Association of Plant Color and Pericarp Color with Colonization of Grain by Members of *Fusarium* and *Alternaria* in Near-Isogenic Sorghum Lines

Deanna L. Funnell, USDA-ARS, Wheat, Sorghum and Forage Research, and Department of Plant Pathology, University of Nebraska, Lincoln 68583-0937; and Jeffrey F. Pedersen, USDA-ARS, Wheat, Sorghum and Forage Research, and Department of Agronomy and Horticulture, University of Nebraska, Lincoln 68583-0937

**ABSTRACT**


White sorghum (*Sorghum bicolor*) grain from tan plants is more desirable for human or animal consumption. Colonization by *Fusarium* and *Alternaria* spp. was assessed for near-isogenic lines differing in wound response (purple or tan) and pericarp color (red or white) in field-grown grain and in greenhouse-grown plants. Seeds were screened on a semi-selective medium for *Alternaria* and *Fusarium*. Significantly fewer fungal colonies were obtained from tan plants with white seed, and fewer numbers of *Alternaria* colonies were obtained from white seed, regardless of plant color, from an irrigated field, while there were no differences in fungal composition of seeds grown at a nonirrigated field. Screening of seed from the nonirrigated field on *Fusarium* semi-selective medium yielded fewer *Fusarium* isolations from seed grown on purple plants compared with seed from tan plants. When inoculated with *Alternaria* sp. and *F. moniliforme*, there can be no differences in lesion lengths on tan/white plants when compared with purple/red plants in most assays; in one assay, tan/white plants had smaller lesion lengths following inoculation with *F. moniliforme*. These results suggest that plants with white seeds were as resistant as plants with the red pericarp trait to colonization by *Alternaria* and *Fusarium* spp. However, the results also suggest that under appropriate environmental conditions seed from tan plants may be more susceptible to *Fusarium* spp. than seed from purple plants.

Additional keywords: food-grade sorghum, *Fusarium verticillioides*, grain mold, mycotoxigenic fungi, wound inoculation

---

**Sorghum (Sorghum bicolor (L.) Moench)** pericarp pigments can affect grain quality. Pigments found in vegetative parts of the plant and in the peduncle and glumes of flowers (12,44) also may indirectly impact sorghum grain quality. Pigments associated with the “purple wound response” accumulate following mechanical injury, insect feeding, or pathogen invasion (22,31). Grain with nonpigmented pericarps (white seed), grown on tan plants can have unfavorable agronomic qualities (39). Among the four color phenotypes (purple, red, purple/white, tan/red, and tan/white), there were no significant differences in germination, seedling vigor, and field performance. However, when plant color and pericarp color phenotypes were considered separately, germination and field emergence were significantly better for plants having either purple plant color or red pericarp phenotypes (39). It was proposed that these differences may be due to greater colonization and/or infection by fungi of plants with the white-grain or tan plant color phenotypes (39). Therefore, it was incumbent to test the hypothesis that sorghum with white grain and tan plant-color are more susceptible to colonization by fungi than plants carrying seed with red pericarps with the purple wound response.

In this work, we assessed colonization of field-grown sorghum grain by *Alternaria* spp. and *Fusarium* spp. Isolates of these genera were obtained from field-grown grain using two different semi-selective media (1,28). These fungal species were chosen because of prevalence in both diseased and asymptomatic sorghum grain (23,43,49). Additionally, greenhouse inoculation assays (18) were conducted using an *Alternaria* sp. isolated from sorghum seed and a *Fusarium moniliforme* sensu lato isolate pathogenic on sorghum.

