Seed-applied Fungicide and Inoculant Interactions for Late-planted Soybean in the Mid-southern United States

Bobby R. Golden,* Tom W. Allen, and John M. Orlowski

Abstract

Soybean [Glycine max (L.) Merr.] production has recently replaced cotton (Gossypium hirsutum L.) production on a large number of acres in the mid-southern United States. Seed-applied inoculants and fungicides are common management inputs to help soybean producers deal with limited Bradyrhizobium japonicum populations and soilborne diseases associated with early planting (April) that is standard for soybean production in Mississippi. However, it is unclear if seed-applied inoculants and fungicides are necessary for late-planted (May or June) soybean in fields with a history of non-soybean or following flooded conditions. Studies were conducted at four late-planted locations during the 2011 and 2012 growing seasons in Mississippi, planted between May (2011; \( n = 1 \)) and mid-to late June (2011, 2012; \( n = 3 \)). Three common seed-applied fungicide products (ApronMaxx RTA, ApronMaxx RFC, and Trilex 6000) and two seed-applied inoculant products (Optimize and Vault-HP) were applied alone and in combination. Within-season plot evaluations were conducted to determine the effects on soybean stand and yield. Postharvest evaluations considered important soybean seed quality characteristics (e.g., damaged seed, mold). Seed-applied inoculant products did not affect soybean stands, but ApronMaxx RFC increased soybean stands by \( >8\% \) compared with nontreated soybean and soybean treated with the other fungicide products. An inoculant × fungicide interaction was observed for soybean yield. ApronMaxx RFC without an inoculant and Trilex 6000 with Optimize increased yield by 7% over nontreated soybean. Performance of seed-applied fungicides appeared to vary depending on the type of inoculant used.

Over the past decade, cotton acreage in Mississippi has significantly declined. In 2006 1.23 million acres of cotton were planted in Mississippi, but in 2009 only 305,000 acres of cotton were sown. In contrast, in 2006, 1.8 million acres of soybean were planted and in 2009 planted soybean acreage increased to 2.2 million acres (USDA–NASS, 2006, 2009). This shift in acres created a number of problems with soybean nodulation on land that had historically been cropped...
with cotton. Soybean producers in Mississippi have the potential to routinely produce soybean yields >70 bu/acre when grown under irrigation. However, in soils that have a limited history of soybean in rotation, the bacterium *Bradyrhizobium japonicum*, responsible for proper soybean nodulation and subsequent N₂ fixation, may be absent or limited in number (Pedersen, 2004). Additionally, flooding, drought, and extended periods of high temperatures may limit soilborne *B. japonicum* populations (Pedersen, 2004; Albaraeda et al., 2009). In situations where *B. japonicum* populations may be limited, seed-applied bacterial inoculants can be used to increase the chances of adequate nodulation and yield.

Seed-applied fungicides, in addition to inoculant products, have become an important management practice for soybean producers who choose to plant soybean using the Early Soybean Production System (ESPS) common to the mid-southern United States (Heatherly and Spurlock, 1999). The ESPS recommends that producers plant soybean in April when soils can remain cool and wet for extended periods. Seed-applied fungicides can reduce the risks associated with populations of specific soilborne organisms considered to be a complex of fungi that include several species of *Fusarium*, several species of *Pythium*, *Phytophthora sojae*, and *Rhizoctonia solani* (Bowers and Russin, 1999; Grau et al., 2004; Urrea et al., 2013). Soybean in Mississippi is generally treated with a broad-spectrum quinone outside inhibitor (strobilurin) class fungicide and often include more narrow-spectrum products such as mefenoxam or metalaxyl (McGee, 1992; Munkvold, 2009; Urrea et al., 2013).

While soybean inoculants and seed-applied fungicides can be beneficial for soybean planted in the recommended April planting window in the Mid-South, there are a number of events that may cause soybean to be planted much later in the months of May, June, and even July. Excessive rainfall and subsequent flooding and soybean double-cropped after wheat (*Triticum aestivum* L.) or oats (*Avena sativa* L.) can shift soybean planting much later than normal in the growing season. Limited information is available regarding the effects of inoculants and seed-applied fungicide combinations on soybean in the Mid-South. Additionally, some manufacturers suggest that certain inoculant formulations are incompatible with specific seed-applied fungicide products, and several recent studies have reported a negative yield response when bacterial inoculants are combined with specific seed-applied fungicide products (Hiltbold et al., 1980; Schulz and Thelen, 2008; Campo et al., 2009). However, information to determine specific inoculant by seed-applied fungicide production combinations is currently lacking in the mid-southern United States.

