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Is magnesium deficiency a causal agent of sugarcane Yellow Canopy Syndrome?

O Tippett[1], DJ Olsen[1] and Z Ostatek-Boczynski[2]

[1]*Sugar Research Australia Limited, Brandon, Qld 4808*; otippett@sugarresearch.com.au
[2]*Sugar Research Australia Limited, Indooroopilly, Qld 4068*

Abstract Yellow Canopy Syndrome (YCS) is a disorder affecting sugarcane in the Australian industry, the cause of which is unknown. This paper reviews YCS research focusing on magnesium imbalance as a possible cause of the condition. Four studies were undertaken to evaluate the role of Mg in YCS incidence and severity. In Trial 1 sugarcane leaves were collected at multiple locations in the Burdekin and Herbert with samples taken from sugarcane blocks with both YCS symptomatic and asymptomatic plants. Despite adequate soil-Mg, leaf-Mg concentrations were significantly lower ($p \leq 0.05$) in leaves 2, 3, 4, 5 and 6 of YCS symptomatic plants in both regions suggesting an imbalance of this critical nutrient. Trial 2 measured Mg concentrations in sugarcane leaves before, during, and after YCS symptom expression. Symptomatic cane showed decreased leaf-Mg concentrations, but this returned to normal levels once the cane recovered. Trial 3 treated YCS symptomatic cane with foliar and soil applications of Mg in an attempt to mitigate the condition. Neither treatment resulted in alleviation of the YCS symptoms. Trial 4 treated sugarcane with foliar-Mg and soil-Mg prior to onset of symptoms. Despite elevating the Mg concentration in leaves, these pre-symptomatic treatments did not prevent YCS expression and plants exhibited YCS symptoms similar to that of the untreated control. We conclude that YCS affected cane is associated with reduced leaf-Mg concentrations, but it is unlikely that this is the cause of YCS *per se*, as concentrations were well above critical thresholds for plant health. YCS occurs independently of Mg and low Mg is an indirect effect rather than a cause. Given that disruption to plant nutrient balance has been described as a symptom of some plant diseases, we speculate that these findings suggest a biotic causal agent.

Key words YCS, deficiency, magnesium

INTRODUCTION

Yellow Canopy Syndrome (YCS) is a disorder currently affecting sugarcane crops in Queensland (Braithwaite *et al.* 2017). It was first reported in 2012 and has increasingly spread from the north of Cairns to areas south of Mackay (Joyce *et al.* 2016). The symptoms typically present in the mid to lower canopy leaves as a characteristic yellow-orange colour and cause reduced photosynthesis and subsequent yield losses (Olsen and Brownlee 2017). Extensive research has been conducted to determine the cause of YCS, but this remains unknown.

Magnesium (Mg) is a critical mineral nutrient involved in photosynthesis and sugar production in sugarcane. Soils deficient in Mg occur in some high-rainfall areas of north Queensland and plant-deficiency symptoms will first appear in the older leaves (Calcino *et al.* 2018). Severe Mg deficiency is typically characterised by clear interveinal chlorosis and decreased growth. However, in production agriculture, latent deficiencies are more common in which symptoms are not visible but there are associated yield penalties (Gransee and Fuhrs 2013).

Non-structural carbohydrate build-up and chlorosis of sugarcane leaves are characteristic of Mg deficiency and these symptoms have also been observed in YCS plants (Gransee and Fuhrs 2013; Marquardt *et al.* 2016). It takes about 1 week following Mg unavailability for these symptoms to appear. The mechanism that occurs because Mg deficiency involves several impairments, such as the accumulation of starch and sucrose,

decreased photosynthetic capacity and generation of reactive oxygen species (ROS) (Natsuko and Tanoi 2015). Of these, the build-up of sucrose was identified to be directly associated with decreased Mg concentration as phloem loading of sucrose depends on sufficient Mg (Waters 2011). Accumulation of carbohydrates in the leaves results in a reduction in photosynthesis. Once the photosynthetic rate is disrupted, unused light energy produces ROS which in turn causes photo-oxidative impairment to both the chlorophyll and chloroplast membrane (Natsuko and Tanoi 2015).