**MATERIALS AND METHODS**

**Field experiment.** Twenty near-isogenic lines differing in plant color and pericarp color had been previously developed (39). The lines (~97% identical) are S8 segregates of a single S3 family from the BC1 generation of the cross (BTx386 ms5 × BTx630)(ms3 × BTx630) (39). The lines were grown in the field during 2002 at

**Table 1.** Rainfall (Ithaca, NE, 2002 and Lincoln, NE, 2003) and irrigation (Ithaca, 2002) monthly totals (cm)

<table>
<thead>
<tr>
<th>Water (cm)</th>
<th>Ithaca, 2002&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lincoln, 2003&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall, May</td>
<td>3.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rainfall, June</td>
<td>4.06</td>
<td>16.89</td>
</tr>
<tr>
<td>Irrigation, June</td>
<td>5.00</td>
<td>NA&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rainfall, July</td>
<td>3.15</td>
<td>2.59</td>
</tr>
<tr>
<td>Irrigation, July</td>
<td>5.00</td>
<td>NA</td>
</tr>
<tr>
<td>Rainfall, August</td>
<td>16.74</td>
<td>3.30</td>
</tr>
<tr>
<td>Irrigation, August</td>
<td>5.00</td>
<td>NA</td>
</tr>
<tr>
<td>Rainfall, September</td>
<td>2.84</td>
<td>9.68&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rainfall, October</td>
<td>9.27</td>
<td>NA</td>
</tr>
<tr>
<td>Rainfall, November</td>
<td>0.50</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup> Planting date: May 21. Harvest date: November 8.<br><sup>b</sup> Planting date: May 21. Harvest date: September 24.<br><sup>c</sup> From date of planting.<br><sup>d</sup> Not applicable.<br><sup>e</sup> Until date of harvest.
Ithaca, NE and 2003 in Lincoln, NE, for assessment of fungal colonization of grain. Among the 20 near-isogenic lines, there were five of each phenotype: purple (plant color)/red (pericarp color), purple/white, tan/red, and tan/white. Irrigation was applied at Ithaca; fields at the Lincoln location were not irrigated (Table 1). At each location, two-row plots for each of the 20 lines were randomized within each of four repetitions. Seed source (produced in 1999) was the same for both plantings. Irrigation and rainfall totals for Ithaca, 2002 and rainfall totals for Lincoln, 2003 are shown in Table 1. Nitrogen fertilizer was applied prior to planting at both locations at 157 kg ha\(^{-1}\). At the Lincoln location, propachlor \((2\text{-chloro}-\text{N}-(1\text{-methyl-ethyl})-\text{N}^-\text{phenylacetamide}\)) and atrazine \((6\text{-chloro}-\text{n-ethyl-N}^-\text{1-(methyl-ethyl)-1,3,5-trazine-2,4-diamine})\) were applied at 3.36 and 1.1 kg ha\(^{-1}\), respectively, approximately 14 days postemergence. In 2002, bentazon \((2\text{-}(1\text{-methylethyl})\text{-IH}-2,1,3\text{-benzothiadiazin 4(3H)}\text{-one-2,2-dioxide})\) was added to the postemergence application at 0.28 kg ha\(^{-1}\) for velvetleaf \((Abutilon theophrasti\) (Medik)) control. Grain hardness, assessed using a wheat single kernel characterization system, determined that mean hardness ranged from 80 (purple/red plants) to 82 (tan/white plants) (90 to 110 is defined as hard and 40 is defined as soft) (38).

### Isolation of fungi from field-grown seed. Grain was removed from heads of 10 randomly chosen plants within each plot. Seeds were surface-sterilized in 95% ethanol followed by 1% sodium hypochlorite with 0.01% Tween 20 (51). From 14 to 20 seeds per plot were plated onto DCPA medium (1) containing dichloran (Ultra Scientific, North Kingston, RI) and chloramphenicol (Sigma Chemical Co., St. Louis, MO). A randomly chosen subset of 16 plots from Lincoln, 2003, also were plated onto PCNB medium (28), which contains streptomycin (Sigma) and the fungicide pentachloronitrobenzene (Terrachlor, Uniroyal Company, Middlebury, CT). A note was made of seed that appeared molded, discolored, or nonuniform in shape or size to assess possible differences between seed from different plots or locations.

