A field trial with peanut cv 'Florunner' was established April 13, 1990 at the Wiregrass Substation of the Alabama Agricultural Experiment Station. The trial consisted of 32 plots designed to evaluate foliar fungicides for disease control. All samples were taken prior to application of any foliar fungicides. Three sampling dates representing different stages of physiological maturity (Boote, 1982) were used: June 13, R3 stage (swelling of peg); July 5, R5 stage (full size pod with visible seed cotyledon); and July 26, R7 stage (testa coloration on seed). Six replicate plants were selected at random from throughout the field trial on each sampling date. Whole selected plants were dug from the plot; excess soil was shaken from the roots; plants were placed into plastic bags and transported to the laboratory in an ice chest. The maximum time interval between digging of plants and subsampling for plating was 15 h at each sampling date.

For soil samples, an approximately cylindrical sample was taken with a hand trowel from the top 15 cm of soil for a total volume of 600 mL per sample. Six replicate soil samples were taken in nonplanted buffer zones adjacent to the plots.

Three individual pods and three subsamples of roots were removed from each replicate plant in

the laboratory. Each replicate soil sample was mixed prior to removing three subsamples. Subsamples of pods, roots, and soil were weighed, placed into 10 mL 0.2*M* phosphate buffer, pH 7.0 (PB), and agitated for 1 h at 150 rpm on an orbital shaker. After agitation, root and pod samples were triturated with a sterile mortar and pestle.

Determination of microbial populations

The processed samples were serially diluted in PB to 10^{-4} and plated with an automated plating system (Spiral Systems, Inc., Bethesda, MD) onto test media. Total bacterial populations were estimated by plating on 5% TSA (Difco) and *Pseudomonas* agar F (PAF) (Difco) to include a low- and high-nutrient medium respectively. Populations were determined for coryneform bacteria by plating on CNS medium (Gross and Vidaver 1978), for fluorescent pseudomonads by plating on PAF and examining for fluorescence under u.v. light, and for spore-forming *Bacillus* spp. by plating on TSA after placing dilutions in a water bath at 80°C for 20 min. Populations of total fungi were determined by plating on potato dextrose agar amended with 50 μg mL^{-1} streptomycin and erythromycin. *Aspergillus flavus* was quantified by plating on M351B medium (Griffen and Garren, 1974).

Plates were incubated for 24 h for bacilli and fluorescent pseudomonads, 48 h for total bacteria, and 72 h for coryneform bacteria; all incubations for bacteria were at 28°C. Plates for fungi were incubated up to 7 days at 21°C. Bacteria were enumerated with a laser colony counter and Bacterial Enumeration software (both from Spiral Systems). Fungi were visually identified and counted.

Mean population densities were calculated based on log cfu g^{-1} fresh weight sample. The calculated means for each microbial group from pods, roots, and soil were analysed for statistically significant differences using SAS ANOVA procedures (SAS Institute, Inc., Cary, North Carolina). R/S ratios (Katznelson, 1946) and G/S ratios (population of microorganisms in geocarposphere (G)/population in soil (S)) were calculated for each group of microorganisms using the antilog of mean populations.

Results

Microbial populations in the geocarposphere, rhizosphere, and soil

The mean densities of total bacteria, calculated on both 5% TSA and PAF, were significantly larger for pod and root samples than for soil samples at the R3 and R5 developmental stages (Table 1). By the R7 stage, the mean density of total bacteria from pods was not different from soil, while populations from roots were still significantly higher than from soil. Similar mean populations were calculated with the two media for all samples at the R3 and R5 stages, while at the R7 stage calculated means were lower for all samples on the nutrient-rich PAF medium than on nutrient-limited 5% TSA.

Overall population densities of fluorescent pseudomonads were low from all samples, and they were not detected from soil at two of the three sample times (Table 1). The highest calculated mean of fluorescent pseudomonads was 3.98×10^2 cfu g^{-1}(log 2.6 cfu g^{-1}) for pods at the R5 stage. Significantly higher mean densities of fluorescent pseudomonads compared to soil occurred for roots at the R3 and R5 stages and with pods at the R7 stage.

For coryneform bacteria, there were no differences in mean densities among pods, roots, and soil at the R3 stage (Table 1). At both the R5 and R7 stages, mean populations were significantly higher from pods and roots than soil. Coryneform populations decreased from all samples during the season. The largest decrease was 6.3×10^4 cfu g^{-1} (4.8 log units) from soil; decreases were 3.2×10^1 (1.5 log units) and 2×10^2 (2.3 log units) for pods and roots, respectively.

Spore-forming bacilli in soil had a strikingly consistent mean density of 6.3×10^5 to 7.9×10^5 cfu g^{-1} (log 5.8 to 5.9) at all sample times (Table 1). Significantly fewer bacilli were detected on pods and roots than in soil at all three sample times. Populations from pods and roots changed less than 10-fold (1 log unit) over the sample period.