This paper reports on the findings of four trials established to evaluate whether Mg imbalance is a causal agent of YCS. Comprehensive leaf-nutrient testing on YCS-affected sugarcane in the Burdekin and Herbert regions in Trial 1 in 2015 revealed decreased Mg concentrations of YCS symptomatic cane relative to asymptomatic, healthy plants. In Trial 2 nutrient testing was conducted on plants before, during and after YCS expression. Trial 3 was initiated to test whether soil and foliar applications made to YCS symptomatic plants would mitigate the effects of YCS. In Trial 4, soil and foliar magnesium was applied to the cane prior to onset of YCS to assess preventative management strategies.

METHODS AND MATERIALS

Chemical analyses

Analytical procedures for leaf nutrient testing were done as per industry standard. All samples were labelled and processed prior to further chemical analysis. The midrib was removed from all fresh leaf samples, followed by drying at 65°C until a constant weight was reached. The dry samples were ground to a particle size of less than 0.5 mm using a Culatti MFC micro-hammer mill (Kinematica AG, Lucerne, Switzerland).

Analysis of ground, dry leaf samples was conducted following standard analytical procedures. The traditional method of nitric-perchloric acid digestion was utilised for sample decomposition, followed by elemental analysis using ICP-OES (Zasoski and Borau 1977). This method principally provides the analysis of major nutrient elements (phosphorus, potassium, sulfur, calcium and magnesium) as well as trace elements (copper, zinc, iron and manganese). The results are reported in % dry mass (DM) for major elements: P, K, S, Ca and Mg, and in mg/kg DM for trace elements: Cu, Zn, Fe and Mn.

YCS assessment

Five stalks were selected at random in each plot and barcode labelled. Monitoring was conducted weekly on the same barcode labelled stalks over a 6-month period. YCS severity was scored for leaves +1, +2, +3, +4, +5, +6 and +7, where leaf +1 was the first fully expanded leaf corresponding to the top visible dewlap (Kuijper 1915). Ratings were assigned according to a severity-rating key (Figure 1).

Severity Rating	Description (degree of yellowing on leaves)
0	No YCS symptoms evident
1	Yellowing is present in approximately 25-50% of the leaf. It may be presented in a solid yellow form or as mottling either along the leaf edges, tips or on one side of the midrib only.
2	Yellowing is present in approximately 50% of the leaf in either solid yellow or mottling form. Yellow colour exhibits a stronger orange hue than rating 1. Typically found on both leaf margins and leaf tip although symptoms can occur on one side of the midrib only.
3	Yellowing is present in at least 75% of the leaf. Advanced yellowing across the entire leaf blade, with mottling now developed into solid colouring.

Figure 1. YCS severity rating key.

2015 Trial 1

Sampling

Plant samples were collected at six commercial crops from healthy, asymptomatic sites and YCS symptomatic sites in the Burdekin and Herbert regions during 2015 (Table 1). These two regions were selected as sugarcane crops displayed YCS prevalence in the current and previous seasons. These regions also represented contrasting soil types. The Herbert district predominantly comprises low-pH soils and limited irrigation, while the Burdekin region has primarily neutral to alkaline soils and full irrigation as a standard agronomic practice. Soil-nutrient supply to all blocks were sufficient and confirmed with soil testing. Leaf 0 to leaf +6, where L0 is located above the top visible dewlap and L+6 is lower in the canopy, were collected from three or four randomly selected stalks and analysed for leaf-tissue nutrient concentrations (Table 1).

Table 1. Summary of sample sites for symptomatic and asymptomatic leaves 0 to +6.

