



Independent Trial

USA Cotton Trials 2013 - 2018

| | |
|--------------------------|------------------------|
| Memphis Tennessee, USA | 2013 - 2018 |
| Location | Year |
| Agricenter International | American Upland Cotton |
| Conducted by | Crop |
| Small plot replicated | |
| Trial Type | |

Aim

To evaluate the impact on Cotton yields when applying BioAg liquid biostimulants to fertiliser regimes representing Grower Standard Practice (GSP) and GSP less 15% nitrogen.

Introduction

In-field use and demonstrations in Australia's cropping regions had identified positive yield responses when applying BioAg's fermented liquid cultures on a range of crops.

To better evaluate the implications of a biostimulant program over cotton we decided to run independent, small plot replicated trials.

Agricenter International was engaged to perform a range of trials on behalf of BioAg to evaluate the impacts of applying BioAg's liquid biostimulants over a range of crop and fertiliser regimes.

This report details the outcomes of trials performed on Cotton.

Each year, a full biostimulant treatment was applied to a fertiliser application representing GSP, and another representing GSP less 15% Nitrogen. Several additional biostimulant programs were performed over the five years to evaluate the outcomes of alternate biostimulant programs.

Method

Trial Design

The analysis was done using small plot replicated trials. The first treatment was always GSP with additional treatments incorporating BioAg biostimulants at and post planting.

The table below summarises the basic trial information. A strip within the plot was harvested and yields measured.

Table 1: Summary of Trial Parameters

| Year | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 |
|----------------------|---------------------------|------|-------------|------|------|------|
| Crop | American Upland Cotton | | | | | |
| Study Design | Randomised Complete Block | | | | | |
| Plot Size | 8 x 200ft | | 12.6 x 30ft | | | |
| Number of Treatments | 4 | | | | | |
| Plots per Treatment | 4 | | | | | |

Soil Characteristics

Each year new sites were utilised. Soil parameters for each year are provided in the table below:

Table 2: Summary of Soil Parameters

| Year | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 |
|------------------|------|------|------|------|------|------|
| % Sand | 13.6 | | | | 29.6 | 13.6 |
| % Silt | 61.6 | | | | 51.6 | 61.6 |
| % Clay | 24.6 | | | | 18.6 | 24.6 |
| % Organic Matter | 1.3 | | | | 1.8 | 1.4 |
| pH | 6.5 | 7.3 | | | 6.5 | 6.5 |
| CEC | 9.5 | 6.8 | | | 7.8 | 9.5 |
| Fertility | Good | | | | | |
| Drainage | Good | | | | | |

Treatments

Each plot had fertiliser applied prior to planting as well as nitrogen fertiliser side dressed prior to flowering.

Fertiliser rates were based on standard practice in the Tennessee area.

GSP had solid fertiliser applied at planting at the rate of; 22kg of nitrogen, 39kg of phosphorus and 74kg of potassium per hectare; plus 67kg per hectare of nitrogen side dressed.



Plots where the fertiliser applied was GSP less 15% nitrogen (GSP -15%N), had the same amount of fertiliser applied at planting, but a reduced amount of nitrogen fertiliser applied as side dressing, being 57kg of nitrogen per hectare.

Per year the following treatments were performed:

Table 3: Treatments Performed per Year

| Year | T1 | T2a | T2b | T3a | T3b | T3c | T4 |
|------|----|-----|-----|-----|-----|-----|----|
| 2013 | ✓ | ✓ | | ✓ | ✓ | | |
| 2014 | ✓ | | ✓ | | | ✓ | ✓ |
| 2015 | ✓ | | ✓ | | | ✓ | ✓ |
| 2016 | ✓ | | ✓ | | | ✓ | ✓ |
| 2017 | ✓ | | ✓ | | | ✓ | ✓ |
| 2018 | ✓ | | ✓ | | | ✓ | ✓ |

The treatments are detailed in Table 10 in the appendix, including rates of fertiliser and biostimulants, as well as application timing in days post planting and growth stage.