Individual fungal colonies growing out from seed onto the medium were trans-

### Table 2. Isolations of fungi and enumeration of Alternaria species, Fusarium moniliforme, and Fusarium species from seed grown at Ithaca, NE, in 2002 and selected on DCPA medium

<table>
<thead>
<tr>
<th>Plant color</th>
<th>Pericarp color</th>
<th>No. of seeds tested</th>
<th>Fungal isolations/seed</th>
<th>Alternaria spp./seed</th>
<th>F. moniliforme/seed</th>
<th>Other Fusarium spp/seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>By plant and pericarp color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple</td>
<td>Red</td>
<td>335</td>
<td>1.185 ± 0.051</td>
<td>0.709 ± 0.057</td>
<td>0.018 ± 0.011</td>
<td>0.039 ± 0.018</td>
</tr>
<tr>
<td>Purple</td>
<td>White</td>
<td>333</td>
<td>1.165 ± 0.051</td>
<td>0.610 ± 0.057</td>
<td>0.031 ± 0.011</td>
<td>0.089 ± 0.018</td>
</tr>
<tr>
<td>Tan</td>
<td>Red</td>
<td>343</td>
<td>1.192 ± 0.051</td>
<td>0.655 ± 0.057</td>
<td>0.020 ± 0.011</td>
<td>0.064 ± 0.018</td>
</tr>
<tr>
<td>Tan</td>
<td>White</td>
<td>341</td>
<td>0.997 ± 0.051*</td>
<td>0.542 ± 0.057</td>
<td>0.023 ± 0.011</td>
<td>0.055 ± 0.018</td>
</tr>
<tr>
<td>By plant color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple</td>
<td>–</td>
<td>668</td>
<td>1.175 ± 0.044</td>
<td>0.660 ± 0.046</td>
<td>0.024 ± 0.008</td>
<td>0.064 ± 0.013</td>
</tr>
<tr>
<td>Tan</td>
<td>–</td>
<td>684</td>
<td>1.094 ± 0.044*</td>
<td>0.598 ± 0.046</td>
<td>0.022 ± 0.008</td>
<td>0.060 ± 0.013</td>
</tr>
<tr>
<td>By pericarp color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>Red</td>
<td>678</td>
<td>1.188 ± 0.044</td>
<td>0.682 ± 0.046</td>
<td>0.019 ± 0.008</td>
<td>0.051 ± 0.013</td>
</tr>
<tr>
<td>–</td>
<td>White</td>
<td>674</td>
<td>1.081 ± 0.044*</td>
<td>0.576 ± 0.046*</td>
<td>0.027 ± 0.008</td>
<td>0.072 ± 0.013</td>
</tr>
</tbody>
</table>

\* Least squares means (LSM) and standard errors are shown.
\* Indicates number is significantly less than other(s) in comparison at \(P \leq 0.05\).

---

**Fig. 1.** Response of purple plants and tan plants to wound inoculation with *Fusarium moniliforme* (A, B, and C) or sterile broth (C and D). A, Whole sorghum head peduncles 22 days following wound inoculation with toothpicks incubated in broth with *F. moniliforme*. Peduncles from a purple plant (P) with red seed (left) and from a tan plant (T) with red seed (right) are shown. B, Sorghum head peduncles split longitudinally for measurement of plant response 22 days following wound inoculation with *F. moniliforme*. Brackets labeled “1” indicate measurement 1, brackets labeled “2” indicate measurement 2. Peduncles from a purple plant with white seed (left), and a tan plant with white seed (right) are shown. C, Example of lesion extending throughout peduncle. Both sides of a dissected peduncle from a purple plant with red seed are shown. D and E, Longitudinally split peduncles exhibiting response of plants to inoculation with sterile broth. All plants shown have grain with white pericarp. D, Peduncles from plants showing a common response to mock inoculation. The tan plant is on the left and the purple plant is on the right. E, Range of responses to mock inoculation in peduncles from two purple plants.
ferred to half-strength potato dextrose agar (1/2× PDA; made from potato dextrose broth purchased from EM Scientific, Gibbstown, NJ) to assess colony morphology. Fungal isolates were transferred to water agar, with an approximately 0.5 cm² sterile filter paper placed over the inoculation site on the agar surface (34). *Fusarium* spp. were identified, whenever possible, using morphological features, spore types, and conidiophore structures (20,29). Isolates with characteristics and structures consistent with *Fusarium verticilloides* (Sacc.) Nirenberg (=*F. moniliforme*) (33) were defined as belonging to *Fusarium moniliforme* sensu lato (23,24,40), which was referred to as *F. moniliforme* in the text. For purposes of statistical analyses, all other *Fusarium* spp. were counted as a single group.