District	Cultivar	Number of replicates	Number of samples*
Burdekin	Q208ф	3	21
	Q208ф	3	21
	KQ228ф	4	28
	KQ228ф	4	28
Herbert	Q208ф	3	21
	Q208ф	3	21

*each sample is a composite of a minimum of 10 leaves.

Statistics

Analysis of variance was undertaken on the analytical data associated with leaves 0 to +6. Values from the two regions were statistically different ($p<0.05$), likely due to the different nutrient-availability profiles resulting from the different soil pH values of each region. The regional data were therefore analysed separately rather than as a combined data set. This was undertaken using Statistix 10 at a 95% confidence level. Data were checked for normality by Shapiro-Wilk test and transformations made where required. Differences among treatments within regions were determined using a paired t-test at a 95% confidence level.

2016 Trial 2

Sampling

A commercial block of Q240ф first-ratoon sugarcane was selected at Home Hill in February 2016. It displayed characteristic YCS symptomatic cane and very green asymptomatic plants at opposite ends of the same block. We wanted to study this phenotypic difference and to try to identify key agronomic and physiological parameters in order to determine the cause of the phenotypic variation. In May, the YCS plants appeared to have recovered as they no longer exhibited YCS symptoms. In response, the same parameters tested previously were used to determine any significant differences or similarities between the previously symptomatic and asymptomatic cane.

Leaf-tissue nutrient analyses as per industry standard were undertaken on 20 randomly selected stalks of YCS symptomatic and 20 stalks of YCS asymptomatic cane. Leaf + 1 and leaf + 4 with three replicates for YCS symptomatic and asymptomatic plants were analysed. YCS symptomatic samples were collected in early February and post-YCS samples were collected in late May. The February and May data were analysed separately.

Statistics

For each month analysis of variance ($\alpha=0.05$) was determined using split-plot model with treatment the main-plot factor and leaf number the sub-plot factor. LSD pairwise analysis was then conducted to determine significant differences.

2016 Trial 3

Sampling

A field trial on a commercial farm in the Burdekin region was established on 25 February 2016. It was a fourth-ratoon crop of KQ228$^{\phi}$, 6 months old and expressing YCS symptoms. Three treatments were applied with three replicates of each; untreated control, magnesium sulphate 50 kg/ha applied as a foliar spray, and magnesium sulphate 50 kg/ha applied as a soil drench. Baseline leaf-nutrient analysis was undertaken on 19 February 2016 prior to treatment application. Leaf samples were collected from three stalks per treatment and leaf-nutrient analysis was performed as per industry standard. YCS monitoring and assessment was also performed as above. Post-treatment leaf-nutrient analysis was conducted on 31 March 2016 to determine any treatment response.

Statistics

Analysis of variance between treatments was performed using Statisix 10 at a 95% confidence level. Data were first checked for normality by Shapiro-Wilk test and transformations made where required.

2018 Trial 4

Sampling

The trial was established in September 2017 at Sugar Research Australia Brandon in a first-ratoon crop of KQ228$^{\phi}$. There were three treatments with four replicates of each in a randomised complete-block design. The first was a bifenthrin insecticide that was foliar applied using a knapsack sprayer each week from November 2017 through March 2018. The second was foliar applications of $MgSO_4$ at 20 kg/ha every 2 weeks from November 2017 through March 2018 to assess whether additional available Mg would prevent the onset of YCS. The third treatment was an untreated control.

Leaf tissue nutrient measurements were undertaken on 10 randomly selected stalks. Leaf + 1 and leaf + 4 with 4 reps for each treatment were analysed. Samples were collected on 23 February 2018.

Statistics

Analysis of variance among treatments and leaf number was performed using Sigmaplot 14 at a 95% confidence level. Data were first checked for normality by Shapiro-Wilk test. All pairwise multiple comparisons (Bonferroni t-test) were made to determine significant differences.