Total fungal populations were significantly higher from pods and roots than soil at all sample times (Table 1). Densities of fungi in soil increased 7.9×10^0 cfu g^{-1} (0.9 log units) at the

Table 1. Population densities of bacteria and fungi from peanut pods, roots and soil

Week after planting	Developmental stage	Mean population (Log cfu g^{-1} sample) of various microbial groups[a]							
		Sample source	Total bacteria on 5% TSA[b]	Total bacteria on PAF[c]	Fluorescent pseudomonads[d]	Coryneform bacteria[e]	Bacilli[f]	Fungi[g]	*Aspergillus flavus*[h]
6	R3	Pod	7.07**	7.11**	0.38	5.51	3.78**	3.83*	0.63
		Root	7.27**	7.53**	0.96*	5.71	4.39**	4.62*	0.26
		Soil	5.83	5.89	0	5.61	5.76	2.73	0
		LSD 0.05	0.35	0.28	0.77	NS	0.45	0.87	NS
		0.01	0.49	0.40	NS	NS	0.64	NS	NS
9	R5	Pod	7.07**	7.00**	2.60	5.38**	2.58**	3.76*	0.65
		Root	8.12**	8.23**	1.60*	5.81**	4.14**	4.84**	0.26
		Soil	5.95	6.11	0.80	4.25	5.80	3.08	0.30
		LSD 0.05	0.34	0.52	NS	0.65	0.53	0.51	NS
		0.01	0.48	0.74	NS	0.92	0.76	0.73	NS
12	R7	Pod	6.93	5.96	1.46*	4.10**	3.08**	3.95*	0
		Root	8.33**	7.27	0.56	3.38**	4.73*	5.46**	0.58
		Soil	6.61	5.92	0	0.77	5.98	3.60	0
		LSD 0.05	0.67	NS	0.77	1.28	1.04	0.35	NS
		0.01	0.95	NS	NS	1.82	1.47	0.49	NS

[a] Mean of 6 replications with 3 samples/rep for the first sample date and 2 samples/rep for the second and third sample dates.
[b] TSA = trytic soy agar.
[c] PAF = Pseudomonas agar F.
[d] Determined by isolation of PAF and examination for fluorescence under UV light.
[e] Determined by isolation on CNS.
[f] Determined by heating sample to 80°C and plating on TSA.
[g] Determined by isolation on potato dextrose agar.
[h] Determined by isolation on M351B.
* Indicates significant difference from population in soil ($p = 0.05$).
** Indicates significant difference from population in soil ($p = 0.01$).

R3 and R7 stage, while densities from pod and root samples increased 1.6×10^0 cfu g^{-1} (0.2 log units) and 7.9×10^0 cfu g^{-1} (0.9 log units), respectively.

Mean populations of *A. flavus* were below 10 cfu g^{-1} (log 1.0) for all samples at all stages of development (Table 1). These fungi were detected from pods and roots before they were isolated from soil (R3 stage).

G/S and R/S ratios

Positive G/S and R/S ratios were calculated for all microbial groups sampled with the exception of the spore-forming bacilli (Table 2). The geocarposphere effect was greater for bacteria than fungi as evidenced by higher G/S ratios for bacteria at two of the three sample dates. Conversely, the rhizosphere effect was higher for fungi than bacteria, with higher R/S ratios for fungi than bacteria at two of the three sample dates.

The G/S ratios for total bacteria decreased from 18 to 13 and 2.1 at sample dates 1, 2, and 3, respectively. However, G/S ratios of fluorescent pseudomonads increased from 2.4 to 66 from sample date 1 to 2. The R/S ratios showed trends different from G/S ratios for total bacteria, which varied with each sample date, and fluorescent pseudomonads, which decreased with each sample date.

G/S ratios of coryneform bacteria increased dramatically from 0.8 to 13 and 2,171 at sample dates 1, 2, and 3, respectively. R/S ratios for coryneform bacteria also increased from 1.3 to 36 and 413 at sample dates 1, 2, and 3, respectively.

With *A. flavus*, G/S ratios were higher than

Table 2. Rhizosphere : soil and geocarposphere : soil ratios of microorganisms from peanut

Sample time[b]	Calculated ratio[c]	Microbial group[a]					
		Total bacteria[d]	Fluorescent pseudomonads	Coryneform bacteria	Bacilli	Total fungi	*Aspergillus flavus*
1	G/S	18	2.4	0.8	0.01	13	4.3
	R/S	28	9.1	1.3	0.04	78	1.8
2	G/S	13	66	13	0.6×10^{-3}	4.8	2.2
	R/S	148	6.6	36	0.2×10^{-1}	58	0.9
3	G/S	2.1	29	2171	0.1×10^{-2}	2.2	1.0
	R/S	52	3.6	413	0.6×10^{-1}	72	3.8

[a] See footnotes on Table 1 for media used.
[b] See Table 1 for the corresponding developmental stage of peanut.
[c] G/S = (mean population of pod samples) (mean population of soil samples)$^{-1}$.
R/S = (mean population of root samples) (mean population of soil samples)$^{-1}$.
[d] Based on counts with 5% TSA.