Assessments of colonization by fungi in grain from the complete set of field-grown plants were analyzed using Proc MIXED (SAS Institute, Cary, NC). Plant color, pericarp color, line, and environment (due to application of irrigation at Ithaca, 2002) were considered fixed, and replication was considered random in the model. The experimental design was a randomized complete block with four replications with lines nested in phenotype. Preliminary analyses indicated that environmental interactions with line or phenotype were significant for multiple traits. Therefore, separate analyses were conducted for each environment. Because multiple plots had seed with no *F. moniliforme* or other *Fusarium* spp. recovered, data were normalized using arcsine transformation. The results were equivalent for both transformed and untransformed. Therefore, only nontransformed least squares means (LSM) with standard errors are presented.

Apparent effectiveness of field blocking was lost upon random selection of the small subset of 16 random lines used for the PCNB experiment. These results were analyzed using Proc GLM (SAS) since only fixed effects, line, plant color, and pericarp color were included in the model. Means and standard deviations are reported.

**Greenhouse experiments.** For each assay, plants were grown and inoculated in the following way. Seed from each line was sown into a standard mix in 25-cm pots, grown in a greenhouse, and thinned to two plants per pot. Two weeks following

| Table 3. Isolations of fungi and enumeration of *Alternaria* spp., *Fusarium moniliforme*, and *Fusarium* species from seed grown at Lincoln, NE, in 2003 and selected on DCPA medium

<table>
<thead>
<tr>
<th>Plant color</th>
<th>Pericarp color</th>
<th>No. of seeds tested</th>
<th><em>Fusarium</em> isolations/seed</th>
<th><em>Alternaria</em> spp./seed</th>
<th><em>F. moniliforme</em>/seed</th>
<th>Other <em>Fusarium</em> spp./seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple</td>
<td>Red</td>
<td>317</td>
<td>1.141 ± 0.083</td>
<td>0.476 ± 0.107</td>
<td>0.009 ± 0.009</td>
<td>0.029 ± 0.011</td>
</tr>
<tr>
<td>Purple</td>
<td>White</td>
<td>339</td>
<td>1.068 ± 0.083</td>
<td>0.526 ± 0.107</td>
<td>0.009* ± 0.009</td>
<td>0.011 ± 0.011</td>
</tr>
<tr>
<td>Tan</td>
<td>Red</td>
<td>296</td>
<td>1.069 ± 0.083</td>
<td>0.546 ± 0.107</td>
<td>0.021 ± 0.009</td>
<td>0.036 ± 0.012</td>
</tr>
<tr>
<td>Tan</td>
<td>White</td>
<td>306</td>
<td>1.084 ± 0.083</td>
<td>0.525 ± 0.107</td>
<td>0.012 ± 0.009</td>
<td>0.027 ± 0.011</td>
</tr>
</tbody>
</table>

* Least squares means (LSM) and standard errors are listed. P value for fungal isolations per seed is 0.371, for *Alternaria* spp./seed is 0.507, for *F. moniliforme*/seed is 0.893, and for isolations of other *Fusarium* spp./seed is 0.705.

* 0.000 Indicates no *F. moniliforme*.

Table 4. Isolations of fungi and enumeration of *Alternaria* spp., *Fusarium moniliforme*, and *Fusarium* species from seed grown at Lincoln, NE, in 2003 and selected on PCNB medium

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. of seeds tested</th>
<th><em>Fusarium</em> isolations/seed</th>
<th><em>Alternaria</em> spp./seed</th>
<th><em>F. moniliforme</em>/seed</th>
<th>Other <em>Fusarium</em> spp./seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>By plant color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple</td>
<td>132</td>
<td>0.660 ± 0.320</td>
<td>0.341 ± 0.249</td>
<td>0.000* ± 0.000</td>
<td>0.066 ± 0.090*</td>
</tr>
<tr>
<td>Tan</td>
<td>119</td>
<td>0.580 ± 0.285</td>
<td>0.168 ± 0.150</td>
<td>0.076 ± 0.056</td>
<td>0.143 ± 0.089</td>
</tr>
<tr>
<td>By pericarp color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>117</td>
<td>0.701 ± 0.276</td>
<td>0.296 ± 0.241</td>
<td>0.037 ± 0.054</td>
<td>0.155 ± 0.103</td>
</tr>
<tr>
<td>White</td>
<td>134</td>
<td>0.493 ± 0.277</td>
<td>0.235 ± 0.216</td>
<td>0.029 ± 0.054</td>
<td>0.045 ± 0.042</td>
</tr>
</tbody>
</table>

* Totals of a subset of seed reported in Table 3 that was plated onto both DCPA and PCNB at the same time.

