# Evaluation of *Arachis* Species and Interspecific Tetraploid Lines for Resistance to Aflatoxin Production by *Aspergillus flavus*

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## ABSTRACT

Aflatoxins are carcinogenic and extremely toxic secondary metabolites produced primarily by two fungi, *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare. Elimination of aflatoxin contamination in peanut (*Arachis hypogaea* L.) is a high priority of the peanut industry. Resistant cultivars should be an effective and low-cost part of an integrated aflatoxin management program. To date, no cultivated peanut has been reported with stable high levels of resistance to aflatoxin production. *Arachis* species and interspecific tetraploid lines have been evaluated for resistance to several peanut diseases and insect pests, and highly resistant accessions have been reported. Seven accessions of *A. cardenasii* Krapov. and W.C. Gregory, 29 of *A. duranensis* Krapov. and W.C. Gregory, and 17 interspecific tetraploid lines derived from *A. hypogaea* × *A. cardenasii* were inoculated with *A. flavus* strain NRRL 3357 and analyzed for aflatoxin content after incubation. On average, *A. duranensis* and *A. cardenasii* accumulated significantly less aflatoxin than *A. hypogaea* checks. The mean difference between the two wild species was not significant. *Arachis duranensis* accessions PI 468319 (GKBSPSc 30073), PI 468200 (GKBSPSc 30064), and PI 262133 (GKP 10038 sl.); and *A. cardenasii* accessions PI 262141 (GKP 10017) and PI 475997 (KSSc 36018) had reduced levels of aflatoxin accumulation and should be valuable sources of resistance to aflatoxin contamination. Of the interspecific tetraploid lines, only GP-NC WS 2 supported aflatoxin production not significantly different from resistant parent *A. cardenasii* GKP 10017, and it appears to be a line with reduced capacity for aflatoxin accumulation.

Key Words: Peanut, wild species, germplasm selection.

Peanut (*Arachis hypogaea* L.) is cultivated worldwide in tropical, sub-tropical, and warm temperate regions. It is a major oil crop in many parts of the world, especially in Asia. In North America, peanut is grown as a cash crop and used mainly as a food source. Various diseases and insect pests limit peanut production, and the quality of peanut products is affected by many biotic and/or environmental factors. Aflatoxin contamination is a major problem in most peanut producing countries. Aflatoxins are toxic and carcinogenic secondary metabolites produced primarily by two fungi, *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare. Aflatoxin contamination can occur at any time from preharvest through storage. Elimination of aflatoxin contamination

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is a high priority of the peanut industry because of human health concerns.

Resistant cultivars should be an effective and low-cost part of an integrated aflatoxin management program, and much effort has been put into identifying host plant resistance. Significant genetic variation in resistance to *Aspergillus* infection or preharvest aflatoxin contamination has been identified, and a few breeding lines have been reported to possess resistance to seed colonization and seed infection (Mixon, 1983; Mehan *et al.*, 1991; Isleib *et al.*, 1994; Rao *et al.*, 1995; Holbrook *et al.*, 1998; Upadhyaya *et al.*, 2001). However, progress in developing cultivars with reduced capacity to support aflatoxin production by *A. flavus* has been slow. Mehan *et al.* (1986) screened 502 genotypes and found only two lines, U4-7-5 and VRR 245, that supported very low levels of aflatoxin production. U4-7-5 is the only low-aflatoxin producing genotype that has been used in breeding programs; two lines derived from U4-7-5 were released as germplasm for resistance to seed colonization and seed infection (Rao *et al.*, 1995; Upadhyaya *et al.*, 2001). In spite of these efforts, no cultivated peanut has been reported with stable, high levels of resistance to aflatoxin production.

Possible alternative sources of resistance to aflatoxin production are the wild species of *Arachis*. Only a few reports have been made on the value of wild *Arachis* species for resistance to aflatoxin production. Ghewande *et al.* (1989) reported that seed samples from five *Arachis* species (*A. cardenasii* Krapov. and W.C. Gregory, *A. duranensis* Krapov. and W.C. Gregory, *A. monticola* Krapov. and Rigoni, *A. pusilla* Benth., and *A. stenosperma* Krapov. and W.C. Gregory) were analyzed for aflatoxin content after inoculation and incubation. *Arachis cardenasii* and *A. duranensis* were highly resistant to *in vitro* seed colonization by *A. flavus* (IVSCAF) and supported aflatoxin production in only trace levels, but the particular accessions used among the many studied were not identified. Mehan (1989) evaluated 16 *Arachis* species (nine in section *Arachis*, three in section *Erectoides*, two in section *Rhizomatosae*, and one each in sections *Extranervosae* and *Triseminatae*), and reported that all supported production of aflatoxin B1 (34-110 μg/g seed). Thakur *et al.* (2000) observed wide variation both for seed colonization and aflatoxin production among 35 accessions belonging to 24 species in six different sections. Accessions of the three species *A. pusilla* [ICG 13212 (PI 497572, VSW 6773)], *A. chiquitana* Krapov., W.C. Gregory and C.E. Simpson [ICG 11560 (PI 476004, KSSc 36025)], and *A. triseminata* Krapov. and W.C. Gregory [ICG 8131 (PI 338449, GK 12922) and ICG 14875 (VfaPzSv 130800)] recorded low seed colonization and produced reduced levels of aflatoxin compared with the control *A. hypogaea* cultivar J11.

Although introgression of wild species genes into improved cultivars has been difficult, successful progress in utilization of wild species in peanut improvement has been made (Simpson *et al.*, 1993; Stalker and Simpson, 1995). Interspecific tetraploid breeding lines derived from hybridization between *A. hypogaea* PI 261942 with *A. cardenasii* GKP 10017 (PI 262141) have been identified with very high levels of resistance to several peanut diseases. Fifteen of these interspecific tetraploid germplasm lines have been released with high resistance to early leafspot (*Cercospora arachidicola* S. Hori) (Stalker and Beute, 1993; Stalker *et al.*, 2002b), insects (Stalker and Lynch, 2002) and root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood] (Stalker *et al.*, 2002a). All the resistance traits are believed to be directly derived from *A. cardenasii*. Garcia *et al.* (1995) studied 46 introgression lines (including GP-NC WS 1 to WS 10) from the same interspecific cross for the introgression of *A. cardenasii* chromosome segments, detected introgressed segments in 10 out of 11 linkage groups, and were able to identify introgression into both genomes of *A. hypogaea*. The tetraploid germplasm lines represent a possible source of high-levels of aflatoxin resistance that would be immediately accessible for transfer into commercially viable cultivars of *A. hypogaea*. The objective of this study was to identify accessions of *A. cardenasii* and *A. duranensis*, and interspecific tetraploid lines held at NC State Univ. with resistance to aflatoxin production.

