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NEW FUNGICIDES FOR USE AS SEED TREATMENTS ON COTTON

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Abstract

Azoxystrobin offers a new class of chemistry compared to existing fungicide seed treatments. It has even a broader range of activity than the triazoles (triadimenol and myclobutanil are examples). Field tests were conducted with old (Vitavax-PCNB + Allegiance FL), current (Baytan 30 + Argent 30 + Allegiance FL), and future seed treatment combinations (Dynasty [Maxim 4FS + azoxystrobin {Protegé} + Apron XL]). Seed from Paymaster (PM) 2167 RR, PM 2280 BG/RR, and FiberMax (FM) 960 BR were treated with the fungicide combinations or left untreated, and planted in small plots at four sites. Regardless of disease pressure, cultivar germination differences or environmental differences, the three fungicide combinations gave similar and better stands than the seed without fungicide protection. Newer fungicides do not necessarily improve cotton stands over older fungicides. In terms of protection against *Rhizoctonia* and *Pythium*, there are many combinations of products that are effective. Another strategy is to "enhance" the activity of existing fungicides, by inhibiting the ability of cells to transport the fungicides out of the fungal cells. Six "inhibitors" were tested with Baytan 30, Apron XL, and Dynasty, against the pathogens *Rhizoctonia solani*, *Pythium ultimum*, and *Thielaviopsis basicola*. Several of the inhibitors did improve disease protection, particularly with the fungicide Baytan 30.

Introduction

Fungicide seed treatments for cotton must protect seed against a wide array of fungi and also against the Oomycetes. However, the most competitive fungicide treatments, must also have superb protectant and systemic activity against *Rhizoctonia solani* and *Pythium* spp. Fungicides have changed over time from inorganic mercury compounds, to broadly acting nonsystemic organic fungicides like thiram, captan, and chloroneb to broadly acting systemic organic fungicides like carboxin and more recently, triadimenol. Triadimenol is from a group of compounds called the triazoles, which control fungi across three major groups (Ascomycetes, Basidiomycetes, and Deuteromycetes) (Ware, 1994). These systemic fungicides have both protectant and curative properties. Triadimenol captured a great deal of the cotton seed market, in part because of it's superb *Rhizoctonia* activity. This fungicide also provides partial control of the black root rot fungus, *Thielaviopsis basicola*. *Pythium* spp. are also important causal agents of seedling disease. Mefenoxam and its earlier form metalaxyl, captured almost the entire cotton seed market for *Pythium* control. Currently, fungicides applied to cotton seed include: thiram, captan, triadimenol (Baytan 30), myclobutanil (Nuflow M, a triazole), carboxin (Vitavax, an oxathiins), PCNB (benzene derivatives), TCMTB (Argent 30), and fludioxonil (Maxim, pyrrole). Combinations of these products change over time, for example, at one time triadimenol was commonly sold with thiram as a ready to use combination, whereas in recent years, triadimenol is combined with TCMTB. Another combination that had interesting synergy was myclobutanil plus fludioxonil. This combination resulted in better *Rhizoctonia* and *T. basicola* management, then either product alone (T. Wheeler, unpublished). The newest fungicide combination on the market is called Dynasty, and combines fludioxonil, mefenoxam, and azoxystrobin.