The treatments can be grouped as follows:

- T1 was performed each year and represents GSP.
- T2a and T2b represent GSP with a full biostimulants program with variations in the use of soil applied *Soil & Seed* whereby:
 - T2a was with 5.3 and 4.7L/Ha of *Soil & Seed* being applied to the soil and in furrow respectively; and
 - T2b was with 9.4L/Ha of *Soil & Seed* applied to the soil.
- T3a, T3b and T3c represent GSP -15%N with a full biostimulants program with variations in the use of soil applied *Soil & Seed* whereby:
 - T3a was with 4.7 and 3.5L/Ha of *Soil & Seed* being applied to the soil and in furrow respectively;
 - T3b was with 5.3 and 3.5L/Ha of *Soil & Seed* being applied to the soil and in furrow respectively; and
 - T3c was with 9.4L/Ha of *Soil & Seed* applied to the soil.
- T4 represents GSP with only 9.4L/Ha of soil applied *Soil & Seed*.

Background to Treatment Selection

Treatments T2a and b are based upon treatments typically recommended in Australia.

Common practice in the USA is to apply fertiliser at standard rates upfront and at one other growth stage. Little monitoring is done on nitrogen requirements through leaf or tissue testing.

The use of biostimulants in Australia has indicated improved nutrient use efficiency. To evaluate this treatments T3a, T3b and T3c, where all performed with less nitrogen applied as a side dress.

Treatment T4 represents the use of only *Soil & Seed* (a soil applied biostimulant).

This was performed to confirm that greater yield benefits could be achieved with a full program (T2a and T2b).

Results

Yield results for treatments are provide below in tonnes per hectare:

Table 4: Yield Results per Treatment per Year

| Year | T1 | T2a | T2b | T3a | T3b | T3c | T4 | LSD (P=.05) | Std Dev |
|------|------|------|------|------|------|------|------|-------------|---------|
| 2013 | 3171 | 3684 | | 3400 | 3468 | | | 206.2 | 128.9 |
| 2014 | 2089 | | 2277 | | | 2977 | 2349 | 312.3 | 218.8 |
| 2015 | 2480 | | 3135 | | | 3503 | 2774 | 431.1 | 269.5 |
| 2016 | 4138 | | 4429 | | | 4386 | 4301 | 178.4 | 111.5 |
| 2017 | 4148 | | 4317 | | | 3937 | 3875 | 588.2 | 339.93 |
| 2018 | 3046 | | 3526 | | | 3216 | 3216 | 230.3 | 133.1 |

Each seed cotton result is converted to a benefit in bales per hectare by using a turnout of 42% (percentage of ginned lint from seed cotton) and a bale weight of 226.8kg of lint per bale.

Table 5: Yield Response by Treatment in Bales of Lint per Hectare

| Year | T2a | T2b | T3a | T3b | T3c | T4 |
|------|------|------|------|------|-------|-------|
| 2013 | 0.95 | | 0.42 | 0.55 | | |
| 2014 | | 0.35 | | | 1.64 | 0.48 |
| 2015 | | 1.21 | | | 1.89 | 0.55 |
| 2016 | | 0.54 | | | 0.46 | 0.3 |
| 2017 | | 0.32 | | | -0.39 | -0.50 |
| 2018 | | 0.89 | | | 1.00 | 0.32 |

In addition to these results analysis of turn out and other cotton properties were measured. However, these were not done each year and are therefore not included in this report.

GSP (T1) v GSP with a Full Biostimulant Program (T2a and T2b)

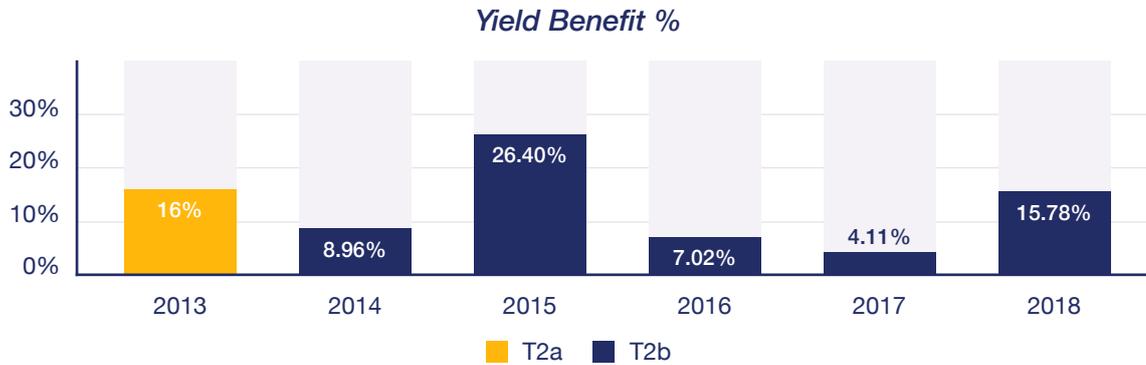
The addition of a full program of biostimulants, *Soil & Seed* as a soil inoculant, and *Balance & Grow* and *Fruit & Balance* as foliar treatments to GSP, provided higher yields than GSP alone in each year of the trials.