## Materials and Methods

***Screening of* Arachis *Species.*** The germplasm evaluated in this study included seven accessions of *A. cardenasii* and 29 of *A. duranensis*. These 36 accessions were divided into two sets with *A. hypogaea* cultivars Perry (Isleib *et al.*, 2003) and Gregory (Isleib *et al.*, 1999) as susceptable checks in each set (Table 1).

The experimental unit was a group of inoculated seed halves in a petri dish. For each experimental unit, whole seeds totaling approximately 5 g were chosen from each line. Seed testas were manually removed to eliminate the potential barrier to *A. flavus* growth. The cotyledons of each seed were separated to permit the seed to rest without rolling. The seed halves were surface sterilized by immersion in a 0.525% (vol:vol) sodium hypochlorite solution (10% vol:vol commercial bleach) for 3 min followed by a rinse in approximately 20 mL of sterile water. The sample was then placed on the surface of four sheets of sterile filter paper moistened with 3 mL sterile water in a 10 cm plastic petri dish. Each seed half was inoculated with 50 μL of a suspension containing approximately $2.5 \times 10^5$ conidia per mL of *A. flavus* strain NRRL 3357 (National Center for Agricultural Utilization Research, Peoria, IL).

A 4 × 5 triple rectangular lattice design was used to test each set of 20 entries. Petri dishes in the same replication were arranged in four rows and five columns on a tray with columns as blocks (Set 1 in trays 1, 3,

and 5; Set 2 in trays 2, 4, and 6). Each of the six trays was independently enclosed in a plastic bag to retain moisture. The six trays were stacked in the incubator at 28 C for 8 d. Short sections of PVC pipe were inserted between adjacent trays in the stack to bear their weight and avoid downward pressure on the petri dishes' lids, allowing air circulation. The trays were rotated in vertical position each of the 8 d of incubation. Petri dishes were checked daily, and sterile water was added as needed to keep the filter paper near saturation, but without free water being evident at the paper surface.

**Table 1. Accessions of *Arachis* species used in the experiments and two *A. hypogaea* checks.**

| Species | Accession no. | PI no. | Collector[a] |
|---|---|---|---|
| *A. cardenasii* | 10017 | 262141 | GKP |
| *A. cardenasii* | 36015 | 475994 | KSSc |
| *A. cardenasii* | 36018 | 475997 | KSSc |
| *A. cardenasii* | 36019 | 475998 | KSSc |
| *A. cardenasii* | 36020 | 475999 | KSSc |
| *A. cardenasii* | 36032 | 476011 | KSSc |
| *A. cardenasii* | 36035 | 476014 | KSSc |
| *A. duranensis* | 7988 | 219823 | K |
| *A. duranensis* | 10038 ll. | 262133 | GKP |
| *A. duranensis* | 10038 sl. | 262133 | GKP |
| *A. duranensis* | 15101 | 468372 | ScBo |
| *A. duranensis* | 21763 | 497262 | ScVa |
| *A. duranensis* | 21764 | 497263 | ScVa |
| *A. duranensis* | 21766 | 497264 | ScVa |
| *A. duranensis* | 21767 | 497265 | ScVa |
| *A. duranensis* | 30060 | 468197 | GKBSPSc |
| *A. duranensis* | 30061 | 468198 | GKBSPSc |
| *A. duranensis* | 30064 | 468200 | GKBSPSc |
| *A. duranensis* | 30065 | 468201 | GKBSPSc |
| *A. duranensis* | 30067 | 468202 | GKBSPSc |
| *A. duranensis* | 30068 | 468203 | GKBSPSc |
| *A. duranensis* | 30069 | 475844 | GKBSPSc |
| *A. duranensis* | 30070 | 475845 | GKBSPSc |
| *A. duranensis* | 30071 | 475846 | GKBSPSc |
| *A. duranensis* | 30072 | 475847 | GKBSPSc |
| *A. duranensis* | 30073 | 468319 | GKBSPSc |
| *A. duranensis* | 30074 | 468320 | GKBSPSc |
| *A. duranensis* | 30075 | 468321 | GKBSPSc |
| *A. duranensis* | 30077 | 468323 | GKBSPSc |
| *A. duranensis* | 30078 or. fl. | 468324 | GKBSPSc |
| *A. duranensis* | 30078 yl. fl. | 468324 | GKBSPSc |
| *A. duranensis* | 36002 | 475882 | KSBScC |
| *A. duranensis* | 36003 | 475883 | KSBScC |
| *A. duranensis* | 36005 | 475885 | KSBScC |
| *A. duranensis* | 36006 | 475886 | KSBScC |
| *A. duranensis* | 36036 | 475887 | KSSc |
| *A. hypogaea* | Gregory | | |
| *A. hypogaea* | Perry | | |

[a]B = D.J. Banks; Bo = E. Bordas; C = C.L. Cristobal; G = W.C. Gregory; K = A. Krapovickas; P = J.R. Pietrarelli; S = C.E. Simpson; Sc = A. Schinini; Va = S.E.S. Valente.