RESULTS AND DISCUSSION

2015 Trial 1

Leaves 0–6 in the Burdekin region showed a consistent trend of higher Mg in asymptomatic plants than in YCS plants (Figure 2)., with symptomatic YCS cane having statistically lower levels ($p \leq 0.05$) across leaves +2, +3, +4, +5 and +6. This trend was occurred in the Herbert where YCS symptomatic plants showed statistically lower Mg concentrations ($p \leq 0.05$) across leaves +2, +3, +4, +5 and +6 (Figure 3. Mean magnesium concentrations (±SE) of YCS symptomatic and asymptomatic cane from Q208A in the Herbert in 2015. YCS cane showed statistically lower magnesium levels ($p \leq 0.05$) across leaves +2, +3, +4, +5 and +6, denoted by an asterisk (*).).

The soils on which the control and YCS plants were grown had sufficient nutrients available in both the Burdekin and Herbert regions. Burdekin soils characteristically tend towards an alkaline pH and the soils of the Herbert are more acidic. Reduced Mg uptake may occur in acidic soils as the solubility of Mg decreases and it becomes less available (Guo *et al.* 2016). Therefore, the reduced uptake in YCS affected cane is unlikely attributable to soil pH, since this occurs in both acidic and alkaline conditions. These findings suggest the possibility that YCS affects the uptake and distribution of Mg within the plant.

A study into the growth and nutrition of mollicute-infected maize (De Oliveira *et al.* 2002) found that spiroplasma-

infected plants had reduced nutrient uptake, potentially the result of pathogen interference. The interference of Mg uptake could potentially cause a decrease in photosynthesis, as this element forms part of the chlorophyll molecule. It is also a critical mineral element required for all cellular metabolism. Mg deficiency is characterised by yellow striping on leaves, which is comparable to YCS symptom expression. The mechanism as to how the pathogen induces a decrease in uptake remains unclear. One theory is that the pathogen secretes a toxin. An alternative theory by Huber and Jones (2013) considered it a consequence of competition for Mg between the pathogen and the plant. Contrastingly, it was found that maize plants infected with a phytoplasma and healthy controls took up similar amounts of nutrients, although growth and production of the diseased plants was highly compromised (De Oliveira *et al.* 2002).

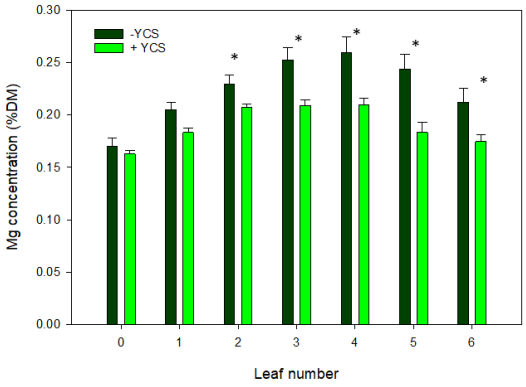

Figure 2. Mean magnesium concentrations (±SE) of YCS symptomatic and asymptomatic cane from Q208ф and KQ228ф in the Burdekin in 2015. YCS cane showed statistically lower magnesium levels (p≤0.05) across leaves +2, +3, +4, +5 and +6, denoted by an asterisk (*).

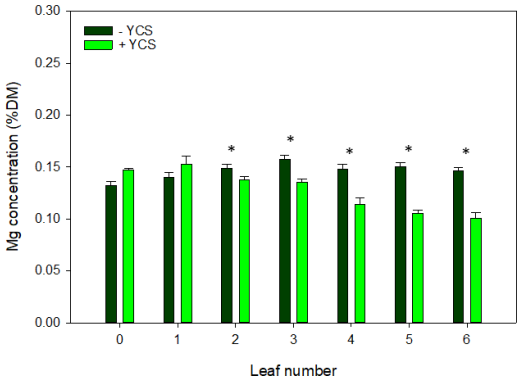

Figure 3. Mean magnesium concentrations (±SE) of YCS symptomatic and asymptomatic cane from Q208ф in the Herbert in 2015. YCS cane showed statistically lower magnesium levels (p≤0.05) across leaves +2, +3, +4, +5 and +6, denoted by an asterisk (*).