Azoxystrobin is part of the strobilurin class of fungicides. Strobilurins are produced naturally by a number of Basidiomycetes and *Bolinea lutea* (an Ascomycete). This natural product was found to have fungicidal activity as a result of their ability to inhibit mitochondrial respiration in fungi. They interfere with the function of cytochrome bc1 complex located in the inner mitochondrial membrane of fungi by binding at a specific site on cytochrome b (Clough and Godfrey, 1998). What was so exciting about this class of chemistry, was that they acted on a unique site, meaning that resistance to other fungicides should not affect the strobilurins (Clough and Godfrey, 1998). Recently, a correlation was found between resistance to azoxystrobin and myclobutanil, which is a sterol C_{14}-demethlation inhibitor, similar to triadimenol (Wong and Wilcox, 2002). At the time that strobilurins were discovered, resistance to the triazoles was becoming more prevalent, so finding a new class of fungicides with a different mechanism of control was important. After experimentation with new, artificially produced strobilurins, azoxystrobin was discovered (Clough and Godfrey, 1998). It has activity against Ascomycetes, Basidiomyces, Dueteromycetes, and Oomycetes. It is systemic and active at low rates, and has demonstrated preventative, curative, eradicant, and antisporulant activity (Clough and Godfrey, 1998). Both oral and dermal toxicities are quite safe compared with many other pesticides. Resistance has developed to strobilurins rapidly (Avila-Adame and Koller, 2003; Ishii et al., 2001; Vincelli and Dixon, 2002), though there are no published reports of resistance to *Rhizoctonia*. Once resistance develops to any strobilurin, then all the fungicides in this class become less reliable, since they all share the same mode of action (Godfrey and Clough, 1998). Azoxystrobin also suppresses *Pythium*, though not as effectively as mefenoxam (T. Wheeler, unpublished). Unfortunately, the two fungicides when used together do not act synergistically to control *Pythium*, so it is not possible to reduce the dosage of either fungicide when used together (T. Wheeler, unpublished). However, the use of azoxystrobin and mefenoxam together, as well as fludioxonil, may slow down any development of resistance from cotton seed and seedling pathogens.

Initially, captan or thiram were the primary fungicides, then metalaxyl for *Pythium* control became popular, and carboxin-PCNB for *Rhizoctonia* control. In recent years the metalaxyl/carboxin/PCNB combination has given way to triadimenol/metalaxyl/TCMTB. Combinations with azoxystrobin, such as Dynasty, may be the next serious competitor for cotton seed protection. Dynasty has recently received its cotton label. A series of fungicide tests were conducted in 2004 on several cultivars in the northern High Plains of Texas. The objective was to compare if newer fungicides were more effective than older fungicides.

The current chemistries available for cotton seedling disease protection are effective. However, in the future, rather than development of new fungicide chemistries, there may be development of new products that improve existing chemistries. Fungi, as well as other organisms are able to pump foreign materials (pesticides for example) out of their cells by a transporter. The company Entercel, LTD (http://www.entercel.com) has discovered an enzyme which provides the energy to drive this transporter pump. They also discovered a number of small molecule inhibitors of this enzyme. These inhibitors are capable of reducing transport out of the cell of foreign materials. This means that pesticides may stay in the cells longer, or at higher concentrations.

Materials and Methods

Field tests with cultivars/seed treatments

Seed without fungicides was obtained for FiberMax (FM) 960 BR, Paymaster (PM) 2167 RR, and PM 2280 BG/RR. Fungicide seed treatments were applied to each of these cultivars. The treatments were none, Vitavax-PCNB + Allegiance FL (6 + 0.75 oz/100 lb seed), Baytan 30 + Argent 30 + Allegiance FL (0.50 + 2.0 + 0.75 oz/100 lb seed), and Dynasty CST 125 FS + Systhane 40 WSP (3.1 + 0.84 oz/100 lb seed). Fungicides were applied using a Hege 11 Seed Treater (Hege Maschinen GmbH, Waldenburg Germany). Small plot test sites were selected in five counties (Carson, Deaf Smith, Gray, Moore, and Randall). Plots were 35.5 ft long, two rows wide, and were planted at a rate of five seed per ft row. Cultivars were blocked separately at each site, and treatment was randomized within each cultivar. Stand counts were taken weekly, though only the final counts, taken at 14 and 28 days after planting are presented. Stand counts were analyzed for affect of site, cultivar, seed treatment, cultivar by site, and cultivar by seed treatment.