After 8 d, petri dishes were removed from the incubator and samples were rated separately for mycelial growth, color, and development of "fluffy" colonies using a proportional scale of 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies). Samples were then dried for 1 d at 60 C and for another 3 d at 50 C, then ground to a friable meal in a coffee mill and stored in scintillation vials until analyzed for aflatoxin content in the NCSU Mycotoxin Lab in the Dept. of Poultry Science. Aflatoxin was extracted from a 2 g ground sample with acetonitrile-water (21:4 vol:vol) in a 5:1 ratio of extractant volume to sample weight. The extract was purified using a Mycosep 224 column (Romer Labs, Union, MO). Aflatoxin was measured by fluorescence high performance liquid chromatography as the post-column-generated bromide derivative (Traag *et al.*, 1987; Trucksess *et al.*, 1994). HPLC analysis was conducted using a Brownlee SPHERI-5 ODS, 5 mm 100 × 4.6 mm column fitted with a Brownlee NewGuard RP-18, 7 mm 15 × 3.2 mm column (Perkin-Elmer Corporation, Norwalk, CT). The mobile phase was 4/1/1 water/acetonitrile/methanol, containing 10 mg potassium bromide/L and 100 mL nitric acid/L, pumped at 2 mL/min with a LC-6A Solvent Delivery Module (Shimadzu Scientific Instruments, Columbia, MD). Samples were injected with a SIL-9A Automatic Sample Injector (Shimadzu Scientific Instruments, Columbia, MD) and detected using a RF-551 Fluorescence Detector (Shimadzu Scientific Instruments, Columbia, MD) with excitation at 360 nm and emission at 440 nm. Post-column derivatization was carried out using a KOBRA-cell post-column bromination unit (Vrije Universiteit, Amsterdam, The Netherlands) and 110 V power supply (Lamers & Pleuger, Den Bosch, The Netherlands). Data were recorded and calculations relative to pure aflatoxin standard were performed using a microcomputer equipped with SS-420 analog/digital converter boards and the EXCHROM 6.2 chromatography software system (Scientific Software, Inc., San Ramon, CA).

Aflatoxins B1, B2, and total aflatoxin were measured. Aflatoxin data were log-transformed [$Y' = \ln(Y + 0.5)$] to stabilize error variance. Both raw and transformed data were subjected to analysis of variance using the general linear model procedure (PROC GLM) of SAS version 8.2 (SAS Institute, Cary, NC). Data were analyzed initially using the incomplete block experimental design structure, but if there were no significant block effects, they were re-analyzed as a randomized complete block design. Adjusted means were separated by Fisher's protected t-test. Means of the transformed data were "back-transformed" with the inverse of the

transformation function ($Y = e^{Y'} - 0.5$) to present values in parts per billion (ppb).

***Screening Interspecific Tetraploid Lines.*** The germplasm evaluated in this study included 17 interspecific tetraploid lines (GP-NC WS 1 to GP-NC WS 15, N96074L, and N96076L). The 17 interspecific tetraploid lines originated from a triploid interspecific hybrid made by Smartt and Gregory (1967) between *A. hypogaea* PI 261942 and *A. cardenasii* GKP 10017. *Arachis cardenasii* is a diploid ($2n = 2x = 20$) species collected near Roboré, Bolivia. It is highly resistant to several diseases and insects (Stalker and Simpson, 1995). The *A. hypogaea* parent PI 261942 ($2n = 4x = 40$) is a purple-seeded valencia-type (subsp. *fastigiata* Waldron *var. fastigiata*) line introduced from the Guaraní region of Paraguay. This genotype is highly susceptible to several diseases and insects (Stalker, 1984; Guok *et al.*, 1986). Interspecific lines GP-NC WS 1 to GP-NC WS 4 were released as resistant germplasm to early leafspot (Stalker and Beute, 1993), GP-NC WS 5 and GP-NC WS 6 as root-knot nematode-resistant germplasm (Stalker *et al.*, 2002a), and GP-NC WS 7 to GP-NC WS 10 as insect-resistant germplasm (Stalker and Lynch, 2002). GP-NC WS 11 to GP-NC WS 15 involve more complicated ancestry, including *A. hypogaea* parents NC 5, NC 6, NC 3033, and PI 270806, and were released as leafspot-resistant germplasm (Stalker *et al.*, 2002b). *Arachis hypogaea* N96074L and N96076L have GP-NC WS 4 as a parent, so the lines have *A. cardenasii* ancestry. *Arachis* species donor *A. cardenasii* GKP 10017 (PI 262141), *A. hypogaea* parent PI 261942, and *A. hypogaea* cultivar Perry were included in the test as checks.

Seed preparation, inoculation, and incubation procedures were as described above except each piece was inoculated with 50 µL of a suspension containing approximately $1 \times 10^6$ conidia per mL of *A. flavus* strain NRRL 3357, and a $4 \times 5$ triple rectangular lattice design with two repetitions was used to test these 20 entries. Evaluation of fungal growth related traits, aflatoxin analysis, and data analysis procedures were as described above.

## Results and Discussion

**Arachis *Species*.** Blocks were not a significant source of variation for color and fluffy ratings, so they were not included in the analysis of variance for those traits. Significant variation was observed among the genetic entries for each trait, but some showed variation only among species (aflatoxin B1, aflatoxin B2, and total aflatoxin), while others showed only variation only among accessions within species (growth, color, and fluffy ratings). Species and accession within species had significant effects on log-transformed aflatoxin B1, log-transformed aflatoxin B2, and log-transformed total aflatoxin (Table 2).

On average, growth, color, and fluffiness of colonies were not measurably different across the *Arachis* species and *A. hypogaea*. Gregory and Perry accumulated the largest amounts of aflatoxin in both sets. The two *Arachis* species averaged significantly less aflatoxin B1, B2, and total aflatoxin than *A. hypogaea* (Table 3). However, reduced and high aflatoxin accumulators were observed within each species (Table 4). This indicates a need to evaluate many accessions per species, and differences between species observed in this experiment may not necessarily represent the entire species.

Vigorous fungal growth, dark green color, and few fluffy colonies were observed in this experiment. Significant differences were observed among the entries. *Arachis duranensis* 30073 was distinguished from all other entries by supporting less fungal growth and color development. Great variation also occurred for aflatoxin B1, B2, total aflatoxin, and their log-transformed values (Table 4). Identification of the "best" *Arachis* species accessions was dependent on the manner of expressing aflatoxin production. Based on raw (untransformed) data for aflatoxins B1 and B2, *A. duranensis* 36002, 30071, 36006, 30072, 30070, 30074, 10038 sl., and *A. cardenasii* 36032, 36018, and 10017 were the 10 lowest aflatoxin accumulators. It should be noted that some means had negative values due to the adjustment for statistically significant block effects. This does not indicate that there were negative values among the data, but that the means for these accessions were sufficiently close to zero that the block adjustment carried them below zero. Based on log-transformed data, *A. duranensis* 30073, 30064, 10038 sl., 30070, 15101, 21766, 7988, 30068, and *A. cardenasii* 10017 and 36018 had very low-level aflatoxin accumulation. Log transformation resulted in substantial reduction in the coefficient of variation for aflatoxin data (Table 4) and gave more precise mean separation.

