Study into the use of two novel bio stimulants in the Australian turf industry to improve plant health and stimulate root growth.

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Authors Jerry Spencer BSc Hons, and Cameron Smith CSTM.

jerry@gilbasolutions.com

Abstract: The use of biostimulants and turf pigments is gaining traction in the professional turf industry. Combinations of salicylic acid, copper phthalocyanine, EO-PO block copolymers, 2,3- Butanediol and β-cyclocitral were researched for their use in turfgrass and their effects on turf quality, colour or root growth.

A replicated block pot trial was run for 133 days on perennial ryegrass, looking at using these as stand-alone applications and incorporating them with a combination of copper phthalocyanine pigment, salicylic acid and a novel soil wetting agent formulation. Results were as follows:

1. Pigment applications gave significantly higher DGCI and Colour Quality readings than all other treatments.
2. 2,3 Butanediol gave a significantly lower Colour Quality result than the untreated control.
3. The pigment and pigment plus bio stimulants gave a significantly higher Percent Cover and Cover Quality readings than all the other treatments.
4. At a depth of 0-2cm no significant differences in organic matter content were observed but all the treatments gave higher levels than the untreated control.
5. At a depth of 2-4cm, although not significantly higher than the untreated control, the pigment plus bio stimulant gave approximately 2x greater organic matter content than the untreated control.
6. Although not being significantly higher than the untreated control, the Wetting agent + bio stimulant gave the highest organic matter contents at a depth of 4-6cm.
7. At a depth of 6-8cm, with the Wetting agent + bio stimulant and pigment plus bio stimulant treatments gave the highest organic matter contents.

Keywords: biostimulant; turfgrass; organic matter; root growth; 2-3-butanediol; β-cyclocitral; salicylic acid

1. Introduction

Australian turf managers are constantly under pressure to produce the best possible turf surfaces within budget, and while having minimal environmental impact. This is all whilst being subjected to increasing levels of use and expectations from end users.

A key requirement to producing turf to the highest standard is a vigorous root system, with ideally this being a result of minimal use of phosphorus. This is for two key reasons:

Firstly, heavy applications of phosphorus are thought to stimulate the growth of the undesirable weed grass winter grass (Poa annua).

Secondly, excess phosphorus can run to waste and cause issues with algal blooms, and pollution of waterways.

The use of bio stimulants to stimulate root growth is not new, and the use of seaweed-based products containing high levels of cytokinins is a common approach adopted by many turf managers.

In recent years there have been major advances in bio stimulant technology. 2,3-Butanediol and β-cyclocitral are two of these and are of particular interest as they have been shown to stimulate root growth even when used at very low concentrations.

2,3-Butanediol

2,3-Butanediol, is a Bacterial Volatile Compound, and has been reported to enable plants to survive biotic and abiotic stresses better.

Biotic Stress Management:

Synthetic Butanediol induced plant defence against ***R. solani*** for creeping bentgrass [1].

A two-year study in Canada [2] showed that Butanediol provided enhanced resistance to creeping bent grass against the dollar spot pathogen in lab tests, with disease reduction in the range of 50% to almost 100%.

When combined with turf fungicides, 2,3-Butanediol increased efficacy at half rates [3].

Butanediol with half rates of fungicides significantly increased the disease control efficacy against dollar spot and summer patch disease compared to the half concentration of azoxystrobin, and tebuconazole alone.

Abiotic Stress Management:

Bent grass treated with 2,3-Butanediol shows faster recovery from high temperature stress than the untreated control and shows increases in diameter/height/leaf index compared to untreated grass. [4]

In contrast under drought or cold temperature stress, an application of Butanediol in creeping bent grass and tomato plants significantly reduced the negative impact of these abiotic stresses [5].

2,3-Butanediol treatment improves the survival rate of Arabidopsis, 3 times more than water treatment in drought conditions [6].

Growth Response:

2,3-Butanediol increases the growth and size of red pepper [7].

Trials on Kentucky Bluegrass and Creeping Bent have shown a greening response when Butanediol is applied every two weeks. The chlorophyll index was increased by 18% in Kentucky Blue grass and 8% in Creeping Bent compared to the non-treated group.

