Effects of Bacillus cereus on Cotton Growth and Yield and the Development of PGR-IV Plus

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RESEARCH PROBLEM

The use of plant growth regulators (PGRs) in cotton to control growth and enhance yield is a widely used practice. The most commonly used PGR to control vegetative growth is Pix® (mepiquat chloride). Recently, the bacterium Bacillus cereus was added to Pix to form Pix Plus, a new and improved version of Pix. Research results have shown that Pix Plus has a small yield advantage over Pix while still costing the same. In subsequent research, Bacillus cereus has also been added to PGR IV.

BACKGROUND INFORMATION

There are numerous reports about the use and advantages of mepiquat chloride for controlling plant vegetative growth (e.g. Cothren, 1995). Generally, research has shown that mepiquat chloride controls growth (height) 100% of the time, and results in earlier maturity about 50% of the time and a yield increase about 25% of the time (Oosterhuis et al., 1991).

Pix Plus, formerly MepPlus, is a new PGR first tested in 1994 and registered in 1997 by Microflo (Memphis, TN) and now marketed by BASF (Research Triangle Park, NC). It consists of mepiquat chloride (MC) (4.2%), the bacteria Bacillus cereus (0.05%), and inert ingredients (95.75%). Bacillus cereus was reported to have tolerance exemption on all crops. Recent studies (Oosterhuis et al., 1998; Parvin and Atkins, 1997) have indicted that Pix Plus had a similar effect on plant height control as MC. In addition, Pix Plus has been reported to increased photosynthesis, leaf starch content, dry matter partitioning (Zhao and Oosterhuis, 2000), and lint yield (Parvin and Atkins, 1997) of field-grown cotton compared with the untreated control and MC treated plants. Subsequent research showed that a combination of BC plus a hormone-based formulation enhanced growth characteristics that influenced final yield. Growth chamber and field evaluations initiated in 1998 evaluated several formulations in either single or split

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applications. Favorable results were obtained and additional field trials began in 1999 refining proper rates and timing. Based on initial findings, extensive field trials under the direction of certified crop consultants and university researchers began in 2000. The hypothesis of this study is that the use of a combination of *Bacillus cereus* and mepiquat chloride will lead to increased efficacy of other hormone-based products and improved plant growth and higher yields. The following is a summary of the results of these studies. The objectives were to evaluate the benefits of applying *Bacillus cereus* with: (a) mepiquat chloride on yield and fiber quality; (b) a hormone-based PGR (i.e., PGR-IV Plus) on yield and fiber quality; and (c) a pesticide on yield and fiber quality. In addition, we wanted to determine the optimum rate and timing of BC plus the plant growth regulator PGR-IV Plus.

**RESEARCH DESCRIPTION**

In 1998, formulation studies of *Bacillus cereus* and PGR-IV were conducted at 17 field locations across the Cotton Belt, in addition to a growth room study in Fayetteville, Arkansas. The optimum rate and timing of PGR-IV Plus was investigated in 1999 at 26 locations. In 2000, a single timing was evaluated at 33 field locations. In 2001 the formulation was optimized in a growth room study, and in 2002 field evaluations were continued. In all field studies, cotton (*Gossypium hirsutum* L.) was planted using current state extension recommendations for optimum cotton yield. Treatments included: (1) an untreated control, (2) Pix Plus as needed for height control, and (3) PGR-IV Plus (i.e., PGR-IV with *Bacillus cereus*). The spray applications were made using aerial application or a backpack sprayer calibrated to deliver 10 gal/acre in consultant field trials and University small plot studies, respectively.

**RESULTS AND DISCUSSION**

*Bacillus cereus* and PGR-IV Plus

*Bacillus cereus* (BC) was originally identified as having PGR effects, i.e., improved partitioning and translocation of carbohydrates to fruits (Zhao and Oosterhuis, 2001). BC was subsequently mixed with mepiquat chloride (MC) at rates of 1x to 4x to improve yield while still providing vegetative control. The 2x rate was generally the best, but at some locations yield response was best at the higher rates. Subsequently, combinations of BC + PGR-IV were tested in 1998-2002 on cotton that had been, or would be, treated with Pix Plus. From these studies, PGR-IV Plus was formulated. The overall results support the hypothesis of additional yield from improved partitioning of carbohydrates to the fruit.

1998 Formulation Study

Five formulations of varying concentrations of PGR-IV with *Bacillus cereus* (PGR-IV Plus) were evaluated at 17 sites in either single or split applications. All formu-
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 Twenty sites across the Cotton Belt were used to evaluate the selected formulation from the 1998 trials. The sites included six from Mississippi, six from South Carolina, six from Texas, four from Louisiana, one from Arkansas, one from Missouri, and one from California. Single and split applications of PGR-IV Plus were made to cotton that had been or would be treated with Pix Plus. Timings included pinhead square (PHS), early bloom (EB), and early bloom + 3 weeks (EB + 3). Split applications of the single applications were also made. Single applications at PHS and EB had the highest yield as compared to the Pix Plus treatment (data not shown). Average yield increase from Pix Plus across 20 sites ranged from 30 to 62 lb lint/acre. Bloom applications were the most consistent across all twenty locations with the PGR-IV Plus treatment ranking in the top three treatments for each experiment at 75% of the locations.