*Arachis duranensis* 30073 had the least fungal growth and lowest aflatoxin accumulation based on the log-transformed mean and may be a good source of resistance for breeding for reduced aflatoxin accumulation.

*Arachis cardenasii* 10017 ranked third for reduced aflatoxin content based on log-transformed total aflatoxin and was not significantly different ($P \leq 0.05$) from *A. duranensis* 30073. *Arachis cardenasii* 10017 is the *Arachis* species accession from which there has been the greatest amounts of introgression into tetraploid populations via hybridization with *A. hypogaea*.

***Interspecific Tetraploid Lines.*** In the second experiment, a large amount of variation was observed among the 20 entries for all traits measured (Table 5). *Arachis* species parent *A. cardenasii* 10017 had vigorous fungal growth and fluffy colonies but the lowest production of aflatoxins B1 and B2, and total aflatoxin, before and after log transformation. The color score was relatively low, perhaps because of the high incidence of fluffy colonies, which do not develop green color as readily as normal colonies. While *A. cardenasii* 10017

**Table 2. Mean squares fungal growth and aflatoxin production traits for 36 accessions of *Arachis* species and two *A. hypogaea* checks.**

| Source | df | *A. flavus* Growth | *A. flavus* Color | *A. flavus* Fluffy | Aflatoxin B1 | ln (B1+0.5) | Aflatoxin B2 | ln (B2+0.5) | Aflatoxin (B1+B2) | ln (B1+B2+0.5) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ---- 0-10 rating[a] ---- | | | ppb | | ppb | | ppb | |
| Set | 1 | 0.21 | 0.33 | 0.33 | 251,084 | 0.7340 | 7 | 2.6371 | 253,828 | 0.7508 |
| Tray (set) | 4 | 5.33** | 14.33** | 3.03† | 7,196,552* | 9.0178** | 2,581* | 10.4708** | 7,470,704* | 9.0897** |
| Block (set, tray) | 24 | 1.03* | | | 3,322,373† | 2.6811† | 1,397† | 4.7712** | 3,457,078† | 2.7077† |
| Species | 2 | 1.00 | 0.57 | 1.81 | 38,440,664** | 14.9129** | 11,254** | 21.6792** | 39,767,385** | 15.0006** |
| Accessions (species) | 35 | 2.87** | 5.00** | 4.30** | 1,971,370 | 3.4281** | 815 | 3.6349* | 2,049,683 | 3.4493** |
| Error | 77(53) | 0.6 | 1.27 | 1.30 | 2,013,263 | 1.5917 | 860 | 1.9083 | 2,095,631 | 1.6019 |
| CV (%) | | 8.2 | 12.4 | 65.8 | 105.8 | 20.3 | 130.5 | 81.1 | 106.2 | 20.3 |

[a]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

†,*,** Denote mean squares significant at the 10%, 5%, and 1% levels of probability, respectively.

**Table 3. Adjusted means for fungal growth and aflatoxin production traits for *Arachis* species and *A. hypogaea* checks.[a]**

| Species | *A. flavus* Growth | *A. flavus* Color | *A. flavus* Fluffy | Aflatoxin B1 | ln (B1+0.5) | Back-transformed B1 | Aflatoxin B2 | ln (B2+0.5) | Back-transformed B2 | Aflatoxin (B1+B2) | ln(B1+B2+0.5) | Back-transformed (B1+B2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ----- 0-10 rating[b] ----- | | | ppb | | ppb | ppb | | ppb | ppb | | ppb |
| *A. cardenasii* | 9.65[a] | 9.40[a] | 1.17[a] | 656[a] | 6.1718[a] | 479 | 11[a] | 1.6865[a] | 5 | 666[a] | 6.1828[a] | 484 |
| *A. duranensis* | 9.38[a] | 8.97[a] | 1.90[a] | 1114[a] | 5.9818[a] | 396 | 19[a] | 1.4118[a] | 4 | 1133[a] | 5.9913[a] | 399 |
| *A. hypogaea* | 8.96[a] | 9.17[a] | 1.50[a] | 4187[b] | 7.9975[b] | 2973 | 71[b] | 3.8490[b] | 46 | 4258[b] | 8.0130[b] | 3020 |

[a]Means followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

was one of the most aflatoxin-resistant accessions analyzed, *A. hypogaea* parent PI 261942 had high fungal growth, dark green color, and low mean fluffy score and was among the genotypes with high aflatoxin accumulation. There were large variances among the 17 interspecific tetraploid lines for fungal growth and aflatoxin contamination. Several lines had more vigorous fungal growth and supported more aflatoxin production than the *A. hypogaea* parent PI 261942, but none accumulated less aflatoxin than *A. cardenasii* 10017. Based on the log-transformed result, GP-NC WS 2 was the only line that was not significantly different from the resistant *Arachis* species parent *A. cardenasii* 10017. All other tetraploid lines except N96074L contained more aflatoxin than check Perry.

*Arachis* species appear to be a valuable source of resistance to aflatoxin production. However, it is not unexpected that none of the 17 interspecific tetraploid lines was superior to *A. cardenasii* 10017 because they were initially selected for other resistances and not for resistance to aflatoxin production. Garcia *et al.* (1995) studied 46 introgression lines (including GP-NC WS 1 to WS 10) from the same interspecific cross and reported that the aggregate of the several introgressed segments represented approximately 360 cM or 30% of the diploid peanut genome. No single introgression line contained all the introgressed segments.

*Arachis duranensis* accessions should be introgressed into *A. hypogaea* to provide additional lines for *A. flavus* evaluation. Because inoculation and analysis of aflatoxin content is time consuming and expensive, the use of molecular markers tightly linked to aflatoxin resistance gene(s) would improve selection efficiency. It would be useful to identify polymorphic markers in GP-NC WS 2 and develop populations for marker-assisted selection. Garcia *et al.* (1995) reported both RFLP and RAPD markers were efficient in the detection of alien chromosome introgression. Because these markers are representative of *A. cardenasii*, they may be useful if *A. flavus* resistant populations are developed. It might then be possible to use marker-assisted selection in breeding for resistance to aflatoxin production.

## Acknowledgments

The authors wish to thank Dr. Winston Hagler and Hunter Edwards of the NCSU Mycotoxin Lab. for their help in conducting the aflatoxin assays. This work was supported in part by a grant from The Peanut Foundation.