With the exception of 2014 and 2017 the yield gain was greater than the Least Significant Difference (LSD).

2014 and 2017 were extremes in growing conditions, with 2014 being a poor year and 2017 a very good year.

While still providing a positive yield response it is probable that:

- In the poor growing year there were high levels of abiotic stresses which could not be offset to any significant degree through the use of biostimulants, whilst
- In the very good growing year abiotic stresses were minimal and therefore the benefit of the biostimulant program was curtailed.

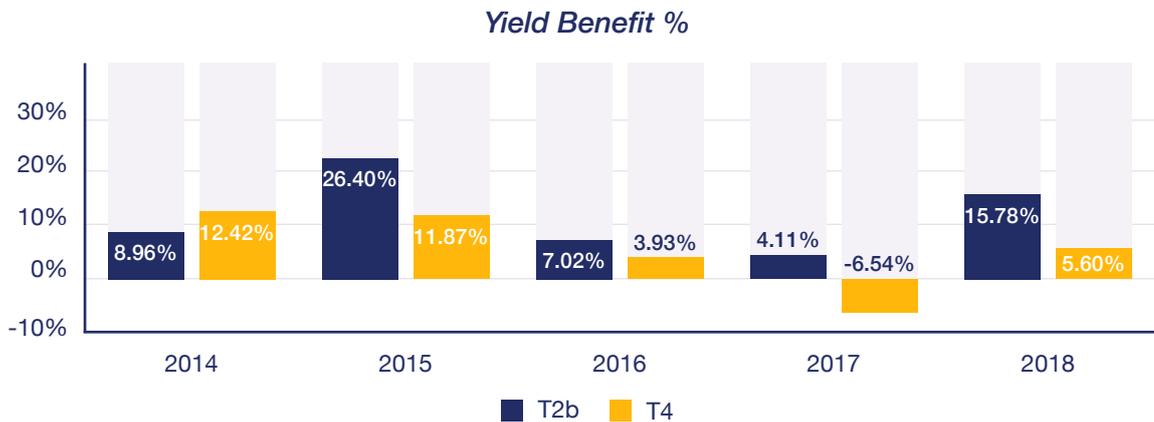


GSP (T1) v GSP with a Full Biostimulant Program (T2b) v GSP with Soil Only Biostimulant (T4)

With the exception of 2017, the 'Soil Only Biostimulant' treatment delivered higher yields than GSP alone.

However, none of the results were greater than the LSD.

With the exception of 2014 the 'Full Biostimulant' program delivered higher yields than a 'Soil Only Biostimulant' program.



GSP (T1) v GSP -15%N Full Biostimulant Program (T3a, T3b and T3c)

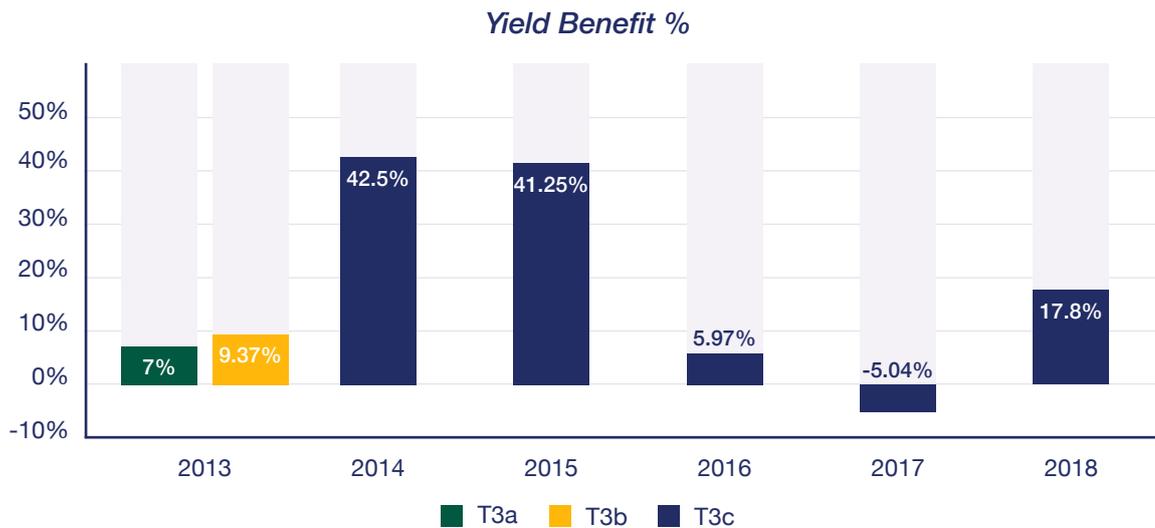
The results achieved from these treatments varied across years.

