PHYSIOLOGICAL ASPECTS OF *BACILLUS CEREUS* ON COTTON

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Abstract

Foliar-application of *Bacillus cereus* caused some numerical, but not significant, differences in photosynthesis, stomatal conductance and transpiration. Dry matter accumulation was significantly influenced with reproductive organs being favored. Increases in various metabolites were also recorded, noticeably in myo-inositol. The bacterium seems to effectively influencing plant metabolism and growth resulting in improved carbohydrate partitioning to the reproductive structures. The mode of action appears to be via metabolites produced by the bacterium influencing the biochemistry of the leaf, particularly carbohydrate relationships, culminating in improved efficiency of translocation to the major sinks. The end product is improved dry matter partitioning to the fruit.

Introduction

The new plant growth regulator MepPlus contains Mepiquat Chloride and the bacteria *Bacillus cereus* (BC). Recent studies have indicated that applying MepPlus have similar effect on plant height control as applying Mepiquat chloride which has been widely used to control cotton (*Gossypium hirsutum* L.) plant vegetative growth and to improve lint yield. Additionally, MepPlus can more efficiently improve leaf photosynthesis, dry matter partitioning (Wells, 1997; Oosterhuis et al., 1998), and lint yield (Parvin and Atkins, 1997; Wells, 1997). However, the physiological mechanisms of BC functions are not clear. Studies were conducted in a growth chamber in 1998 to determine physiological responses of cotton plant growth to foliar application of BC.

Methods and Materials

Three experiments were conducted in a growth chamber at the University of Arkansas, Fayetteville. The chamber was programmed for a 12 h photoperiod, with day/night temperatures of 30/25 °C and relative humidities of 60/80%. Cotton (cv. Suregrow 125) seeds were planted in 2-L pots filled with sand. After emergence, seedlings were thinned to one plant per pot and watered with half-strength modified Hoagland's nutrient solution to maintain a sufficient nutrient and water supply.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:566-568 (1999)
National Cotton Council, Memphis TN

In experiments 1 and 2, five treatments were included: (1) a control without BC, (2) foliar application of BC-2 at 3 weeks after planting, (3) foliar application of BC-4 at 3 weeks after planting, (4) foliar application of BC-2 at 5 weeks after planting, and (5) foliar application of BC-4 at 5 weeks after planting. Experiment 3 consisted of (1) a control without BC, (2) foliar application of BC-2 at 4 weeks after planting, (3) foliar application of BC-4 at 4 weeks after planting, and (4) foliar application of BC-8 at 4 weeks after planting . A rate equivalent to 8 (Exp. 1 and 2) or 4 (Exp. 3) once BC per acre in 10 gallons of water was sprayed using a calibrated backpack sprayer.

Stomatal conductance, photosynthetic rate, transpiration, and dark respiration of uppermost expanded main-stem leaves were measured 5 and 10 days after application of BC. Two weeks after application, concentrations of leaf ATP, and leaf and petiole soluble sugars (sucrose, fructose, glucose and myo-inositol) were determined. Thereafter, plants were harvested to determine plant height, the numbers of main-stem nodes, fruiting branches, squares, leaf area, and dry weights of different tissues. Additionally, a culturing bacterium technique was used to investigate changes in the number of bacteria on the leaf surface at different times after foliar application of BC(Fig. 1).

Experiments were arranged in a complete randomized design with 6 replications. Data were analyzed statistically with an ANOVA and LSD tests.

Results and Discussion

Leaf Gas Exchange and Transpiration

Application of BC numerically improved leaf net photosynthetic rate (Fig. 2). Of the three BC treatments, BC-2 treatment showed the greatest photosynthetic rate. Effects of BC on leaf stomatal conductance and transpiration rate were the same as on photosynthesis. However, these differences were not statistically significant ($P > 0.05$). BC application did not affect leaf dark respiration (data not shown).

Leaf ATP Concentration

ATP is directly involved in plant carbon metabolism. Leaf ATP content may be related to leaf photosynthesis, respiration, and carbohydrate translocation. Therefore, leaf ATP concentration was measured 7 days after BC application in Experiment 2 to investigate if differences existed in ATP content between treatments. Results indicated that foliar application of BC to cotton did not affect leaf ATP concentration (Table 1).

Soluble Sugars

The sucrose, glucose, fructose and myo-inositol contents of both the upper most expanded main-stem leaves and their petioles were determined using Hendrix's methods (1993) (for leaves) or HPLC (petioles) 2 weeks after applying BC at 3 and 5 weeks after planting. Results indicated that BC

application did not affect leaf sugar concentrations of 3-week-old plants although some numerical changes in the sugar concentrations were observed (Table 2). However, when plants were 5 weeks old, applying BC-2 significantly increased leaf sucrose, glucose, and total sugar concentrations compared with the untreated control. The BC-4 treatment also had higher total sugar concentration than the control ($P < 0.05$).