**Table 4. Adjusted means for fungal growth and aflatoxin production traits for *Arachis* species and *A. hypogaea* checks.[a]**

| Set | Species | Acces. no. | *A. flavus* Growth | *A. flavus* Color | *A. flavus* Fluffy | Afla-toxin B1 | ln (B1+0.5) | Back-trans-formed B1 | Afla-toxin B2 | ln (B2+0.5) | Back-trans-formed B2 | Afla-toxin (B1+B2) | ln(B1+ B2+0.5) | Back-trans-formed (B1+B2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ----- 0-10 rating[b] ----- | | | ppb | | ppb | ppb | | ppb | ppb | | ppb |
| 1 | *A. cardenasii* | 10017 | 8.42$^{bc}$ | 8.67$^{cde}$ | 1.33$^{abc}$ | 327$^{a-d}$ | 4.1990$^{abc}$ | 66 | 0$^{a-d}$ | -0.5311$^{ab}$ | 0 | 327$^{a-d}$ | 4.1994$^{abc}$ | 66 |
| 1 | *A. cardenasii* | 36015 | 8.87$^{b-f}$ | 8.67$^{cde}$ | 2.00$^{bcd}$ | 849$^{a-d}$ | 6.0908$^{b-i}$ | 441 | 5$^{a-d}$ | 0.8946$^{a-f}$ | 2 | 854$^{a-d}$ | 6.0929$^{b-i}$ | 442 |
| 1 | *A. cardenasii* | 36018 | 9.43$^{c-h}$ | 8.67$^{cde}$ | 0.67$^{ab}$ | 312$^{a-d}$ | 4.7929$^{b-d}$ | 120 | 1$^{a-d}$ | -0.4818$^{ab}$ | 0 | 314$^{a-d}$ | 4.7959$^{b-e}$ | 121 |
| 1 | *A. cardenasii* | 36019 | 10.07$^{e-i‡}$ | 10.00$^{e}$ | 1.33$^{abc}$ | 1831$^{b-g}$ | 7.0623$^{e-j}$ | 1167 | 33$^{b-d}$ | 2.2842$^{c-j}$ | 9 | 1864$^{b-g}$ | 7.0737$^{e-j}$ | 1180 |
| 1 | *A. cardenasii* | 36020 | 9.37$^{c-h}$ | 9.33$^{de}$ | 0.00$^{a}$ | 1122$^{a-e}$ | 6.9287$^{e-j}$ | 1021 | 21$^{a-c}$ | 3.0599$^{f-j}$ | 21 | 1143$^{a-e}$ | 6.9465$^{e-j}$ | 1039 |
| 1 | *A. cardenasii* | 36032 | 9.31$^{c-h}$ | 9.33$^{de}$ | 1.33$^{abc}$ | 180$^{a-d}$ | 5.5770$^{b-h}$ | 264 | 0$^{a-d}$ | 0.4014$^{a-e}$ | 1 | 179$^{a-d}$ | 5.5829$^{b-h}$ | 265 |
| 1 | *A. cardenasii* | 36035 | 10.96$^{i‡}$ | 10.00$^{e}$ | 2.67$^{cd}$ | 1084$^{a-e}$ | 6.5011$^{c-j}$ | 665 | 18$^{a-c}$ | 2.2872$^{c-j}$ | 9 | 1102$^{a-e}$ | 6.5137$^{c-j}$ | 674 |
| 1 | *A. duranensis* | 10038 sl. | 8.96$^{b-f}$ | 6.67$^{b}$ | 2.00$^{bcd}$ | 452$^{a-d}$ | 4.4899$^{bcd}$ | 89 | 7$^{a-d}$ | 0.2937$^{a-d}$ | 1 | 459$^{a-d}$ | 4.4939$^{bcd}$ | 89 |
| 1 | *A. duranensis* | 21763 | 9.44$^{c-h}$ | 8.67$^{cde}$ | 2.67$^{cd}$ | 3655$^{e-h}$ | 6.6788$^{d-j}$ | 795 | 67$^{c-e}$ | 3.0560$^{e-j}$ | 21 | 3722$^{e-h}$ | 6.6928$^{d-j}$ | 806 |
| 1 | *A. duranensis* | 21767 | 8.93$^{b-f}$ | 7.33$^{bc}$ | 1.33$^{abc}$ | 2620$^{c-g}$ | 7.1989$^{f-j}$ | 1337 | 45$^{c-e}$ | 2.7826$^{d-j}$ | 16 | 2665$^{c-g}$ | 7.2127$^{f-j}$ | 1356 |
| 1 | *A. duranensis* | 30060 | 10.48$^{hi‡}$ | 9.33$^{de}$ | 3.33$^{d}$ | 1480$^{a-e}$ | 5.6955$^{b-i}$ | 297 | 26$^{a-d}$ | 1.0310$^{a-g}$ | 2 | 1506$^{a-e}$ | 5.7025$^{b-i}$ | 299 |
| 1 | *A. duranensis* | 30061 | 9.02$^{b-g}$ | 9.33$^{de}$ | 0.00$^{a}$ | 2343$^{b-g}$ | 7.3242$^{f-j}$ | 1516 | 39$^{c-e}$ | 2.4770$^{c-j}$ | 11 | 2382$^{b-g}$ | 7.3364$^{f-j}$ | 1535 |