Growth chamber tests with Entercel inhibitors

Six inhibitors were tested, two in each run and a “no inhibitor” treatment, in combinations with either Baytan 30 at 0, 0.25, and 0.5 oz/100 lb seed, Apron XL at 0, 0.16, and 0.32 oz/100 lb seed, and Dynasty CST 125 FS at 0, 1.95, and 3.9 oz/100 lb seed. The inhibitors were dissolved initially in DMSO (137.5 mg of inhibitor/ml of DMSO) and 0.08 ml of this solution (containing 11 mg of product) was added to the fungicide seed treatment solution, in sufficient water (8 ml) to treat 1 lb of seed. The fungicide treatments without inhibitors were also treated with 0.08 ml of DMSO/lb seed. Soil was autoclaved twice at 240 F for 2 hrs, and then inoculated with no pathogen, *Rhizoctonia solani*, *Pythium ultimum*, or *T. basicola*. *Rhizoctonia solani* was grown on potato dextrose agar (39 g/L) for 4-5 days prior to inoculation. Soil was placed in a pot (Stuewe and Sons, Corvallis, OR) holding 100 cm^3 soil. The pot was filled about 2/3 full and a 9 mm diam. plug of *R. solani* was transferred to the soil. Soil was then added and a seed planted 1” from the surface. *Pythium ultimum* was inoculated similarly to *R. solani*, except the fungus was grown on corn meal agar (17 g/L). *Thielaviopsis basicola* was grown on carrot agar (15 g agar, 50 ml carrot juice, 1 g CaCO3/L) for at least 6 wks prior to the experiment. Spores were washed off the plates and calibrated to a concentration of 20,000 – 25,000/ml. Two mls were added to 100 cm^3 soil and mixed. The soil was added to the pots and planted with the appropriate seed treatment. The 84 treatment combinations (4 pathogens [none, *R. solani*, *P. ultimum*, *T. basicola*], three fungicides [Baytan 30, Apron XL, Dynasty], 3 rates/fungicide [0, ½ rate, full rate], and three inhibitors [none, x, y]) were arranged in a randomized complete block design with nine replications, and kept in a growth chamber set at 67 F for approximately 25 days. At that time each pot was rated for emergence (0 versus 1), hypocotyl damage (0 = no damage, 1 = superficial damage, 2 = sunken lesion, 3 = lesion killing the seedling), root necrosis (0 to 100 % scale), and root rot (0 = none, 1 = rot had stopped the taproot). The experiment was run three times, in order to test all six inhibitors (two tested per run).

Results

Field tests with cultivars/seed treatments

All three seed treatment combinations performed significantly better than cotton seed with no fungicide treatment (Table 1). Carson county data was dropped from the analysis, because of damage to one of the three cultivars, which was unrelated to the fungicide treatments. The remaining four sites represented a range of conditions, with the best stand overall found in Moore (average of 118 plants/plot) and Deaf Smith (average of 102 plants/plot) counties. Randall county had relatively poor stands, averaging 56 plants/plot, and Gray county had very poor stands, averaging 29 plants/plot. There were no interactions between seed treatments and sites or seed treatments and cultivars. There was an interaction between site and cultivar. PM 2280 BG/RR had similar plant stands as PM 2167 RR and FM 960 BR at Deaf Smith, Gray, and Moore counties. However, at Randall county, stands were poorer for PM 2280 BG/RR (average of 42 plants/plot) compared with FM 960 BR (62 plants/plot) and PM 2167 RR (64 plants/plot). Regardless of disease pressure, cultivar germination differences or environmental differences, the three seed treatment combinations gave similar and better stands than the seed without fungicide protection. Both Baytan 30 and Systhane 40 WSP also provide for some protection against *T. basicola* which causes black root rot. In these tests, black root rot was not a factor. Vitavax-PCNB + Allegiance FL have been a seed treatment combination for decades, though in the mid 1990's through the current year, it has been at least partially replaced with combinations using Baytan 30 or Nuflow M (same a.i. as Systhane 40 WSP). The newest competitor is Dynasty, which includes mefenoxam, fludioxonil, and azoxystrobin. As the data suggests, newer combinations do not necessarily improve cotton stands over older materials. The changes have mainly been in terms of environmental issues like the amount of active ingredient being applied to seed. In terms of protection against *Rhizoctonia* and *Pythium*, there are many combinations of products that can be effective.

Growth chamber tests with Entercel inhibitors

Two of the six inhibitors failed to stay in solution at all when added back to water, and the other four where not completely in solution once they were added to water (from DMSO). So, the concentration of inhibitor that actually reached the seed is unknown, though less than was desired. Also, the distribution of inhibitor on the seed was probably poor. As a result, this data is preliminary and not proof of the effect or lack of effect of these inhibitors. In most of the fungicide/pathogen combinations, the inhibitors did not significantly ($P = 0.05$) improve seed protection, though that may have been due to application problems. A few combinations were interesting, and merit further testing, with many more replications or a better formulation of the inhibitors.