2016 Trial 2

Analysis of Mg concentrations of YCS symptomatic and asymptomatic cane leaves in the same block situated in Home Hill in February 2016 showed decreased levels, although not statistically significant, of Mg in YCS symptomatic plants compared to YCS asymptomatic plants in both leaf +1 and leaf +4 (Figure 4). Later in May when the YCS symptomatic plants appeared to have recovered, leaf Mg concentrations were very similar in both YCS symptomatic plants and YCS asymptomatic plants. Magnesium concentrations were significantly higher in leaf +4 than in leaf +1 for both YCS symptomatic and asymptomatic plants at both times.

This field was subjected to the same agronomic practices and site-specific seasonal conditions and pressures, so it is possible to attribute these differences to YCS. The previously infected cane appeared to recover in May

when Mg levels had returned to very similar concentrations in both sets of plants. The soil test results show that Mg levels in the soil remained the same throughout the growing period which further suggests that there appears to be a disruption in Mg uptake either within the roots or the plant during an outbreak of YCS and what we recorded was not a typical nutrient deficiency.

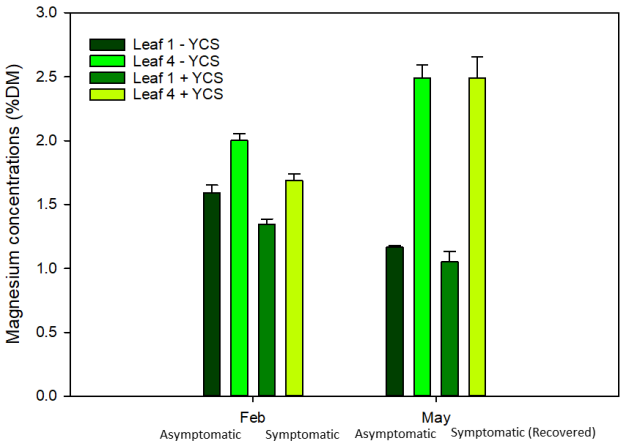

Figure 4. Mean magnesium concentrations (±SE) in leaves +1 and +4 in YCS symptomatic and asymptomatic cane in February at peak YCS expression and in May when the cane had appeared to recover.

2016 Trial 3

Following the application of soil and foliar applied $MgSO_4$ there were no significant differences among the treatments for both leaf +1 and leaf +4 (Figure 5). There were statistically higher Mg concentrations in leaf +4 than in leaf +1 across all treatments.

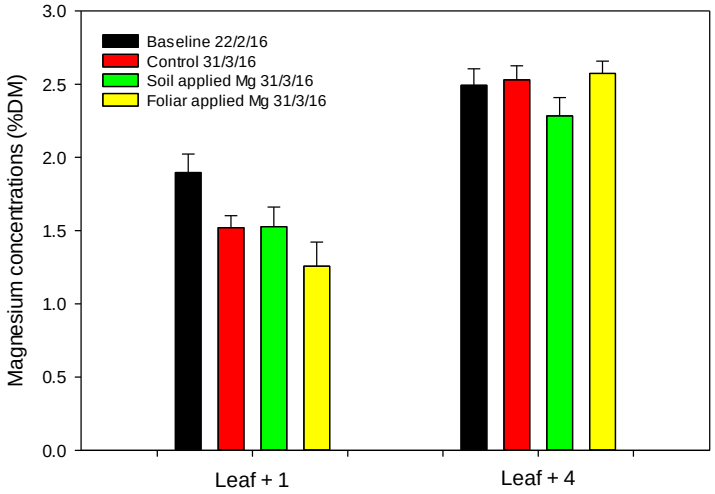

Figure 5. Mean magnesium concentrations (±SE) of KQ228$^{(l)}$ YCS symptomatic cane treated with soil and foliar applied magnesium in the Burdekin.