* 0.000 Indicates that no *F. moniliforme* were isolated from grain of purple plants on PCNB.

* Indicates number is significantly less at P ≤ 0.05.

*The number of seeds tested for *Fusarium* isolations/seed was 400, for *Alternaria* spp./seed was 200, and for *Fusarium* moniliforme*/seed was 200.

* For assay 1, one plant in each pot was inoculated with *F. moniliforme* and the other plant with the *Alternaria* sp. isolate. Up to four plants per line, five lines per phenotype, for up to 20 plants per phenotype, were inoculated with each isolate. Lesions were measured 18 dpi.

For assay 2, plants were inoculated with *F. moniliforme* or mock inoculated. Up to four plants per line (up to 20 plants of each phenotype) were inoculated with *F. moniliforme* and up to two plants per line (up to 10 plants of each phenotype) were mock inoculated. Both plants in a given pot were inoculated with the same culture. Plant lines with the white pericarp phenotype were tested in assay 3; lines having the red pericarp phenotype were tested in assay 4. Measurements in both assays 3 and 4 were made 22 dpi.

Assays 1 and 2 were conducted as split-plot experiments, with lines being whole-plots and inoculum being subplots. The design was completely random, with line, pericarp color, plant color, and inoculum considered fixed effects, and pot considered random. Due to plant mortality, unequal numbers of treatment combinations were available for both inoculation and assessment. Analyses were conducted using Proc MIXED (SAS), considering lines nested within phenotype. The design for assays 3 and 4 was again completely random, but it differed in that subsampling of identical treatment combinations occurred in each pot. Plant color, line, and inoculum were considered fixed effects, and pot was considered random. Again, due to mortality, unequal numbers of treatment combinations were available for
inoculation and assessment. Appropriate error degrees of freedom were calculated by specifying the KENWARDROGER option in the model to account for unequal sample sizes.

RESULTS

Fungal colonization of seed from plant color/pericarp color near-isogenic lines. Field-grown seed harvested from plants grown at Ithaca during the 2002 season and at Lincoln during 2003 were screened for fungal outgrowths onto DCPA medium (Tables 2 and 3, respectively). When considering combined data from both fields and years, grain with red pericarp color yielded significantly greater ($P = 0.028$) numbers of mean fungal isolations ($1.147 \pm 0.043$) on this medium than white grain ($1.079 \pm 0.043$). Environmental interactions with plant color and pericarp color were frequently significant. Therefore, the two environments were analyzed separately. For seed grown at Ithaca (irrigated) in 2002, tan plants having seeds with white pericarp had significantly fewer numbers of total fungal colonies isolated ($P = 0.022$) than the other three phenotypes (Table 2). Numbers of *Alternaria* colonies isolated were significantly less from lines having white seeds than from lines having red seeds ($P = 0.031$) (Table 2). There were no significant differences in numbers of *Fusarium* isolations or for isolations of *Fusarium* species other than *F. moniliforme* when comparing the four plant color/pericarp color phenotypes or when comparing plant color or pericarp color (Table 2). For field-grown seed collected from Lincoln (nonirrigated) in 2003, there were no significant differences in any of the response variables: mean number of fungal isolations per seed, mean number of *Alternaria* colonies per seed, mean numbers of *Fusarium* isolations per seed, or mean number of other *Fusarium* spp. isolated per seed, following plating of seed and selection of fungal outgrowths on DCPA medium when considering the four plant color/pericarp color phenotypes (Table 3) or when considering only plant color or pericarp color.