2000 PGR-IV Plus Dry Formulation Evaluation

To insure product efficacy and extend shelf life, a dry formulation containing the same active ingredients was developed. Thirty-three sites under the supervision of independent cotton consultants evaluated this product. Twenty-five consultants in the Tri-State Delta and eight consultants in Texas made single applications of PGR-IV Plus at early bloom at 1 oz/acre or 1.5 oz/acre to cotton that had been or would be treated with Pix Plus. Average yields for the 1.0 oz/acre rate were 1062 lb lint/acre versus 982 lb lint/acre. The average yields for the 1.5 oz/acre rate were 1048 lb lint/acre versus 992 lb lint/acre. The dry formulation of PGR-IV Plus proved to be highly efficacious with regards to both yield and consistency of positive plant yield response. Cotton treated with Pix Plus and subsequently treated with PGR-IV Plus had increased lint yields of 9.2% at the 1.0 oz/acre rate, and 5.9% at the 1.5 oz/acre rate. Consistency was greatly increased with 93.9% of locations exhibiting yield increases with the 1.0 oz/acre rate.

2001 Growth Chamber and Rate Response

Previous growth chamber and rate response studies were evaluated and further testing was initiated. Both growth chamber and field trials have indicated that the Bacillus cereus component increases carbohydrate partitioning to the fruiting structures, i.e., squares and bolls (Zhao and Oosterhuis, 2000). Field rate response trials conducted in 2001 again proved that applications of PGR-IV Plus at PHS, EB or EB + 3 weeks have a positive yield effect on cotton that has been treated with Pix Plus or
mepiquat chloride. Percent yield increases by application timing of PGR-IV Plus were as follows: pinhead square, 6.0%; early bloom, 7.2%; and early bloom + 3 weeks, 8.7% versus the more traditional mepiquat-chloride applications.

2002 Field Studies - PGR-IV Plus Alone and in Combination with a Pesticide

Field studies in 2002 showed that PGR-IV Plus out-yielded the control by 70 lb lint/acre (+6.1%) for the 1.0 oz/acre rate and by 83 lb lint/acre (+8.2%) for the 1.5 oz/acre rate (Table 1). These results are in agreement with those from the previous three years. Field studies in 2002 showed that Bacillus cereus combined with acephate out-yielded the control by 41 lb lint/acre (+3.4%) at the 0.5 oz/acre rate and by 45 lb lint/acre (+3.7%) at the 0.75 oz/acre rate (Table 2). These results show the positive results from combining Bacillus cereus with a pesticide, presumably through improved translocation. These positive yield responses from combining Bacillus cereus with mepiquat chloride, PGR-IV, and pesticides will necessitate further field testing with additional emphasis on improving our understanding of the mechanism of these combinations.

Increased Yields from PGR-IV Plus Over the Area Standard

There were positive yield increases each year from PGR-IV Plus applications over the area standard (i.e. whatever rate of mepiquat chloride was used as a standard treatment to control vegetative growth):

- 1999 University and Consultants: average yield increase 46 lb lint/acre.
- 2000 MS Delta Consultants: average yield increase 70 lb lint/acre.
- 2000 TX Consultants: average yield increase 67 lb lint/acre.
- 2002 MS Delta University and Consultants: average yield increase 76 lb lint/acre.

In general, over all five years and 91 locations, an average 69.3 lb lint/acre yield increase was achieved.

Theory Behind the Bacillus cereus Hormone Combination

The theory behind the Bacillus cereus hormone combination is that maximum plant uptake rates of nutrients and carbohydrate occur during the squaring and early flowering period (Oosterhuis and Hickey, 2003). Therefore enhancement of partitioning and translocation during this period should benefit yield, particularly under stressful conditions. Earlier studies by Zhao and Oosterhuis (2000) showed that the new plant growth regulator Pix Plus, consisting of Bacillus cereus and mepiquat chloride, improved translocation of photoassimilates from leaves to fruits and partitioning of dry matter among plant tissues, resulting in improved yields (Fig. 1).
PRACTICAL APPLICATION

An important objective of Plant Growth Regulators (PGRs) is to balance vegetative and reproductive growth as well as improve yields and fiber quality. Various PGRs have been used to achieve these objectives with varying successes. Recently, *Bacillus cereus* was added to mepiquat chloride for additional yield advantage. Addition of *Bacillus cereus* to mepiquat chloride resulted in improved partitioning and carbohydrate translocation to the fruit. Subsequent combinations of *Bacillus cereus* and the hormone PGR-IV (forming PGR-IV Plus) resulted in additional yield increases averaging 69.3 lb lint/acre in field and growth room studies over a four year period at 71 locations across the Cotton Belt (that had received standard Pix Plus applications). Optimum timing of PGR-IV Plus application was achieved with early- to mid-bloom applications. These studies have shown activity of *Bacillus cereus* with growth retardants (mepiquat chloride), growth enhancers (PGR-IV), and insecticides (acephate). Future studies will continue to evaluate *Bacillus cereus* in PGR-IV Plus for growth and yield advantages as well as in combinations with insecticides.

LITERATURE CITED


Table 1. Effect of PGR-IV Plus on yield averaged over 20 locations in 2002.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1.0 oz/acre</th>
<th>1.5 oz/acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1127</td>
<td>1009</td>
</tr>
<tr>
<td>PGR-IV Plus</td>
<td>1196</td>
<td>1092</td>
</tr>
<tr>
<td>Difference</td>
<td>70 (+6.1%)</td>
<td>83 (8.2%)</td>
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</tbody>
</table>
Table 2. Effect of *Bacillus cereus* with Acephate (AcePlus) on yield averaged over 18 locations in 2002.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5 oz/acre</th>
<th>0.75 oz/acre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthene</td>
<td>1204</td>
<td>1216</td>
</tr>
<tr>
<td>AcePlus</td>
<td>1245</td>
<td>1261</td>
</tr>
<tr>
<td>Difference</td>
<td>41 (+3.4%)</td>
<td>45 (+3.7%)</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of mepiquat chloride and Pix Plus on dry matter accumulation (left) and partitioning (right) of field-grown cotton (From Zhao and Oosterhuis, 2000).