β-cyclocitral.

β-cyclocitral is an effective root growth promoter in monocots and potentially a valuable tool to enhance growth under environmental stress.

In turfgrass systems strong root growth and branching are highly desirable, as they enhance the plants ability to take up nutrients and water. β-cyclocitral enhances root growth and branching [8].

Salicylic acid.

Plants rich in SA and its derivatives, have been used in medicine for 1000's of years. For example. aspirin is based on a derivative of Salicylic acid.

Salicylic acid (SA) is found in plants, and plays a role in plant growth, and helps fight against both biotic and abiotic stress such as heat stress recovery [9].

Salicylic acid is a plant growth regulator [10], that modulates the antioxidant defence system and decreases oxidative stress [11].

Plant-water relations in abiotic stress affected plants were regulated by SA [12], and it was found that SA regulates plant water relations and increases heat tolerance [13].

Salicylic acid also reduced ***Alternaria solani*** in tomatoes and enhanced plant growth [14].

Turf specific research.

SA has also been shown to enhance heat tolerance of Kentucky Bluegrass, which regrew faster following heat stress. An SA concentration of 0.25 mmol [15] was most effective in enhancing heat tolerance.

In perennial ryegrass [16] research looked at how SA affected this under drought stress. It was found that foliar SA increased chlorophyll a, b content, and reduced electrolyte leakage, proline accumulation and antioxidant enzyme activity. All these factors suggested that SA can be used to reduce the negative impacts of drought stress.

2.Materials and Methods

A tube trial was established on October 1st 2023, using perennial ryegrass var Grand Slam GLD sown at the equivalent of 35g/m2 into a clean USGA specification sand. This was a replicated block trial with 6 treatments including an untreated control and 5 blocks (30 tubes in total). The treatments were 1) 2,3-butanediol 1L/Ha, 2) β-cyclocitral 300ml/Ha, 3) wetting agent plus β-cyclocitral and 2,3-butanediol at 12.5L/Ha, 4) Vertmax Duo plus β-cyclocitral and 2,3-butanediol at 12.5L/Ha, 5) Vertmax Duo at 1L/Ha and (6) an untreated control.

The 2,3-butanediol was supplied by GS Caltex Corporation, the β-cyclocitral by JH Chemical HK Co., Ltd Hangzhou and the Vertmax Duo from Gilba Solutions, Australia.

Following seeding, the pots were allowed to establish until the first treatment was made on the 29th October 2023. Subsequent applications were made on the 7th of November 2023, 17th November 2023, 2nd February 2024 and 1st March 2024.

For the duration of the trial irrigation was applied daily via an onsite misting system and no fertilizer was applied.

Assessments were as follows:

1. Turf DGCI.
2. Turf Colour Quality.
3. Density Quality.
4. Percent Cover.
5. Cover Quality.
6. Organic Matter by Loss on Ignition.

Digital Image Analysis.

Turf Quality Analysis was carried out using a light box. Images were taken with a Panasonic DMC-TZ80, and then analysed using Turf analyzer software to determine the DGCI, Colour Quality, Density Quality, Percent Cover and Cover Quality.

Loss on Ignition (LOI).

The individual rootzones were taken and carefully removed from their respective tubes. The top 1cm was then cut off and removed. This was to ensure that any organic matter results were due to root development rather than surface thatch.

These rootzones were then split into sections 0-2 cm, 2-4 cm, 4-6 cm and 6-8 cm. The samples were then dried in a muffle furnace for 48 hours at 60°C, and then ashed at 440°C for 4 hours. Weights were taken after drying and ashing, with the loss of ignition then being calculated. This corresponds to the organic matter content at the respective depth. The ash content is expressed as a percentage of the mass of the oven-dried sample.

% Ash Content = (C X 100)/B
where: C = ash weight in g, and B = oven-dried test specimen, weight in g.
The calculation to determine the amount of organic matter by difference, is as follows:
% Organic matter = 100.0 - D
where: D = ash content, %.
If these products were increasing root growth, organic matter contents would increase over the control. The assumption was that as this was initially a clean USGA spec sand, any increases in organic matter would be due to increases in root growth.