Seeds from the random subset of plants sampled from plots at Lincoln, 2003, plated onto DCPA medium, also were plated onto PCNB medium (Table 4). When fungal outgrowths from seed were selected on PCNB medium, mean *Fusarium* spp. other than *F. moniliforme* were significantly less ($P = 0.047$) in seed grown on plants with the purple color phenotype than those grown on tan plants (Table 4). There were no significant differences among the four plant color/pericarp color phenotypes for any of the measurements: colonies isolated per seed, numbers of *Alternaria* colonies, numbers of *Fusarium* spp., and numbers of *Fusarium* species other than *F. moniliforme* (Table 4). For selection of fungal outgrowths on DCPA for seeds from the random subset, there were no significant differences in the measured traits for plant color, pericarp color, or the four plant phenotypes (Table 3 and data not shown).


---

**Fig. 2.** Response of plant color/pericarp color lines to inoculation with an *Alternaria* species field isolate and a *Fusarium moniliforme* isolate pathogenic on sorghum assay 1. Mean (LSM) lesion lengths (mm) for the first measurement and the second measurement 18 days following inoculation of plant color/pericarp color lines. Positive standard errors are shown. Bars marked with different letters for each measurement are statistically significant at $P \leq 0.05$. A, Inoculation of 20 purple/red, 20 purple/white, 20 tan/red, and 19 tan/white plants with *Alternaria* species. There were no significant differences between lines for measurement 1 or measurement 2. B, Inoculation of 20 purple/red plants, 20 purple/white plants, 19 tan/red plants, and 18 tan/white plants with *Fusarium moniliforme*. 

---

414 Plant Disease / Vol. 90 No. 4
from plants grown at Lincoln in 2003 included: _F. acuminatum_ Ell. & Ev. sensu Gordon (15), _F. equesti_ (Corda) Sacc. sensu Gordon (15), _F. solani_, _F. proliferatum_, _F. subglutinans_, and _F. thapsinum_.

Response of plant color/pericarp color plants to wound inoculation with an _Alternaria_ isolate and an _F. moniliforme_ isolate. Following inoculation of peduncles of purple and tan plants, lesions on tan plants were different in color from purple plants, due to the lack of pigments that cause the purple wound response. Although a discoloration of the peduncle was apparent in _F. moniliforme_-inoculated tan plants, lesions were lighter in color than lesions on purple plants infected with _F. moniliforme_ (Fig. 1A). Upon splitting the peduncle, dark red lesions were apparent in the purple-colored plants that included the entire width of the peduncle (measurement 1), with lesion edges often lighter in color and not spanning the peduncle (measurement 2) (Fig. 1B). The relatively dark lesion that encompasses the diameter of the peduncle (measurement 1) following inoculation of tan plants with fungi is light brown to tan, while measurement 2, which includes the less discrete portion of the lesion, is lighter though still discernible and may be woody in appearance (Fig. 1B). Lesions can extend beyond the peduncle and into the stalk. This was observed in both tan and purple plants; a purple plant is shown in Figure 1C.

Assay 1: inoculation with an _Alternaria_ isolate and an _F. moniliforme_ isolate. Mean lesion lengths resulting from inoculation with _F. moniliforme_ were significantly greater than those resulting from inoculation with an _Alternaria_ isolate (H02-773-s-3) ($P = 0.0001$; Fig. 2). Inoculum interactions with plant color (for both measurements, $P < 0.0001$) or pericarp color for measurement 2 ($P = 0.004$) also were significantly different. There were no significant differences among phenotypes for either measurement following inoculation with the _Alternaria_ sp. isolate (Fig. 2A).

Inoculation with _F. moniliforme_ in assay 1 resulted in significant differences among the four plant color/pericarp color phenotypes when considering both the first and the second lesion measurement (Fig. 2B). Both measurements yielded significantly greater mean lengths following inoculation of purple plants when compared with all tan plants ($P < 0.0001$). Pericarp color did not appear to affect mean lesion length following inoculation of tan plants (for measurement 1, $P = 0.7540$, for measurement 2, $P = 0.401$), but red-seeded plants exhibited longer mean lesion lengths when compared with lesions produced on white-seeded plants inoculated with _F. moniliforme_ (for measurement 1, $P = 0.016$; for measurement 2, $P = 0.001$) (Fig. 2B).