Therefore, the objectives of this research were to (i) determine the effect of seed-applied inoculants and fungicides on soybean stands and yield under late-planted conditions, and (ii) investigate the interactions between popular seed-applied inoculant and fungicide products in late-planted soybean in Mississippi.

### SEED-APPLIED FUNGICIDES AND INOCULANTS

The selected seed treatments were developed by various manufacturers and are commonly available from retail outlets in Mississippi. The three fungicides evaluated were ApronMaxx RFC, ApronMaxx RTA, and Trilex 6000. Two soybean inoculants, Optimize and Vault-HP, were evaluated alone or in combination with the above-mentioned fungicides (Table 1).

ApronMaxx RFC was developed by Syngenta Crop Protection (Research Triangle Park, NC) and includes the active ingredients fluixuronil [4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile] (2.31%) and mefenoxam ([(R)-2-[(2,6-dimethylphenyl)-methoxycetyl]aminomethyl]proionic acid methyl ester) (3.46%). ApronMaxx RTA includes the active ingredients mefenoxam (1.10%) and fluixuronil (0.73%) and is also manufactured by Syngenta. Trilex 6000 is a multicomponent seed treatment fungicide that includes triloxystrobin [benzenacetic acid, (E,E)-α-(mehoxyimino)-2-[[1-[3-(( trifluoromethyl)phenyl)ethylidene]amino]oxy]methyl]-methyl ester] (7.12%) and metalaxyl [methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate] (5.69%) as well as an insecticide, imidacloprid (48.7%), and is produced by Bayer CropScience (Research Triangle Park, NC).

The two biological seed treatment inoculants were Optimize (Monsanto BioAg, St. Louis, MO) and Vault-HP (BASF Crop Protection, Research Triangle Park, NC). Optimize contains multiple strains of proprietary rhizobia as well as a lipo-chitooligosaccharide (LCO) growth promoter, which is a natural signaling molecule added to maximize the plant’s genetics. Optimize has a minimum guarantee of 5 × 10⁹ viable *B. japonicum* cells/g and 2 × 10⁸% LCO/g of product. Optimize is labeled to have a 120-day window between treatment and planting. Vault-HP contains at minimum (3.0 × 10⁹ colony-forming units/ml) of proprietary

<table>
<thead>
<tr>
<th>Table A. Useful conversions.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>To convert Column 1 to Column 2, multiply by</strong></td>
</tr>
<tr>
<td>0.304</td>
</tr>
<tr>
<td>0.405</td>
</tr>
<tr>
<td>28.4</td>
</tr>
<tr>
<td>67.19</td>
</tr>
<tr>
<td>1.12 × 10⁻²</td>
</tr>
</tbody>
</table>
rhizobia strains as well as a growth promoter. Unlike Optimize, Vault-HP contains a bio-fungicide (Integral; <1% Bacillus subtilis, MBI600).

**SITE DESCRIPTION AND STUDY ESTABLISHMENT**

Field experiments were established during the 2011 and 2012 growing seasons at two locations in Mississippi to evaluate the effect of fungicidal seed treatments and inoculants on soybean yield and quality. In 2011 one study was established at the Mississippi State University Delta Research and Extension Center (DREC; 33.419192, —90.91022) on a Bosket very fine sandy loam (fine-loamy, mixed, active, thermic Fluvaquentic Endoaquept) on 18 June, while the other site was established in a grower field near Redwood, MS, on a Commerce silt loam (fine-silty, mixed, active, thermic Mollic Hapludalf) on 30 June. In 2012 studies were established at two separate locations at the DREC. One study was planted on Tunica clay (clayey over loamy, smectitic over mixed, superactive, nonacid, thermic Fluvaquentic Endoaquept) on 9 May following corn (Zea mays L.). Cotton or corn was the previous crop grown at the experimental locations located at DREC. The Redwood site in 2011 was established following the 2010 soybean crop; however, an extended flood delayed planting into late June.