Tubes were monitored regularly using digital image analysis in combination with Image J. Statistical analysis was carried out using Studio R, agricolae package. All data were subjected to two-way ANOVA (analysis of

variance) to determine the Treatment effects. Treatment means were separated using the Duncan's multiple range test at the P = 0.05 level of probability.

3. Results

Image 1. (A) Image taken on 29th October 2023 before treatments applied. (B) Image taken on the 7th November 2023.

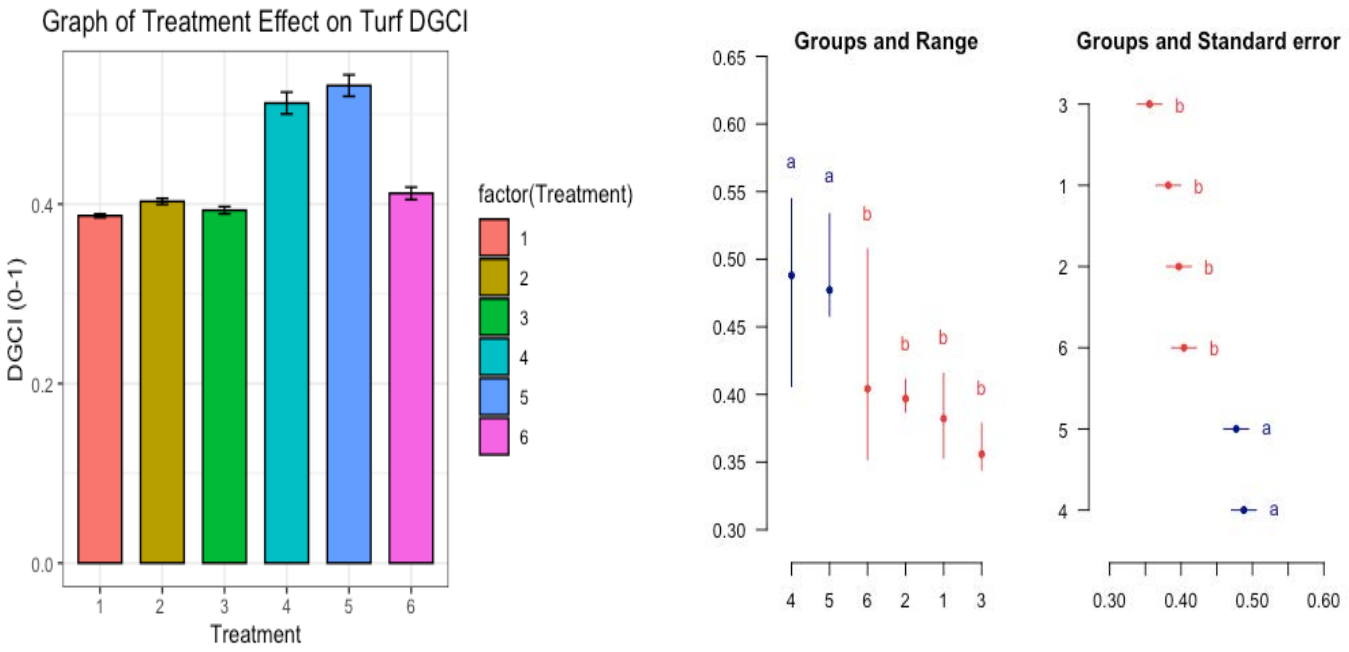
A

B

Digital Image Analysis.