| 1 | *A. duranensis* | 30064 | 9.90$^{c-i}$ | 10.00$^{e}$ | 2.00$^{bcd}$ | 814$^{a-d}$ | 3.7916$^{ab}$ | 44 | 21$^{a-c}$ | 0.7345$^{a-f}$ | 2 | 836$^{a-d}$ | 3.7988$^{ab}$ | 44 |
| 1 | *A. duranensis* | 30067 | 10.49$^{ghi‡}$ | 10.00$^{e}$ | 2.00$^{bcd}$ | 1013$^{a-e}$ | 7.1849$^{e-j}$ | 1319 | 16$^{a-c}$ | 2.2976$^{c-j}$ | 9 | 1029$^{a-e}$ | 7.1973$^{e-j}$ | 1335 |
| 1 | *A. duranensis* | 30070 | 7.71$^{b}$ | 6.67$^{b}$ | 0.67$^{ab}$ | 269$^{a-d}$ | 5.0642$^{b-f}$ | 158 | 3$^{a-d}$ | 0.2938$^{a-d}$ | 1 | 272$^{a-d}$ | 5.0682$^{b-f}$ | 158 |
| 1 | *A. duranensis* | 30075 | 9.82$^{c-i}$ | 10.00$^{e}$ | 0.00$^{a}$ | 1529$^{a-e}$ | 7.3231$^{f-j}$ | 1514 | 25$^{a-d}$ | 2.2659$^{c-j}$ | 9 | 1554$^{a-e}$ | 7.3345$^{f-j}$ | 1532 |
| 1 | *A. duranensis* | 36002 | 8.64$^{bcd}$ | 8.00$^{bcd}$ | 0.67$^{ab}$ | -1012$^{a‡}$ | 5.6886$^{b-i}$ | 295 | -25$^{a}$ | -0.4493$^{ab}$ | 0 | -1037$^{a}$ | 5.6908$^{b-i}$ | 296 |
| 1 | *A. duranensis* | 15101 | 9.91$^{d-i}$ | 10.00$^{e}$ | 2.67$^{cd}$ | 509$^{a-d}$ | 5.3636$^{b-g}$ | 213 | 1$^{a-d}$ | 0.5876$^{a-f}$ | 1 | 510$^{a-d}$ | 5.3676$^{b-g}$ | 214 |
| 1 | *A. hypogaea* | Gregory | 7.69$^{b}$ | 9.33$^{de}$ | 1.33$^{abc}$ | 4450$^{gh}$ | 7.6793$^{g-j}$ | 2163 | 66$^{c-e}$ | 2.9615$^{d-j}$ | 19 | 4516$^{g\,h}$ | 7.6892$^{g-j}$ | 2184 |
| 1 | *A. hypogaea* | Perry | 9.91$^{d-i}$ | 8.67$^{cde}$ | 2.00$^{bcd}$ | 4264$^{fgh}$ | 7.7346$^{hij}$ | 2286 | 78$^{de}$ | 3.6351$^{hij}$ | 37 | 4342$^{fg\,h}$ | 7.7492$^{hij}$ | 2319 |
| 2 | *A. duranensis* | 10038 ll. | 9.44$^{c-h}$ | 8.00$^{bcd}$ | 1.33$^{abc}$ | 862$^{a-d}$ | 6.3893$^{c-j}$ | 595 | 18$^{a-c}$ | 1.3406$^{a-h}$ | 3 | 880$^{a-d}$ | 6.4022$^{c-j}$ | 603 |
| 2 | *A. duranensis* | 7988 | 9.82$^{c-i}$ | 9.33$^{de}$ | 0.67$^{ab}$ | 645$^{a-d}$ | 5.4956$^{b-h}$ | 243 | 6$^{a-d}$ | -0.8178$^{a}$ | 0 | 651$^{a-d}$ | 5.4913$^{b-h}$ | 242 |
| 2 | *A. duranensis* | 21764 | 10.70$^{hi‡}$ | 10.00$^{e}$ | 1.33$^{abc}$ | 1111$^{a-e}$ | 5.8989$^{b-i}$ | 364 | 23$^{a-c}$ | 1.7486$^{b-i}$ | 5 | 1134$^{a-e}$ | 5.9153$^{b-i}$ | 370 |
| 2 | *A. duranensis* | 21766 | 8.90$^{b-f}$ | 8.67$^{cde}$ | 0.67$^{ab}$ | 719$^{a-d}$ | 5.4747$^{b-h}$ | 238 | 5$^{a-d}$ | 0.0195$^{abc}$ | 1 | 724$^{a-d}$ | 5.4728$^{b-h}$ | 238 |
| 2 | *A. duranensis* | 30065 | 8.96$^{b-f}$ | 8.00$^{bcd}$ | 1.33$^{abc}$ | 1407$^{a-e}$ | 6.6433$^{d-j}$ | 767 | 33$^{b-d}$ | 2.9121$^{d-j}$ | 18 | 1440$^{a-e}$ | 6.6626$^{d-j}$ | 782 |
| 2 | *A. duranensis* | 30068 | 10.21$^{f-i‡}$ | 10.00$^{e}$ | 2.67$^{cd}$ | 1143$^{a-e}$ | 5.4950$^{b-h}$ | 243 | 22$^{a-c}$ | 1.5549$^{a-i}$ | 4 | 1164$^{a-e}$ | 5.5070$^{b-h}$ | 246 |