YCS symptom expression was highest in February (Figure 6) and gradually decreased across all treatments including the untreated control. Higher YCS expression occurred in the cane treated with foliar applied $MgSO_4$ than in the control, suggesting that this treatment was increasing the symptoms. The soil-applied $MgSO_4$ resulted in statistically similar YCS expression to the control, suggesting that this treatment had no measurable effect.

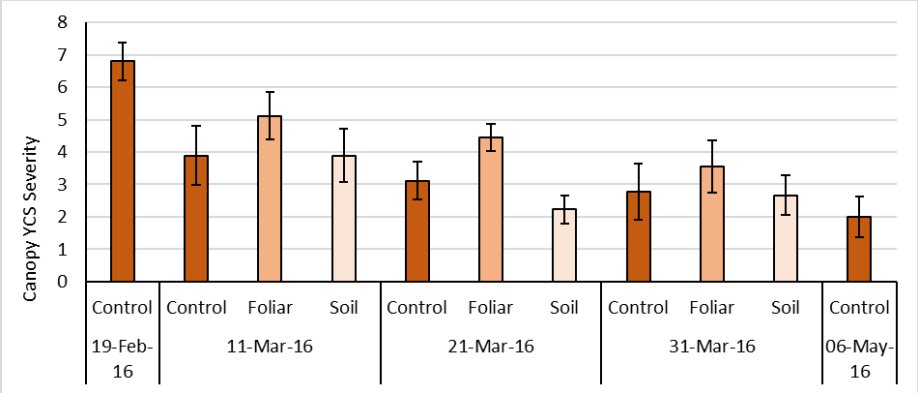

Figure 6. YCS severity (sum of leaf severity ratings) of KQ228$^{(i)}$ with treatments of foliar-applied magnesium (50 kg/ha), soil-applied magnesium (50kg/ha), and untreated control. Bars are the average of 9 stalks and means are ± 1 standard error.

In 2016, we hypothesised that Mg applications might alleviate YCS symptom expression and improve photosynthetic function as nutrient manipulation through soil and foliar application forms an important cultural control against disease in production agriculture. Sufficient nutrition is critical in maintaining disease resistance through the production of inhibitory compounds or defence responses to pathogen infection while also shortening the susceptibility of particular growth stages to plant-pathogen interactions via increased growth. Plant nutrient status can impact disease potential more effectively than inoculum potential in most cases. In turn, the severity of most diseases can be reduced by adequate nutrient management (Huber and Hanekalus 2007). Nome *et al.* (2009) found that maize infected with *Spiroplasma kunkelii* expressed symptoms very much like plants grown on magnesium-deficient soil and demonstrated that the spiroplasma modified the crop's magnesium up-take. Infected maize grown in low magnesium soils were more impaired than infected plants on high magnesium soils. They concluded that symptom expression may be potentially related to a competition for the cation between the crop and the pathogen and that increased magnesium availability can impact the way the pathogen invades the plant by allowing access to young cells and to multiply within their cytoplasm.

However, our results showed that foliar- and soil-applied Mg did not ameliorate the symptoms of YCS. In reality, the foliar-applied Mg treatment expressed higher rates of YCS whilst the soil-applied Mg treatments had symptom expression comparable to the control. The additional Mg applications did not result in higher leaf-Mg concentrations relative to the control which suggests either the cane does not luxury feed on Mg or there was a nutrient uptake interference. Overall, YCS symptom expression declined from the peak observed in February to April across all treatments, which correlates to cooler, more humid and wetter weather conditions. Our findings strongly indicate that Mg deficiency or imbalance is an indirect effect of and not the cause of YCS.

2018 Trial 4

From late October 2017 we recorded low incidences of YCS across all treatments until following a significant rainfall event in late January 2018 (Figure 7) when cane in the untreated control and $MgSO_4$ treatment began to express increasing YCS symptoms, whilst the insecticide treated cane remained green. In early February, there were clear differences between these two groups., with the insecticide-treated cane remaining green and asymptomatic.