When inhibitor 14 was added to Baytan 30 (rates of 0, 0.25, and 0.50 oz/100 lb seed), hypocotyl lesions caused by *R. solani* were less severe (Fig. 1A), particularly at the ½ rate of Baytan 30. The same combination of inhibitor and fungicide also led to a reduction in root necrosis caused by *T. basicola* (Fig. 1B). The combination of inhibitor 2 and Baytan 30 led to an increase in plant stand (Fig. 2) in the presence of *R. solani*.

Table 1. The affect of seed treatment, averaged across three cultivars and four sites, on stand.

Seed Treatment	Oz of product per 100 lbs seed	Stand at 14 days	Stand at 28 days
None	0	66 b	68 b
Vitavax-PCNB + Allegiance FL	6 + 0.75	77 a	79 a
Baytan 30 + Argent 30 + Allegiance FL	0.50 + 2 + 0.75	76 a	78 a
Dynasty + Systhane	3.1 + 0.84	78 a	79 a
Minimum significant difference		4	4

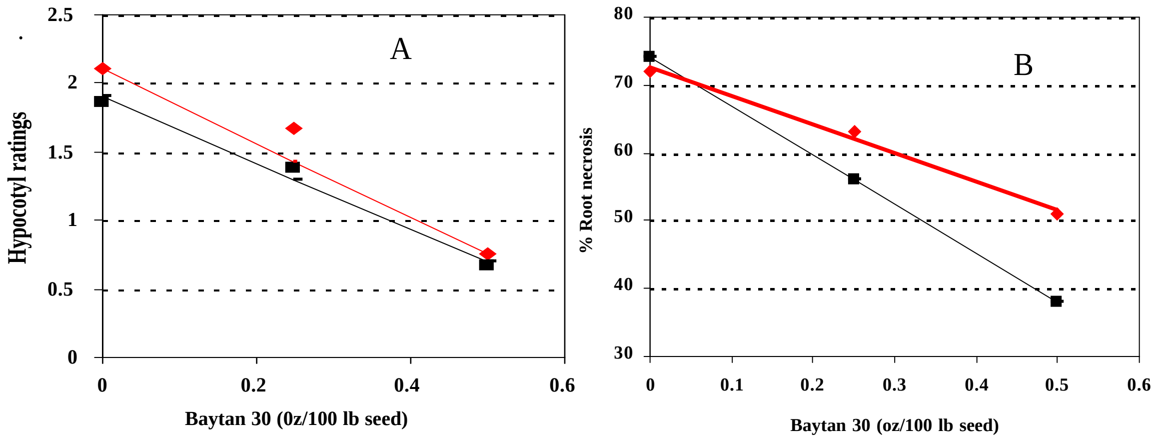

Figure 1. Affect of Baytan 30 on A) hypocotyl injury (0=healthy, 1=superficial lesion, 2= sunken lesion, 3=dying plant) caused by *Rhizoctonia solani*, and B) root necrosis caused by *Thielaviopsis basicola*, when using the fungicide alone and with inhibitor 14

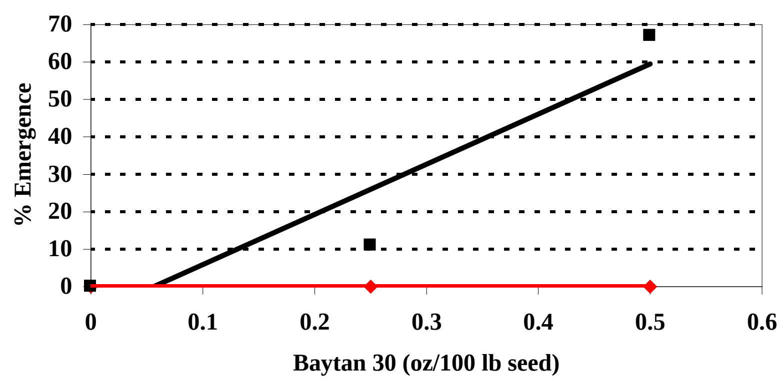

Figure 2. Affect of Baytan 30 on plant emergence in the presence of *Rhizoctonia solani* when using the fungicide alone or with inhibitor 2 .

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