**Fig. 3.** Response of plant color/pericarp color lines to inoculation with a _Fusarium moniliforme_ isolate pathogenic on sorghum and to inoculation with sterile broth: assay 2. Mean (LSM) lesion measurements (mm) 18 days following inoculation of plant color/pericarp color lines with toothpicks incubated in broth with _F. moniliforme_ or with toothpicks incubated in sterile broth by plant phenotype. Twenty-one purple/red plants, 15 purple/white plants, and 18 each of tan/red plants and tan/white plants were inoculated with _F. moniliforme_. Ten plants each of purple/red, purple/white, and tan/white phenotypes and 11 plants of the tan/red phenotype were inoculated with sterile broth. For inoculations with _F. moniliforme_, the first and second measurements are shown while the second measurement is shown for inoculations with sterile broth. Positive standard errors are shown. Bars marked with different letters are statistically significant at $P \leq 0.05$. Larger responses (Fig. 1E) may be the result of a wound response but also could be the result of incidental contamination via the wound or the exposed broth-soaked toothpick. In assay 2, there were significant differences between inocula, _F. moniliforme_ culture versus sterile PDB ($P < 0.0001$), and no significant interaction of inoculum with plant color or pericarp color. The mean lesion lengths following each treatment across all plant color/pericarp color phenotypes for measurement 1 was 101.7 mm versus 32.8 mm, and for measurement 2, 127.6 mm versus 35.3 mm, for inoculation with _F. moniliforme_ or sterile broth, respectively. No significant differences due to the interactions of plant color with pericarp color (for measurement 1, $P = 0.469$, for measurement 2, $P = 0.164$) accounted for significant differences among the four phenotype classes following inoculation with _F. moniliforme_ (for measurement 1, $P = 0.042$, for measurement 2, $P = 0.286$) (Fig. 3). There were no significant differences for measurements 1 and 2 following mock inoculations with sterile broth (Fig. 3, measurement 2 is shown).

Assays 3 and 4: comparison of purple and tan plants inoculated with _F. moniliforme_. Evidence from previous work has indicated that the red pericarp trait can contribute to pigmentation in nonreproductive plant parts (7). Therefore, two assays were conducted to directly assess the effects of pigments involved in the purple wound response. Assay 3 involved only white-seeded plants; assay 4 had only red-seeded plants (Fig. 4). In these two assays, there were no significant differences be-
between the mean lesion lengths resulting from inoculation of purple plants or tan plants with *F. moniliforme* when considering the second measurement (Fig. 4). In assay 4, significant differences between the mean lengths of the first measurement ($P = 0.023$), but not the second ($P = 0.1832$), resulted following inoculation with *F. moniliforme* (Fig. 4B). Inoculation of each plant color/pericarp color phenotype with sterile broth resulted in mean measurements significantly less than those following inoculation with *F. moniliforme* ($P < 0.0001$), but there were no interactions with plant phenotype. There were no significant differences indicated between tan and purple plants following mock-inoculation with toothpicks incubated in sterile broth (Fig. 4).

In summary, a screen of plant color/pericarp color near-isogenic lines for colonization or infection by *Alternaria* and *Fusarium* spp. provided evidence that white sorghum grain grown on tan plants may not have increased susceptibility to colonization by members of these genera as compared to purple plants with red seed. In these controlled greenhouse assays, tan plants yielded lesions not significantly different from those produced on purple plants following inoculation with an *Alternaria* isolate and *F. moniliforme*, or significantly smaller lesions following inoculation with *F. moniliforme*. When screening seed from plants grown at Ithaca in 2002 and at Lincoln in 2003, there were no consistent results suggesting that white seed from tan plants was more susceptible to these fungal groups than red seed from purple plants. However, when seed from Lincoln, 2003, was selected on PCNB, significantly greater numbers of *Fusarium* isolations other than *F. moniliforme* were obtained from seed of tan plants when compared with seed from purple plants. This suggested that pigments involved in conferring the purple wound response may be involved in protecting these lines, and perhaps the developing grain, from colonization by *Fusarium* species.