| 2 | *A. duranensis* | 30069 | 9.90$^{d-i}$ | 10.00$^{e}$ | 2.00$^{bcd}$ | 2157$^{b-g}$ | 6.9006$^{e-j}$ | 992 | 42$^{c-e}$ | 3.0775$^{f-j}$ | 21 | 2199$^{b-g}$ | 6.9185$^{e-j}$ | 1010 |
| 2 | *A. duranensis* | 30071 | 9.85$^{c-i}$ | 10.00$^{e}$ | 1.33$^{abc}$ | -298$^{ab‡}$ | 5.5430$^{b-h}$ | 255 | -17$^{ab}$ | 0.0511$^{abc}$ | 1 | -315$^{ab}$ | 5.5466$^{b-h}$ | 256 |
| 2 | *A. duranensis* | 30072 | 9.72$^{c-i}$ | 10.00$^{e}$ | 5.33$^{e}$ | 97$^{abc}$ | 5.8239$^{b-i}$ | 338 | 4$^{a-d}$ | 1.0976$^{a-h}$ | 2 | 100$^{abc}$ | 5.8373$^{b-i}$ | 342 |
| 2 | *A. duranensis* | 30073 | 4.59$^{a}$ | 4.00$^{a}$ | 0.67$^{ab}$ | 642$^{a-d}$ | 2.0592$^{a}$ | 7 | 15$^{a-c}$ | 1.0598$^{a-h}$ | 2 | 656$^{a-d}$ | 2.0694$^{a}$ | 7 |
| 2 | *A. duranensis* | 30074 | 9.91$^{d-i}$ | 9.33$^{de}$ | 2.00$^{bcd}$ | 399$^{a-d}$ | 6.3338$^{c-j}$ | 563 | -1$^{abc}$ | 1.4839$^{a-h}$ | 4 | 398$^{a-d}$ | 6.3430$^{c-j}$ | 568 |
| 2 | *A. duranensis* | 30077 | 9.54$^{c-i}$ | 10.00$^{e}$ | 5.33$^{e}$ | 1168$^{a-e}$ | 7.4919$^{g-j}$ | 1793 | 23$^{a-c}$ | 3.5349$^{g-j}$ | 34 | 1191$^{a-e}$ | 7.5142$^{g-j}$ | 1833 |
| 2 | *A. duranensis* | 30078 yl. fl. | 10.04$^{d-i‡}$ | 10.00$^{e}$ | 2.67$^{cd}$ | 1127$^{a-e}$ | 6.1339$^{b-j}$ | 461 | 25$^{a-d}$ | 2.5005$^{c-j}$ | 12 | 1152$^{a-e}$ | 6.1521$^{b-j}$ | 469 |
| 2 | *A. duranensis* | 30078 or. fl. | 9.70$^{c-i}$ | 10.00$^{e}$ | 2.67$^{cd}$ | 832$^{a-d}$ | 6.6576$^{d-j}$ | 778 | 13$^{a-c}$ | 1.7226$^{a-i}$ | 5 | 845$^{a-d}$ | 6.6680$^{d-j}$ | 786 |
| 2 | *A. duranensis* | 36003 | 9.34$^{c-h}$ | 9.33$^{de}$ | 2.00$^{bcd}$ | 1380$^{a-e}$ | 6.6604$^{d-j}$ | 780 | 24$^{a-d}$ | 1.6879$^{a-i}$ | 5 | 1405$^{a-e}$ | 6.6716$^{d-j}$ | 789 |
| 2 | *A. duranensis* | 36005 | 9.83$^{c-i}$ | 10.00$^{e}$ | 2.00$^{bcd}$ | 1727$^{b-f}$ | 7.6759$^{g-j}$ | 2155 | 22$^{a-c}$ | 3.1908$^{f-j}$ | 24 | 1749$^{b-f}$ | 7.6890$^{g-j}$ | 2184 |
| 2 | *A. duranensis* | 36006 | 9.28$^{c-h}$ | 8.67$^{cde}$ | 0.67$^{ab}$ | 53$^{abc}$ | 5.5146$^{b-h}$ | 248 | 2$^{a-d}$ | 0.5460$^{a-f}$ | 1 | 55$^{abc}$ | 5.5218$^{b-h}$ | 250 |
| 2 | *A. duranensis* | 36036 | 10.04$^{d-i‡}$ | 10.00$^{e}$ | 2.00$^{bcd}$ | 2347$^{c-g}$ | 7.5283$^{g-j}$ | 1859 | 52$^{d-e}$ | 2.7512$^{d-j}$ | 15 | 2399$^{c-g}$ | 7.5436$^{g-j}$ | 1888 |
| 2 | *A. hypogaea* | Gregory | 8.69$^{b-e}$ | 10.00$^{e}$ | 2.00$^{bcd}$ | 5340$^{h}$ | 8.5415$^{j}$ | 5122 | 93$^{e}$ | 4.6470$^{j}$ | 104 | 5433$^{h}$ | 8.5604$^{j}$ | 5220 |
| 2 | *A. hypogaea* | Perry | 9.54$^{c-i}$ | 8.67$^{cde}$ | 0.67$^{ab}$ | 2694$^{d-g}$ | 8.0346$^{ij}$ | 3085 | 47$^{c-e}$ | 4.1524$^{ij}$ | 63 | 2741$^{d-g}$ | 8.0533$^{i\,j}$ | 3144 |
| | Mean | | 9.38 | 9.07 | 1.73 | 1341 | 6.2166 | 916 | 23 | 1.7036 | 12 | 1364 | 6.2270 | 930 |