In the highest yielding years for GSP (2017, 2016 and 2013) the benefit of the treatments over GSP was minimal to very slightly negative. Potentially due to a shortfall in nitrogen.

In the lowest yielding years for GSP (2014, 2015 and 2018) the benefit of the treatments was significant and in 2014 and 2015, delivered the highest yield of any treatments.

The results cannot determine if yield results are driven by nitrogen over use, nitrogen shortfall or improved nitrogen use efficiency from the use of biostimulants. The variability in growing conditions is also an influence, and additional trials are required to better understand the factors at play.

What is evident is that when using a full program of biostimulants, equivalent or higher yields to GSP were achieved even when using 15% less nitrogen in the side dress application.



Economics

The following is evaluated in the context of the Australian market.

Treatment T2a and T2b

Table 6: Economic Benefit of a Full Biostimulant Program on GSP

| Year | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | Average |
|------------------------|-------|-------|-------|-------|-------|-------|---------|
| Cost of Biostimulants# | \$132 | \$128 | | | | | \$114 |
| Benefit Bales/Ha | 0.95 | 0.35 | 1.21 | 0.54 | 0.31 | 0.89 | 0.71 |
| Yield Benefit \$/Ha | \$418 | \$153 | \$534 | \$237 | \$138 | \$392 | \$312 |
| Net Return \$/Ha | \$286 | \$25 | \$406 | \$109 | \$10 | \$264 | \$183 |

#Based on a delivered cost of \$7 per litre; %based on ex farm price of \$440 per Bale.

Clearly there was an economic benefit achieved with the addition of the treatments. While the benefit was low in the poor (2104) and very good (2017) growing seasons, it was still a positive financial return.

Treatment T4

Table 7: Economic Benefit of a Soil Only Biostimulant Program on GSP

| Year | 2014 | 2015 | 2016 | 2017 | 2018 | Average |
|------------------------|-------|-------|-------|--------|-------|---------|
| Cost of Biostimulants# | \$56 | | | | | \$56 |
| Benefit Bales/Ha | 0.48 | 0.55 | 0.30 | -0.50 | 0.32 | 0.23 |
| Yield Benefit \$/Ha | \$212 | \$240 | \$133 | -\$221 | \$139 | \$100 |
| Net Return \$/Ha | \$156 | \$184 | \$77 | -\$227 | \$83 | \$44 |

#Based on a delivered cost of \$7 per litre; %based on ex farm price of \$440 per Bale.

The financial benefit of *Soil & Seed* only is less clear and is significantly eroded by the result of the 2017 trial year. With the exception of 2017 there was solid financial returns on the use of *Soil & Seed* only.

When considering the use of the foliar biostimulants as incremental to *Soil & Seed* it could be reasoned that they delivered incremental or additional yield. Note in 2017 the foliar are only evaluated as delivering a result above GSP.

Table 8: Economic Benefit of Foliars above the Soil Only Biostimulant Program

| Year | 2014 | 2015 | 2016 | 2017 | 2018 | Average |
|------------------------|--------|-------|-------|-------|-------|---------|
| Cost of Biostimulants# | \$72 | | | | | \$72 |
| Benefit Bales/Ha | -0.13 | 0.67 | 0.24 | 0.31 | 0.67 | 0.33 |
| Yield Benefit \$/Ha | -\$59 | \$294 | \$104 | \$138 | \$253 | \$146 |
| Net Return \$/Ha | -\$131 | \$222 | \$32 | \$66 | \$181 | \$74 |

#Based on a delivered cost of \$7 per litre; %based on ex farm price of \$440 per Bale.