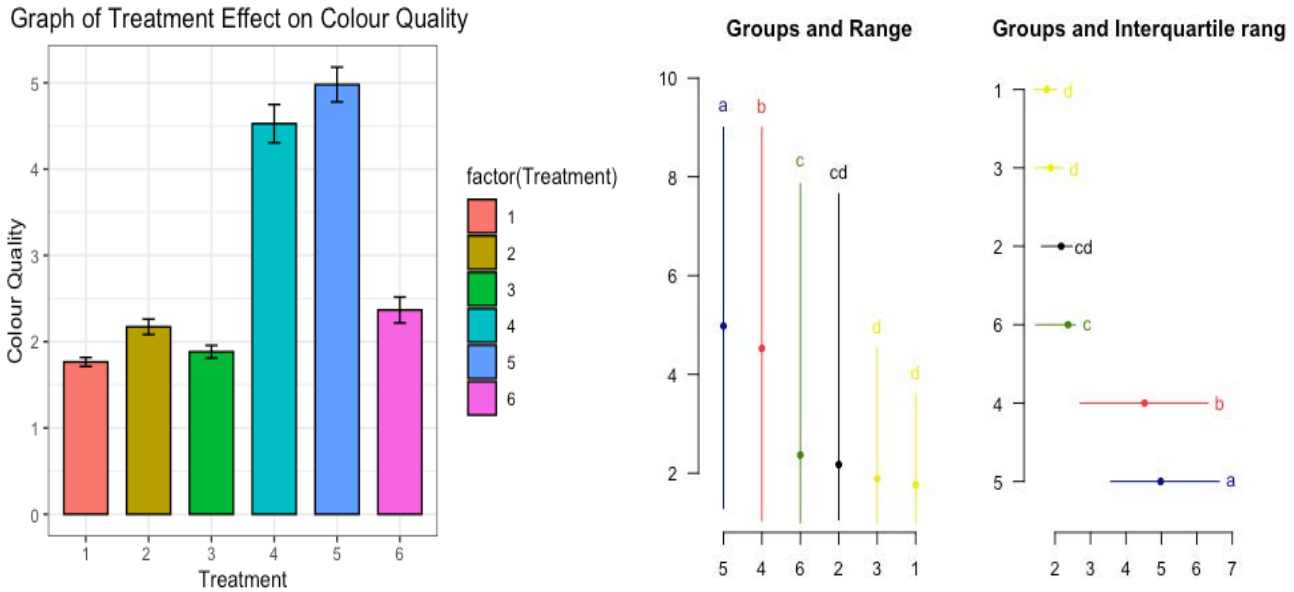
Over the duration of the trial, Digital Image Analysis showed significant P<0.05) differences between the treatments. DGCI readings were highly significant for the Pigment (5) and Pigment plus 2,3-butanediol and cyclocitral (4) treatments compared to the control and other treatments.

Figure 1. Dark Green Colour Index. Values with different letters are significantly different ($P < 0.05$).



With Colour Quality, the Pigment (5) and Pigment plus 2,3-butanediol and cyclocitral (4) treatments showed significant P<0.05) results compared to the control and other treatments.

Figure 2. Colour Quality. Values with different letters are significantly different ($P < 0.05$).



With the Cover Quality both the Pigment (5) and Pigment plus 2,3-butanediol and cyclocitral (4) treatments continued to give significant P<0.05) results compared to the control and other treatments.

Figure 3. Cover Quality. Values with different letters are significantly different ($P < 0.05$).