Glyphosate-resistant soybean cultivar Armor DK 4744 (Armor Seed, Jonesboro, AR) was used at all locations in both years. Before planting, soybean seed was weighed into 5-lb lots for precise application of each individual seed treatment. A 5-lb lot was loaded into a small-batch tabletop rotary drum seed treater (USC, LLC, Sabetha, KS) and the inoculant–fungicide slurry applied by slowly streaming each treatment into the drum. The coated seed was allowed to rotate for 2 min before being transferred to a clean plastic bag and stored overnight at 4°C. The seeds were then counted and placed into packets for planting. Plots were planted with a four-row John Deere (Moline, IL) cone-type planter with 40-inch row spacing and calibrated to deliver a target seeding rate of 145,000 seeds/acre. Plot length was 35 ft. All locations were maintained as weed free, with pest management and irrigation scheduling performed according to Mississippi State University Extension Soybean Management Guidelines.

Soybean stand density was determined by counting the number of plants in a 10-ft section in the middle of each harvest row at V4. The two middle rows of each plot (6.65 ft) were harvested with a Kincaid 8-XP small-plot combine (Kincaid Equipment Manufacturing, Haven, KS) equipped with a HarvestMaster grain weigh system (Juniper Systems, Logan, UT). All yields were adjusted postharvest to 13% moisture assuming 60 lb seed/bu. Harvested grain was analyzed for test weight and damage, including stink bug injury, mold, heat injury, foreign matter, and total grain damage, by Mid-South Grain Inspection Service in Stoneville, MS.

Within each site-year, field experiments were arranged in a randomized complete block with treatments defined by three seed treatment fungicides applied alone or in combination with two seed treatment inoculants, plus a nontreated control. Each treatment was replicated four times. Fungicide and inoculant treatments were classified as fixed effects for analysis. Site-years were initially analyzed separately to evaluate the homogeneity of variance assumption. Preliminary analysis did not indicate a problem with unequal variances; thus, the site-years were combined for analysis, with site-year classified as a random effect. Evaluations of site-year as a random effect allow for inference over a range of environments, and have been used by others (Carmer et al., 1989). Mean separations were performed using Fisher’s protected LSD at the 0.05 level of significance. All statistical analyses were conducted using the general linear model procedure in SAS Version 9.3 (SAS Institute, 2010).
Table 2. Model significance for the ANOVA for the main effects of inoculant and fungicide and the inoculant × fungicide interaction averaged across four locations in Mississippi during the 2011 and 2012 growing seasons.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Inoculant†</th>
<th>Fungicide†</th>
<th>Inoculant × fungicide†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant stands</td>
<td>p = 0.0046</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>0.18</td>
<td>0.19</td>
<td>0.016</td>
</tr>
<tr>
<td>Test weight</td>
<td>0.93</td>
<td>0.61</td>
<td>0.96</td>
</tr>
<tr>
<td>Damaged seed</td>
<td>0.65</td>
<td>0.92</td>
<td>0.12</td>
</tr>
<tr>
<td>Foreign matter</td>
<td>0.87</td>
<td>0.15</td>
<td>0.58</td>
</tr>
<tr>
<td>Mold</td>
<td>0.47</td>
<td>0.47</td>
<td>0.27</td>
</tr>
</tbody>
</table>

† Model significant at \( p \leq 0.05 \).

Table 3. Soybean stand as influenced by the main effect of seed applied fungicide for research trials managed in Mississippi during 2011 and 2012.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>V4 plant stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>106,721</td>
</tr>
<tr>
<td>ApronMaxx RFC</td>
<td>117,014</td>
</tr>
<tr>
<td>ApronMaxx RTA</td>
<td>107,303</td>
</tr>
<tr>
<td>Trilex 6000</td>
<td>108,036</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>6,371</td>
</tr>
</tbody>
</table>