**DISCUSSION**

Traits that previously have been associated with resistance to infection in sorghum grain by fungi are a pigmented wound response (46,50) and grain with a pigmented pericarp (10,48). However, sorghum desirable for livestock and for humans would lack these traits (4,39). Concern had been raised that lines with white grain on tan plants may be more susceptible to grain mold fungi (2,10,17,26,39). However, in this study, there was no evidence that tan sorghum plants that produce white seed were significantly and consistently more susceptible to grain mold fungi (2,10,17,26,39). However, in this study, there was no significant difference between tan and purple plants following inoculation with an Alternaria isolate and *F. moniliforme*, or significantly smaller lesions following inoculation with *F. moniliforme*. When screening seed from plants grown at Ithaca in 2002 and at Lincoln in 2003, there were no consistent results suggesting that white seed from tan plants was more susceptible to these fungal groups than red seed from purple plants. However, when seed from Lincoln, 2003, was selected on PCNB, significantly greater numbers of *Fusarium* isolations other than *F. moniliforme* were obtained from seed of tan plants when compared with seed from purple plants. This suggested that pigments involved in conferring the purple wound response may be involved in protecting these lines, and perhaps the developing grain, from colonization by *Fusarium* species.

![Fig. 4. Comparison of responses of purple plants with tan plants for plants with the same pericarp genotype to inoculation with *Fusarium moniliforme* or sterile broth. Measurements were made 22 days following inoculation. Measurements 1 and 2 following inoculation with *F. moniliforme* and measurement 2 following inoculation with sterile broth are shown. Mean (LSM) lesion measurements (mm) and positive standard errors are shown. Bars having different numbers within a measurement are statistically significant at $P \leq 0.05$. A, Plants with the white pericarp genotype. Twenty-seven plants of each phenotype, purple/white and tan/white, were inoculated with *F. moniliforme*, and 10 plants of each phenotype were inoculated with sterile broth. B, Plants with the red pericarp genotype. Twenty-three purple/red plants and 27 tan/red plants were inoculated with *F. moniliforme*, while 9 purple/red plants and 10 tan/red plants were inoculated with sterile broth.](image-url)
4). Additionally, wounding and infection of tan plants can result in a discernable response (Fig. 1A, B, and D). It is possible that wounding with sterile broth could have resulted in infections with other microorganisms (25). Therefore, this control also can assist in accounting for incidental infections that may affect lesion size following inoculation with a pathogen of interest. In assay 3, secondary infections by what appeared to be bacteria at the point of inoculation were noted in three F. moniliforme— inoculated plants (one purple/white plant and two tan/white plants), but no such infections were visually apparent in plants wound-inoculated with sterile broth in assay 3 or in plants inoculated by fungi or wound-inoculated with sterile broth during other assays (data not shown).

The role that pigments in nonreproductive parts of plants could play in development of grain mold is indirect. It has been shown that some pigments produced in response to pathogen invasion of leaves and other plant parts (31, 47) are inhibitory to resistance to grain mold fungi (17, 26). It has been well-documented that F. moniliforme infection of maize plants can result in colonization of grain (27, 35). Therefore, pigments in plant parts other than in the developing seed could conceivably prevent infection of grain. When seed collected from randomly chosen plots grown at Lincoln in 2003 was plated onto DCPA, white grain had significantly lower total fungal isolations per seed and onto DCPA, white grain had significantly less total fungal isolations per seed and onto DCPA, white grain had significantly less total fungal isolations per seed than from seed grown in Lincoln in 2002. This suggests that fungi isolated from seed grown in the two locations in different years were not identical. There were greater numbers of different Fusarium spp., other fungi isolated in 2002 than from seed grown in Lincoln in 2003. On the other hand, two species (F. acuminatum and F. equiseti) were isolated from seed grown at Ithaca in 2003, but were not detected in seed grown at Ithaca in 2002.

ACKNOWLEDGMENTS

We thank J. Toy for conducting field plantings and B. Tamaka for conducting greenhouse assays. We also thank J. Soper and A. Wagner for technical assistance. We thank D. C. Stenger for providing helpful editorial suggestions and for assistance in figure preparation.

LITERATURE CITED

duction by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 82:341-345.