In four of the five years, the use of foliar biostimulants in addition to *Soil & Seed* delivered a financial return. The 2014 result is an outlier in a year where there was no statistical difference in the results for the treatments (T2b and T4).

This does not indicate that the foliar on their own would perform as per the numbers indicated. The foliar biostimulants likely benefit from the application of *Soil & Seed*. To ascertain yield results when only using the foliar biostimulants additional trials would need to be performed.

Table 9: Economic Benefit of a Full Biostimulant Program on GSP – 15%N

| Year | 2013 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | Average |
|------------------------|-------|-------|-------|-------|-------|--------|-------|---------|
| Cost of Biostimulants# | \$85 | \$124 | \$128 | | | | | \$121 |
| Saving in Fertiliser | \$9 | | | | | | | \$9 |
| Benefit Bales/Ha | 0.42 | 0.55 | 1.64 | 1.89 | 0.46 | -0.39 | 1.00 | 0.80 |
| Yield Benefit \$/Ha | \$187 | \$242 | \$724 | \$834 | \$201 | -\$170 | \$442 | \$307 |
| Net Return \$/Ha | \$111 | \$127 | \$605 | \$715 | \$83 | -\$289 | \$323 | \$209 |

#Based on a delivered cost of \$7 per litre; %based on ex farm price of \$440 per Bale.



Highly variable results were achieved over the six years. Poorer results likely highlighting a lack of nitrogen fertiliser in good growing seasons.

Apart from 2017 all results were financially positive. 2017 was the best growing season and the result likely reflects a depletion of nitrogen.

Significant yield results in 2014 and 2015 where in poorer growing seasons, yet the combination of biostimulants and less nitrogen delivered a result better than all other treatments.

Additional trials need to be performed to determine to what degree biostimulants or less nitrogen were the drivers of better yields.

Conclusion

The overlay of a complete BioAg biostimulant program on GSP for the production of Cotton delivered yield benefits in each year of the six years trialled. In the poor and very good growing seasons the results were not statistically significant, but they remained financially positive.

It is clear that use of a program including soil applied and foliar biostimulants performed consistently better than all other treatments trialled.

Applying *Soil & Seed* only onto GSP provided financially positive results in 4 of the 5 years trialled. Though none of the results were greater than the LSD. To statistically validate these results additional trials using a greater number of replicates would need to be conducted.

When using a full biostimulant program with a reduction in the use of nitrogen fertiliser, the results were variable. Though in the lowest yielding years for GSP (2014 and 2015), these treatments delivered higher yields than all other treatments.

What was clear is that in each year the use of a full biostimulant program, even when 15% less side dressed nitrogen fertiliser was applied; an equivalent or better yield was achieved than GSP. To determine the drivers of these results additional trials need to be performed.

Additional Background – About BioAg

BioAg is an Australian manufacturer of liquid biostimulants and natural phosphate fertilisers. BioAg's liquid biostimulant are a range of proprietary microbial cultures, specifically formulated to support different plant growth stages by improving plant and soil performance.

Each culture / product contains a:

- Balanced food supply of carbohydrates, amino acids, enzymes, vitamins, essential nutrients and growth promoters, that feed both plants and beneficial micro-organisms
- Large and diverse population of beneficial micro-organisms, including fungi, bacteria, yeast and protozoa.



Each product has been developed to:

- Stimulate soil biology and plant processes
- Feed soil biology to ensure it is active and able to interact with the plant
- Improve the balance of beneficial microorganisms in soils, and
- Provides microbial food and microorganisms into soils that are low in microbial activity or diversity due to factors such as, stress (cold, heat or water logging), lack of plant activity (fallow) and/or due to a lack of plant diversity (monoculture).

Applying the appropriate product at the requisite growth stage will support and enhance:

- Structured vegetative growth and enhance root development
- Nutrient cycling and improved plant availability of nutrients
 - Chelation of nutrients, via amino bonds
 - Conversion of in-organic nutrients into a microbial form (becomes part of the biomass)
 - Helps to unlock nutrients previously bound in soil complexes
 - Improves the flow of nutrients through the plant
- Water retention and uptake, and
- Plant vigour and tolerance to abiotic stresses.