The BC application did not affect petiole sucrose and fructose concentrations, but increased petiole myo-inositol concentration (Table 3), especially in treatments of applying BC 5 weeks after planting. It has been reported that myo-inositol content is associated with both carbohydrate translocation and plant tolerance to stress environments (Guo, 1993). Therefore, higher petiole myo-inositol in BC-treated cotton plants might benefit carbohydrate translocation from leaves to fruits.

BC Bacteria Culture and Plate Count

To investigate the fate of BC bacteria on the leaf tissue, the bacteria on a given leaf surface were collected, cultured in petri dishes with agar medium at different times after foliar application of BC, and the number of the bacteria colonies in the petri dishes were counted under sterilized conditions. Results showed that the number of BC on the leaf surface rapidly declined with increasing the period of time after spraying BC (Fig. 3). Most of BC bacteria were disappeared after 8 hours of spraying BC.

Plant Growth and Dry Matter Accumulation

Foliar application of BC did not affect the numbers of main-stem nodes and sympodial fruiting branches and leaf area per plant, but there were some trends to decrease plant height and to increase the number of squares compared with the untreated control (Table 4). The BC-2 treatment had significantly higher square dry weight and total dry weight, and the BC-8 treatment had lower stem dry weight than the control (Table 5). There were no differences between treatments in other tissue dry weights.

Mode of Action

The mode of action is obviously multifaceted due to the diversity of compounds produced and released by *Bacillus cereus*. These could include carbohydrates, proteins, amino acids, vitamins, nucleic acids, purines, pyrimidines, and CO_2. *Bacillus cereus* is also known to produce phosphatidyl lipid enzymes, which are bound by polyols, in particular myo-inositol. The mode of action appears to be via metabolites produced by the bacterium influencing the biochemistry of the leaf, particularly carbohydrate relationships, culminating in improved efficiency of translocation to the major sinks. Additional research on the mode of action of BC is needed.

Future Research

Field and growth room research will continue to address the mode of action and optimum field use of this new compound on cotton growth and development. Research will specifically investigate what metabolites are being released by the bacterium, the changes in plant biochemistry that occur, and the effects on dry matter partitioning.

References

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Table 1. Effect of applying two rates of BC five weeks after planting on leaf ATP concentration of growth chamber-grown cotton plants. Each value presents the mean of 3 replications.

Treatment	ATP concentration	
	mg ATP m^{-2}	mg ATP kg^{-1}DW
Control	0.361	6.70
BC-2	0.371	6.92
BC-4	0.354	6.91
LSD(0.05)	ns[†]	ns

[†] ns = not significant ($P > 0.05$)

Table 2. Effect of BC formulations on leaf nonstructural carbohydrate concentrations of growth chamber-grown cotton. Each value is the mean of 3 samples from six plants.

Treatment	Sucrose	Glucose	Fructose	Total
	---------- g kg^{-1} DW----------			
	(BC application 3 weeks after planting[‡])			
Control	11.37	14.72	2.63	28.73
BC-2	8.68	16.13	5.51	30.51
BC-4	12.09	18.02	5.94	36.05
	(BC application 5 weeks after planting[‡])			
Control	9.22	7.74	5.19	22.15
BC-2	16.83*	14.69*	6.55	38.07*
BC-4	7.98	9.99	6.86	24.84*

[‡] Sampling at 10 a.m. of 2 weeks after spraying for sugar measurements.
* Presents the differences are significant from the control($P \leq 0.05$).

Table 3. Effect of BC formulations on petiole sugar concentrations of growth chamber-grown cotton. Each values point is the mean of 3 samples from six plants.

Treatment	Sucrose	Fructose	Myo-inositol
	---------- g kg^{-1} DW----------		
	(BC application 3 weeks after planting‡)		
Control	16.29	13.20	0.51
BC-2	11.79	9.77	0.64
BC-4	17.30	14.14	0.61
	(BC application 5 weeks after planting‡)		
Control	33.6	14.2	0.72
BC-2	31.1	14.5	1.74 *
BC-4	23.2	7.0	1.41 *

‡ Sampling at 10 a.m. of 2 weeks after spraying for sugar measurements.
* presents the differences are significant from the control($P \leq 0.05$).