On 23 February 2018 (Figure 8), leaf +1 in both the control and $MgSO_4$ treatments had significantly higher concentrations of Mg compared to those in leaf +4. The insecticide treatment had no significant effect on the Mg content of leaf +1 and leaf +4 (Figure 8). The $MgSO_4$ treatment had significantly higher concentrations of Mg in leaf +1 than both the insecticide and control treatments, but in leaf +4 this was only significantly higher than in the control.

Our hypothesis that providing sufficient plant Mg would reduce the incidence and severity of YCS was not supported with the data suggesting that Mg deficiency or imbalance is not a direct cause of YCS. We, therefore, speculate that a biotic agent may be responsible for the nutrient disruptions observed in earlier trials as this is a common symptom of plant diseases (Huber and Jones 2013).

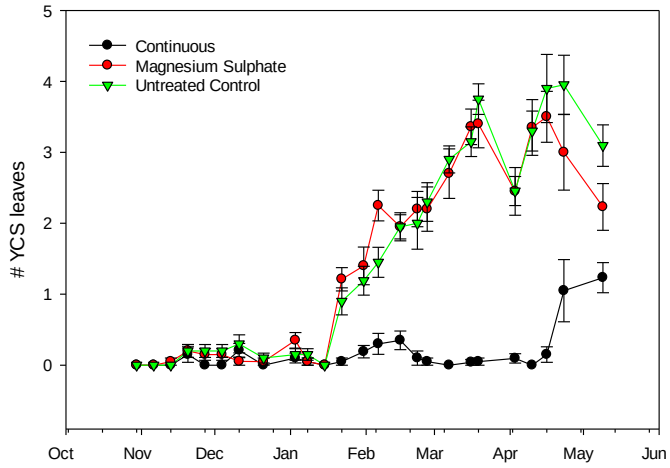

Figure 7. Mean number of symptomatic YCS leaves in the top seven leaves of the canopy across treatments. The Continuous treatment received weekly foliar applications of insecticide.

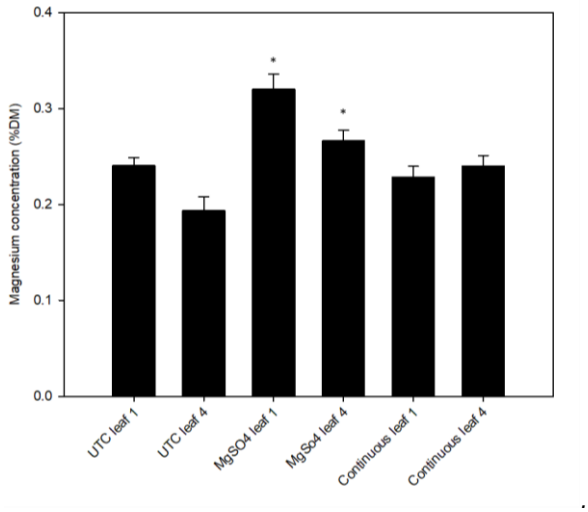

Figure 8. Leaf +1 and +4 mean magnesium concentrations (±SE) of YCS symptomatic cane (UTC (control) and $MgSO_4$) and YCS asymptomatic (Continuous). Statistically higher concentrations are denoted by an asterisk (*).

CONCLUSIONS

We conclude that YCS-affected cane is associated with reduced leaf-Mg concentrations, but that it is unlikely that this is the cause of YCS *per se*, as concentrations in all four trials were well above critical thresholds for plant health. YCS occurs independently of Mg and low Mg is an indirect effect rather than a cause. Given that disruption to plant nutrient balance has been described as a symptom of some plant diseases, we speculate that these findings suggest a biotic causal agent. The observed magnesium imbalances may relate to an interference or blockage, competition between plant and pathogen for cations, or plant defence mechanisms in response to a biotic stress such as a phytoplasma or insect feeding. Further research is being currently conducted to determine the causal agent of YCS.

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