The benefits of biostimulants can be depleted with time. In addition, as plants develop reach their next growth stage the nutritional needs of the plant also change. Applying the appropriate biostimulant, soil inoculant or foliar application, at the right time is a key attribute of any program.

BioAg's three core biostimulant products are:

1. *Soil & Seed* is a broad-spectrum microbial inoculant that assists; nutrient accessibility, nutrient solubilisation, nutrient cycling, enhanced seed germination, root development, disease and drought resistance and residue breakdown.
2. *Balance & Grow* is a broad-spectrum source of foods and stimulants for balanced plant functions, plant health, and vegetative growth including; calcium and phosphate, vitamins, minerals, proteins, enzymes, amino acids and carbohydrates.
3. *Fruit & Balance* is formulated to increase flowering, fruit set and soil microbial activity. It delivers a rich source of plant-available phosphate when the plant is under peak load, stimulating strong fruiting and enhancing yield potential. Fruit & Balance contains a rich source of vitamins, minerals, proteins, enzymes, amino acids, carbohydrates, and growth promoters.

Each product is also available as an organic variant.

Appendices

Table 10: Summary of Applications Performed for Each Treatment

| Year | Day Applied from Planting | Growth Stage# BBCH Index | Product † | T1 | T2 | T2a | T3 | T3a | T4 | T5 |
|----------------|---------------------------|--------------------------|-----------|------|-----|-----|-----------|-----------|-----------|-----|
| Fertiliser | 0 | 00 | | GSP* | GSP | GSP | GSP -15%N | GSP -15%N | GSP -15%N | GSP |
| Application 1 | 0 | 00 | S&S | 5.3 | 9.4 | 9.4 | 4.7 | 5.3 | 9.4 | 9.4 |
| Application 1a | 0 | 00 | S&S | 4.7 | | | 3.5 | 3.5 | | |
| Application 2 | 39 to 81 | 59 to 60 | B&G | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 | |
| Application 3 | 56 to 95 | 65 to 71 | F&B | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | |

Notes:

† S&S is *Soil & Seed*, B&G is *Balance & Grow*, and F&B is *Fruit & Balance* all given as litres per hectare.

* GSP had solid fertiliser applied at planting at the rate of; 22kg of nitrogen, 39kg of phosphorus and 74kg of potassium per hectare; plus 67kg per hectare of nitrogen side dressed (knifed in) at a later growth stage (prior to flowering)

Plots where fertiliser applied was GSP less 15% nitrogen (GSP -15%N), had the same amount of fertiliser applied planting, but a reduced amount of nitrogen fertiliser applied as side dressing, being 57 kg of nitrogen per hectare.

Growth stage is defined by BBCH – scale for cotton (Reference: [https://en.wikipedia.org/wiki/BBCH-scale_\(cotton\)](https://en.wikipedia.org/wiki/BBCH-scale_(cotton))), Growth stages are outlined in Table 11 on the following page.

Table 11: Growth Stages

| Growth Stage | BBCH Index | Description |
|------------------------------------|------------|--|
| Germination | 00 | Dry seed (caryopsis) |
| 6: Flowering | 60 | First flowers opened (sporadically within the population) |
| | 61 | Beginning of flowering ("Early bloom"): 5–6 blooms / 25 ft of row (= 5–6 blooms / 7.5 meter of row) |
| | 65 | Full flowering: ("Mid bloom"): 11 and more blooms / 25 ft of row = 11 and more blooms / 7.5 meter of row |
| | 67 | Flowering finishing: majority of flowers faded ("Late bloom") |
| | 69 | End of flowering |
| 7: Development of fruits and seeds | 71 | About 10% of bolls have attained their final size |
| | 72 | About 20% of bolls have attained their final size |

Conversions Factors:

1lb Seed Cotton = 0.42lb Ginned Lint 1 Bale = 226.8kg Ginned Lint

1 Hectare = 2.47105 Acres

1 Pint = 0.47317 Litres

1 Fluid Oz = 0.02957 Litres

1 unit P2O5 = 0.436 units of P

1 unit K2O = 0.893 units of K

1lb/A = 1.21kg/ha

Raw Data

The trial data is available from the website www.bioag.com.au. For any questions or enquiries please contact your local BioAg Sales Representative.