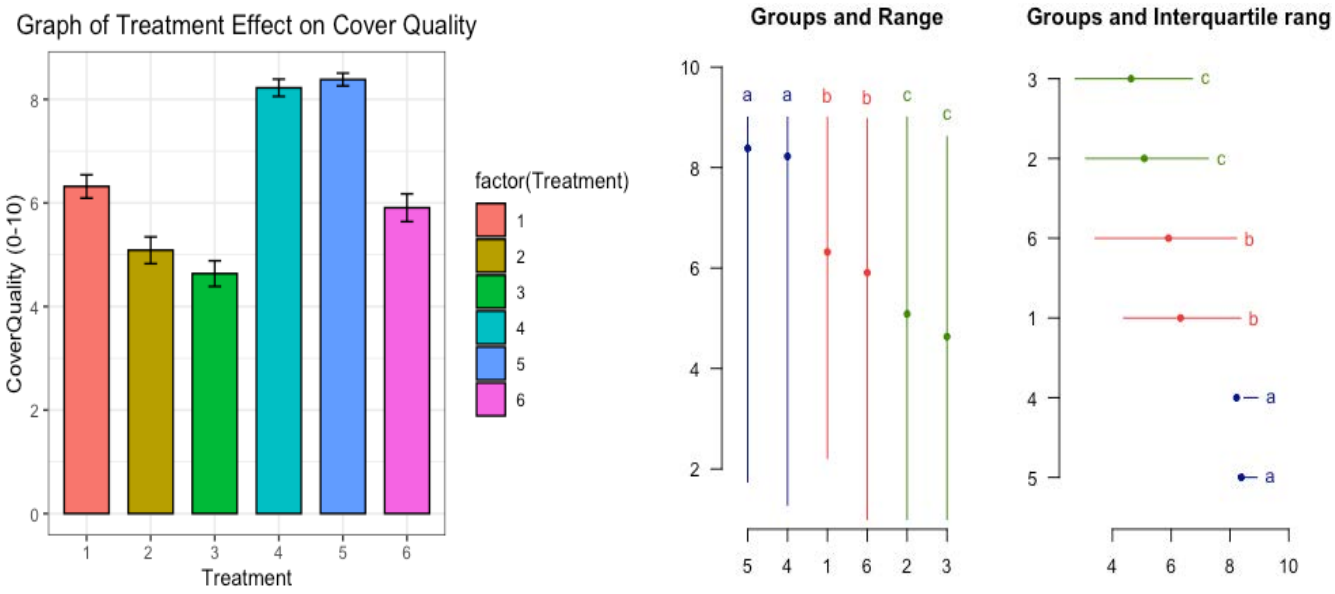
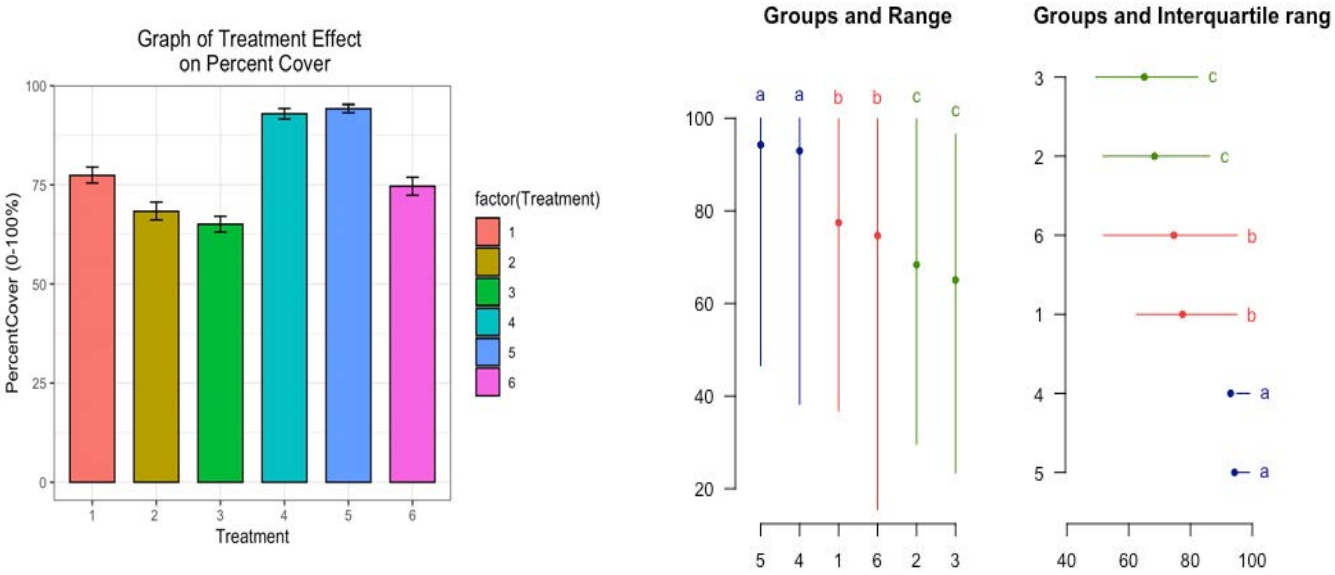