STANDS AND YIELD

Test weight, damaged seed, foreign matter, and mold responses were not observed. However, an inoculant × fungicide interaction was observed for soybean yield when averaged across all site-years (Table 2). Soybean plant population at V4 was unaffected by the fungicide × inoculant interaction (\( p = 0.23 \)), but the main effect of fungicide influenced V4 plant populations (\( p = 0.0046 \)). Averaged over soybean inoculants ApronMaxx RFC seed treatment increased stands by 8.8% compared with the nontreated control (Table 3). Trilex 6000 and ApronMaxx RTA produced similar plant populations when compared with the nontreated control. Gaspar et al. (2014) also failed to observe increased stands for ApronMaxx or Trilex 2000 (Trilex 6000, without the imidacloprid insecticide component) over the nontreated soybean. However, Bradley (2008) observed increased stands for a seed treatment product that contained fluioxynil and mefenoxam (the same active ingredients as ApronMaxx) in three out of 14 environments compared with nontreated soybean. Bradley (2008) also evaluated a seed treatment product that contained azoxystrobin, which is a similar mode of action to the trifloxystrobin found in Trilex 6000, and metalaxyl, the other component of Trilex 6000. The researchers observed increased stands in four out of 14 environments when compared with nontreated seed.

Yields of ApronMaxx RTA with Optimize and Vault-HP were 6.7 and 6.4% greater than ApronMaxx RTA without an inoculant (Table 4). However, all treatments containing ApronMaxx RTA had yields similar to nontreated soybean. ApronMaxx RFC without inoculant increased soybean yield over the nontreated soybean by 7% (Table 4), but yield was similar to ApronMaxx RFC with Optimize and Vault HP. Trilex 6000 with Optimize increased soybean yield compared with the nontreated control by 7%. Furthermore, Trilex 6000 with Optimize yielded more than Trilex 6000 without inoculant by 11% and Trilex 6000 with Vault-HP by 6%. Shulz and Thelen (2008) observed yield increases for seed-applied fungicide in five out of 16 site-years; however, no fungicide × inoculant interactions were observed at site-years where fungicide increased yield.

ApronMaxx RTA and ApronMaxx RFC have the same active ingredients but different concentrations of both mefenoxam and fluioxynil, resulting in different concentrations of each chemical delivered to the seed. ApronMaxx RFC delivers more mefenoxam (0.0519 vs. 0.0365 oz/cwt) but less fluioxynil (0.0347 vs. 0.055 oz/cwt) than ApronMaxx RTA. Stand and yield increases over nontreated soybean were observed with ApronMaxx RFC but not with ApronMaxx RTA. This suggests that seed treatment products containing greater rates of mefenoxam may be beneficial to late-planted soybean in the Mid-South. In contrast to ApronMaxx RFC, Trilex 6000 increased yield over nontreated soybean only when combined with Optimize (6.7%) but not with Vault HP. ApronMaxx RFC without an inoculant yielded similarly to Trilex 6000 with Optimize. Campo et al. (2009) reported negative effects on rhizobial survival, nodulation, and yield with a number different fungicidal seed treatment active ingredients, but also suggested that the effects varied by \( B. japonicum \) strain, while Revellin et al. (1993) observed decreased nodulation and yields for soybean treated with metalaxyl, one of the components of Trilex 6000. All of the fields used in this study had...
a recent history of soybean production and likely contained native B. japonicum, so it is not clear why additional inoculant would result in increased yield with the Trilex 6000. Soybean root nodulation was not accessed between treatments; however, no nitrogen deficiency symptoms were observed in any environment, indicating adequate nodulation across treatments. Perhaps differences in the B. japonicum strains or potentially different biologicals and growth promoters in the two inoculant products affected their interaction with the fungicidal elements of Trilex 6000 but not ApronMaxx RFC.

The results of this study suggest that there are interactions between fungicide-applied seed treatments and inoculants, but these interactions are inconsistent. Given the large number of seed treatment and inoculant combinations available to producers, it would be extremely difficult to create an easily interpretable suggestion for soybean producers. This study and other studies show that stand and yield increases resulting from seed-applied fungicides and inoculants are inconsistent and depend on a number of factors, such as the environment, native soil pathogen levels, as well as the rates of active ingredients that constitute the particular seed treatment product. Soybean growers should choose seed treatment inputs based on their knowledge of field cropping history and previous incidence of soilborne diseases in the field, and should always consult the label for both inoculants and seed-applied fungicides to look for compatibility issues. However, growers should not expect a consistent yield increase from either inoculant or seed-applied fungicides.

References