Table 4. Effect of BC rates on plant height (PH), main-stem nodes (MSN), the number of fruiting branches (FB) and squares, and leaf area (LA) of growth chamber-grown cotton.†

Treatment	PH	MSN	FB	Squares	LA
	cm	----------no. plant^{-1}----------			cm^2 plant^{-1}
	Spray at 3 weeks after planting				
Control	43.8	11.7	6.7	10.8	1291
BC-2	40.7*	11.8	7.0	12.7*	1357
BC-4	41.1	11.8	7.2	12.8*	1272
BC-8	40.4*	11.7	7.0	11.5	1253

†Measurements were taken 2 weeks after foliar application of BC at 4 weeks after planting. Each data point is mean of 6 plants.
‡Means with * within a column are significant from the control ($P \leq 0.05$).

Table 5. Effect of BC rates on dry matter accumulation of different tissues of growth chamber-grown cotton plants.

Treatment	Dry weight (g plant^{-1})				
	Leaves	Stems	Roots	Squares	Total
Control	6.22	3.79	3.38	0.38	13.76
BC-2	6.36	3.79	3.88	0.43*	14.46*
BC-4	6.01	3.61	3.98	0.41	14.01
BC-8	6.00	3.44*	3.19	0.39	13.02

† Measurements were taken 2 weeks after foliar application of BC at 4 weeks after planting. Each data point presents the mean of 6 plants.
‡ Means with * within a column are significant from the control ($P \leq 0.05$).

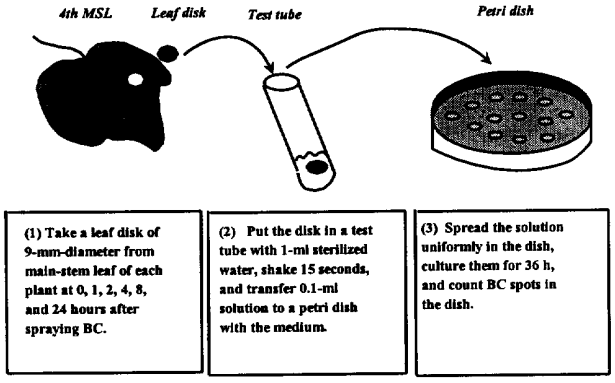

Figure 1. Procedures of culturing and counting BC using petri dish cultural techniques.

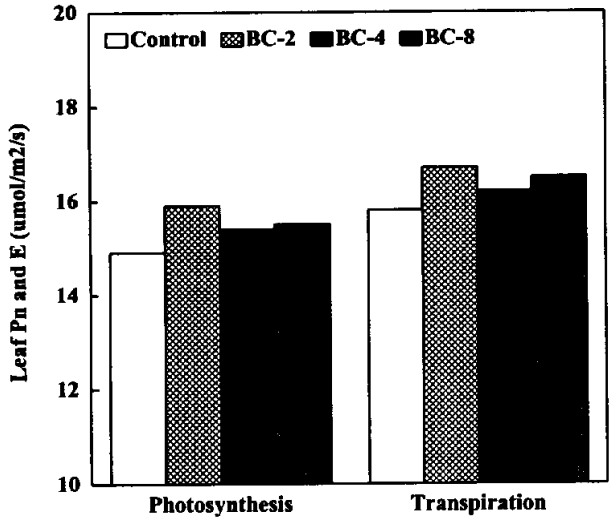

Figure 2. Effect of BC application on leaf net photosynthetic and transpiration rates. Data are means of measurement 5 and 10 days after spraying BC.

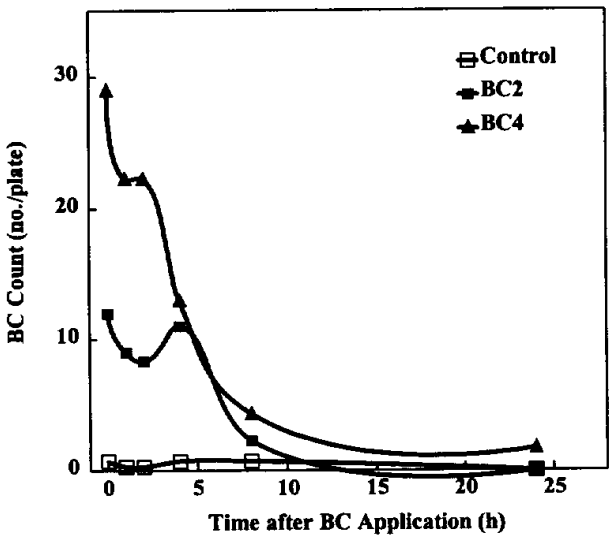

Figure 3. Change in the number of BC colones growing on the leaf surface with time after spraying BC.