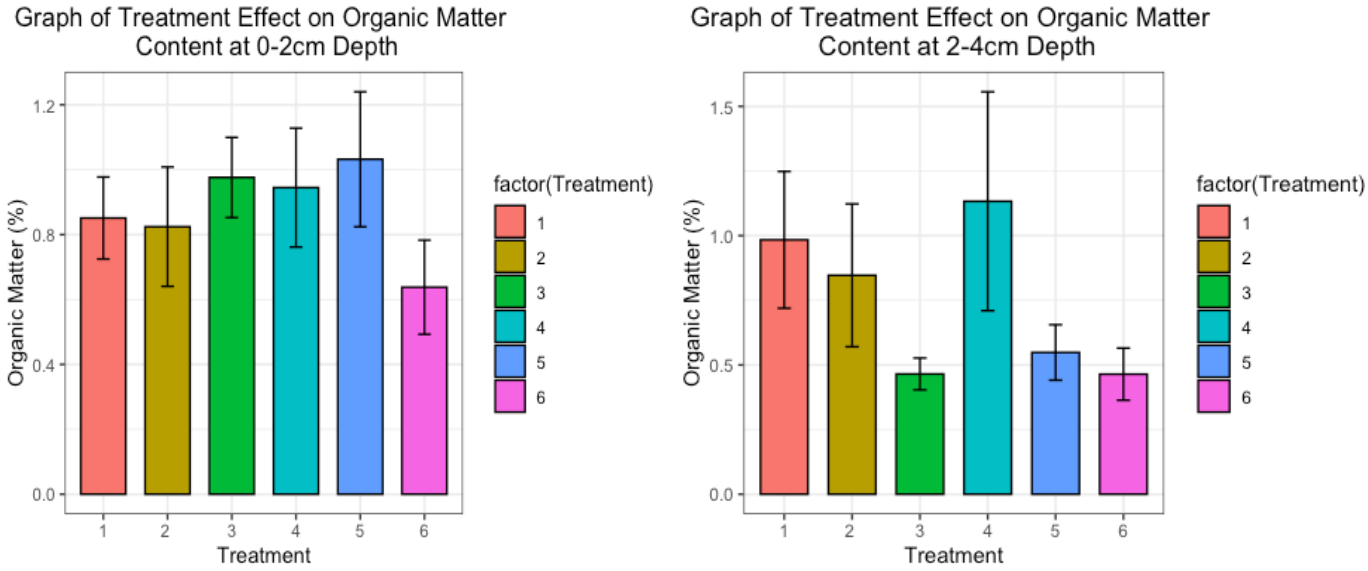
Figure 4. Percent Cover. Values with different letters are significantly different ($P < 0.05$).



Loss on Ignition and Organic Matter %.