R/S ratios at sample times 1 and 2. At sample time 3, the R/S ratio was higher than the G/S ratio. The G/S ratio was highest (4.3) at the first sample time and decreased to 1.0 by the third sample time.

Discussion

Historically, the term 'rhizosphere effect' has been used to denote increased microbial populations on and around roots compared to root-free soil (Curl and Truelove, 1986). In this study, a clear geocarposphere effect was evidenced by increased population densities of bacteria and fungi associated with developing pods compared to pod- and root-free soil. The magnitude of the geocarposphere effect varied with the particular group of microorganisms measured and with the developmental stage of the pods. Similarly, the magnitude of the rhizosphere effect has been shown to depend on microbial groups and the physiological stage of the plant for crops other than peanut (Curl and Truelove, 1986).

The geocarposphere effect on particular microbial groups was quantified by determining the G/S ratios (Table 2), similar to the frequently used R/S ratios (Katznelson, 1946) for the rhizosphere effect. G/S ratios were greater for total bacteria than for total fungi at two of the three sample times, suggesting that bacteria are stimulated more than fungi in the zone around developing pods. In contrast, R/S ratios were higher for total fungi than for total bacteria at two of three sample times, suggesting that fungi are favored over bacteria in the rhizosphere. Together, these observations suggest that exudates from pods may be qualitatively or quantitatively different from root exudates.

G/S ratios for both total fungi and bacteria dropped from 13 and 18, respectively, at the first sample time to 2.2 and 2.1 at the last sample time. This decline in G/S ratios may result from decreased exudation from maturing pods at the R7 developmental stage. Root exudates of mature plants have less carbohydrates, amino acids, and organic acids than exudates from young plants (reviewed in Curl and Truelove, 1986), and peanut pod exudation may follow this same trend, although specific characterizations of pod exudates have not been reported.

Mean population densities of fluorescent pseudomonads were low for pods, roots, and soil at all sample times (Table 1), which contrasts with previous reports on other crops. Fluorescent pseudomonads are considered to be the most abundant bacterial group in the rhizosphere (Curl and Truelove, 1986). Vancura (1980) reported that this group constituted 60–90% of the total bacterial populations on roots of several plants. In our study, the maximum mean density of fluorescent pseudomonads was 3.98×10^2 cfu g^{-1} (log 2.6 cfu g^{-1}) from pods at the second sample time, while the corresponding mean density of total bacteria was 1.0×10^7 cfu g^{-1} (log 7 cfu g^{-1}) (Table 1). This may relate to soil type or differences in root exudates between peanut and other crops.

Coryneform bacteria were affected by the geocarposphere at the last sample period more than any other group investigated. The increase in the G/S ratio from 0.8 to 2,171 for coryneforms from the first to the third sample period (Table 2) suggests that these bacteria are particularly favored by exudates from maturing pods. A review of the data on mean cfu g^{-1} (Table 1) suggests that coryneforms are also dominant on younger pods, as the mean density was 3.2×10^5 cfu g^{-1} (log 5.51) from pods, with a total bacterial population of 1.0×10^7 (log 7). Hence, coryneform bacteria should be included in analysis of possible biological control activity of bacteria from the geocarposphere.

Spore-forming bacilli were not well adapted to either the geocarposphere or the rhizosphere in this study. Mean population densities (Table 1) were generally 1.0×10^0 to 3.2×10^2 cfu g^{-1} (1 to 2.5 log units) lower from pods and roots than soil, resulting in G/S and R/S ratios considerably less than 1.0 (Table 2). This suggests that bacilli would not be the best candidates for testing as biological control agents in the geocarposphere.

A. flavus, was detected only at mean population densities below 10 cfu g^{-1} (1.0 log) from pod, roots, and soil (Table 1). The fungus was detected from pod and root samples before it was found in soil, and the highest G/S ratio was at the first sample period, when the G/S ratio was greater than the R/S ratio. These observations support the concept that the pathogen invades pods at the early stages of development.

One of the principles of biological control stated by Cook and Baker (1983) is that 'an antagonist sought for use in soil, on a plant surface, or within a plant tissue is most likely to be adapted to the niche where needed if it is obtained from that niche originally'. For biological control of *A. flavus*, this means that antagonists should come from the geocarposphere, since this is the site of fungal invasion of the host. The preferential association of fungi and bacteria with early developmental stages of the pod indicates that some microorganisms are particularly well adapted for colonization of the geocarposphere, and these microorganisms are logical candidates for evaluation as biological control candidates for *A. flavus*.

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