[a]Means followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

‡Adjusted means above 10 for rated traits or below 0 for untransformed aflatoxins do not indicate that data values occurred outside the expected range of values, but that the means were sufficiently close to the limit that the adjustment for significant block effects in the experimental design carried the mean beyond the limit.

**Table 5. Adjusted means for fungal growth and aflatoxin production traits for interspecific tetraploid lines, *A. cardenasii*, and the *A. hypogaea* checks.[a]**

| Genotype | *A. flavus* Growth | *A. flavus* Color | *A. flavus* Fluffy | Aflatoxin B1 | ln (B1+0.5) | Back-transformed B1 | Aflatoxin B2 | ln (B2+0.5) | Back-transformed B2 | Aflatoxin (B1+B2) | ln(B1+B2+0.5) | Back-transformed (B1+B2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ----- 0-10 rating[b] ----- | | | ppb | | ppb | ppb | | ppb | ppb | | ppb |
| *A. cardenasii* 10017 | 10.00$^{d}$ | 5.87$^{a}$ | 7.50$^{e}$ | 6,716$^{a}$ | 7.2004$^{a}$ | 1340 | 219$^{a}$ | 4.2738$^{a}$ | 71 | 6,935$^{a}$ | 7.2252$^{a}$ | 1,373 |
| Interspecific lines | | | | | | | | | | | | |
| N96074L | 7.00$^{a}$ | 9.01$^{d-g}$ | 0.83$^{a}$ | 7,002$^{a}$ | 8.7501$^{bc}$ | 6,311 | 171$^{a}$ | 4.9954$^{abc}$ | 147 | 7,174$^{a}$ | 8.7737$^{bc}$ | 6,461 |
| N96076L | 9.67$^{cd}$ | 9.33$^{e-h}$ | 0.83$^{a}$ | 14,207$^{abc}$ | 9.4341$^{b-f}$ | 12,507 | 469$^{abc}$ | 5.9335$^{c-h}$ | 377 | 14,676$^{abc}$ | 9.4643$^{b-f}$ | 12,891 |
| GP-NC WS1 | 9.00$^{bcd}$ | 9.36$^{e-h}$ | 0.67$^{a}$ | 23,060$^{bcd}$ | 10.0168$^{d-h}$ | 22,400 | 604$^{a-e}$ | 6.3768$^{e-i}$ | 588 | 23,664$^{bcd}$ | 10.0432$^{d-h}$ | 22,998 |
| GP-NC WS2 | 6.67$^{a}$ | 6.54$^{ab}$ | 0.50$^{a}$ | 5,796$^{a}$ | 8.4420$^{ab}$ | 4,637 | 162$^{a}$ | 4.6057$^{ab}$ | 100 | 5,957$^{a}$ | 8.4644$^{ab}$ | 4,742 |
| GP-NC WS3 | 8.33$^{b}$ | 8.03$^{cd}$ | 1.00$^{a}$ | 25,721$^{cd}$ | 9.9609$^{d-h}$ | 21,182 | 706$^{a-e}$ | 6.3748$^{e-i}$ | 586 | 26,427$^{cd}$ | 9.9885$^{d-h}$ | 21,774 |
| GP-NC WS4 | 10.00$^{d}$ | 9.02$^{d-g}$ | 1.33$^{ab}$ | 43,376$^{ef}$ | 10.6274$^{fgh}$ | 41,248 | 1434$^{f}$ | 7.2112$^{ijk}$ | 1354 | 44,810$^{ef}$ | 10.6597$^{fgh}$ | 42,605 |
| GP-NC WS5 | 8.67$^{bc}$ | 9.00$^{d-g}$ | 0.83$^{a}$ | 15,631$^{abc}$ | 9.4873$^{b-f}$ | 13,191 | 362$^{ab}$ | 5.7121$^{c-f}$ | 302 | 15,993$^{abc}$ | 9.5100$^{b-f}$ | 13,493 |
| GP-NC WS6 | 9.50$^{cd}$ | 6.68$^{ab}$ | 5.67$^{d}$ | 21,931$^{bcd}$ | 9.8078$^{c-h}$ | 18,175 | 630$^{a-e}$ | 6.2063$^{d-i}$ | 495 | 22,561$^{bcd}$ | 9.8349$^{c-h}$ | 18,673 |
| GP-NC WS7 | 8.17$^{b}$ | 8.09$^{cd}$ | 1.17$^{ab}$ | 11,295$^{ab}$ | 9.2936$^{b-e}$ | 10,868 | 243$^{a}$ | 5.4338$^{b-e}$ | 229 | 11,537$^{ab}$ | 9.3145$^{b-e}$ | 11,098 |
| GP-NC WS8 | 8.67$^{bc}$ | 8.23$^{de}$ | 2.83$^{c}$ | 16,194$^{abc}$ | 9.5752$^{b-g}$ | 14,403 | 403$^{ab}$ | 5.8751$^{c-g}$ | 356 | 16,597$^{abc}$ | 9.5998$^{b-g}$ | 14,761 |
| GP-NC WS9 | 9.17$^{bcd}$ | 7.03$^{bc}$ | 4.83$^{d}$ | 31,848$^{de}$ | 10.2586$^{e-h}$ | 28,527 | 1004$^{c-f}$ | 6.7710$^{g-j}$ | 872 | 32,851$^{de}$ | 10.2892$^{e-h}$ | 29,412 |
| GP-NC WS10 | 9.83$^{d}$ | 8.70$^{def}$ | 2.50$^{bc}$ | 40,345$^{ef}$ | 10.5268$^{fgh}$ | 37,300 | 1143$^{ef}$ | 6.9547$^{h-k}$ | 1048 | 41,488$^{ef}$ | 10.5546$^{fgh}$ | 38,355 |
| GP-NC WS11 | 10.00$^{d}$ | 10.11$^{gh}$ | 1.17$^{ab}$ | 31,886$^{de}$ | 10.3522$^{e-h}$ | 31,327 | 1053$^{def}$ | 6.9265$^{g-k}$ | 1018 | 32,940$^{de}$ | 10.3846$^{e-h}$ | 32,357 |
| GP-NC WS12 | 10.00$^{d}$ | 9.66$^{fgh}$ | 1.33$^{ab}$ | 39,896$^{ef}$ | 10.4776$^{e-h}$ | 35,511 | 1503$^{fg}$ | 7.0498$^{ijk}$ | 1152 | 41,399$^{ef}$ | 10.5108$^{e-h}$ | 36,708 |
| GP-NC WS13 | 10.00$^{d}$ | 9.22$^{e-h}$ | 1.17$^{ab}$ | 61,158$^{g}$ | 10.9813$^{h}$ | 58,764 | 2682$^{h}$ | 7.8181$^{k}$ | 2485 | 63,840$^{g}$ | 11.0231$^{h}$ | 61,274 |
| GP-NC WS14 | 9.50$^{cd}$ | 10.01$^{gh}$ | 1.00$^{a}$ | 16,376$^{abc}$ | 9.4851$^{b-f}$ | 13,161 | 448$^{ab}$ | 5.8451$^{c-g}$ | 345 | 16,823$^{abc}$ | 9.5114$^{b-f}$ | 13,512 |
| GP-NC WS15 | 10.00$^{d}$ | 10.33$^{h}$ | 0.83$^{a}$ | 46,857$^{f}$ | 10.7104$^{gh}$ | 44,821 | 2049$^{g}$ | 7.5058$^{jk}$ | 1818 | 48,906$^{f}$ | 10.7510$^{gh}$ | 46,677 |
| *A. hypogaea* | | | | | | | | | | | | |
| Perry | 9.50$^{cd}$ | 10.08$^{gh}$ | 1.00$^{a}$ | 14977$^{abc}$ | 8.8631$^{bcd}$ | 7066 | 515$^{a-d}$ | 5.3000$^{a-d}$ | 200 | 15493$^{abc}$ | 8.8921$^{bcd}$ | 7274 |
| PI 261942 | 9.83$^{d}$ | 9.00$^{d-g}$ | 1.83$^{abc}$ | 32304$^{de}$ | 10.2459$^{e-h}$ | 28168 | 860$^{b-e}$ | 6.5773$^{f-j}$ | 718 | 33164$^{de}$ | 10.2715$^{e-h}$ | 28895 |
| Mean | 9.18 | 8.67 | 1.94 | 25329 | 9.7248 | 16728 | 833 | 6.1873 | 486 | 26162 | 9.7533 | 17211 |
| CV (%) | 9.7 | 10 | 63.9 | 42.3 | 10.8 | | 57.0 | 14.5 | | 42.5 | 10.8 | |

[a]Means followed by the same letter are not significantly different ($P < 0.05$) by t-test.

[b]Proportional rating scale from 0 (no growth, green color, or fluffy colonies) to 10 (dense mycelium on all quarters, dark green color, or all fluffy colonies) in one-point increments.

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