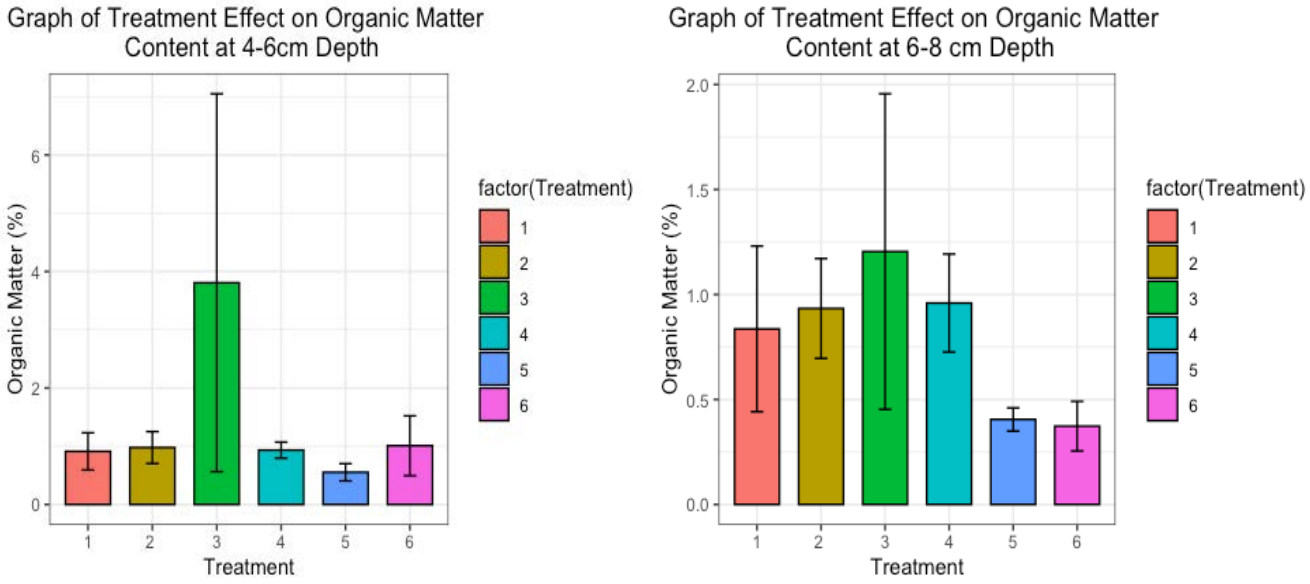
This shows that Wetting agent + Butanediol + β-cyclocitral (3), Vertmax Duo + Butanediol + β-cyclocitral (4), and Vertmax Duo (5) treatments gave the highest organic matter readings at the 0-2cm depth. However, none of these were significantly different from the Control.

Figure 5. Organic Matter % at 0-2 cm and 2-4 cm. Values with different letters are significantly different ($P < 0.05$).



There were no significant differences between the treatments at the 2-4cm depth. The 2,3-Butanediol (1), β-cyclocitral (2) and Vertmax Duo + Butanediol + β-cyclocitral (4) Treatments had the highest organic matter levels at the 2-4cm depth.

Figure 5. Organic Matter % at 4-6 cm and 6-8 cm. Values with different letters are significantly different ($P < 0.05$).



There were no significant differences between treatments at the 6-8cm depth at the 0.05% level. However, the wetting agent + Butanediol + β-cyclocitral (4) and Vertmax Duo + Butanediol + β-cyclocitral (4) showed the highest organic matter contents.

4. Discussion.

2,3-butanediol and β-cyclocitral do appear to stimulate root growth and development, even when used at these low rates. However, the economics of their use must also be taken into consideration.

Currently 2,3-Butanediol is a significantly lower-cost option in comparison to β-cyclocitral.

Bearing in mind Vertmax Duo has already been shown to stimulate root growth and lateral branching of turfgrass the incorporation of 2,3-butanediol has merit for increasing stress tolerance of turfgrass when incorporated into this product.

Most pigment applications are made in the winter months to mask warm season turfgrass dormancy or throughout the year to golf greens in conjunction with fungicides. In the latter case the pigment serves two purposes.

Firstly, it acts as a tracking colourant so you can see where spray is applied, and you can avoid chemical overspray.

Secondly, the biostimulants in the pigment has additional benefit such as stimulating root growth or improving overall plant health.

The fact that 2,3-butanediol has also been shown to reduce fungicide applications by up to 50% is worth serious consideration to its future use in spray applications.

In combination with the Vertmax Duo pigment plus salicylic acid, statistically the results are not different from the Vertmax Duo treatment as a standalone treatment. However, two issues require further testing.

The first is people's perception of colour. What statistically is no different, in the field may result in completely different feedback from the customer.

The second is drying time. A unique property of Vertmax Duo is the fact that once dried it doesn't go back into solution. This trait needs to be tested to ensure that when Vertmax Duo is combined with 2,3-Butanediol or cyclocitral that it is not negatively affected.

5. Conclusions

The results showed that there is significant potential for the use of newer generation biostimulants in the turf industry. Opportunities exist for these as stand-alone applications and to incorporate them with commonly applied products.

Further work needs to be looked at regarding standalone applications at increased rates before moving onto field trials.

The other point worth considering is that is there a similar effect on other grasses such as couch or bentgrass?

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jerry@gilbasolutions.com

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