

# INVESTIGATION INTO THE FLOWERING OF SUGARCANE VARIETY N29 GROWN UNDER DIFFERENT NUTRIENT REGIMES

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## Abstract

Little research has been conducted on the effect of nutrition on flowering of sugarcane plants used for breeding new varieties. Some problems with panicle emergence and survival being experienced at Mount Edgecombe are presumed to be due to incorrect nutrition of the cane plants. A preliminary investigation by the author revealed that nutrient balances within the plant could play an important role in the flowering of potted sugarcane plants. This prompted a further investigation into the impact of nutrition on panicle and seed production in the crossing programme at Mount Edgecombe.

Potted plants of variety N29 were grown in 10 fertiliser regimes involving two calcium, magnesium and micronutrient (CaMgMicro) treatments (-/+) by five macronutrient treatments. Four macronutrient treatments involved the removal of N, NK, NP, or NPK from the fertiliser used during plant establishment, while in the fifth treatment plants received NPK until anthesis. The fertiliser treatments significantly influenced the flowering and seed set of N29. No significant difference was observed between treatments for panicle initiation. Weekly application of nitrogen or CaMgMicro until anthesis significantly increased flowering, and a significant phosphorus by CaMgMicro interaction was observed. The application of potassium when CaMgMicro was not applied delayed flowering significantly. Weekly application of nitrogen until anthesis significantly reduced the percent pollen stained as well as the number of viable seeds per gram of fuzz. However, stalks receiving nitrogen or CaMgMicro until anthesis produced significantly heavier panicles, and significant interactions involving phosphorus, potassium and CaMgMicro were observed. Significantly more viable seeds were obtained per panicle or per gram of fuzz where potassium or CaMgMicro were not applied until anthesis. In addition, more viable seeds per panicle were obtained when no CaMgMicro was applied.

*Keywords:* sugarcane, flowering, nutrients, fertiliser

## Introduction

In South Africa, flowering of sugarcane in the field is markedly seasonal and those flowers obtained are invariably male infertile. In addition, many desirable parent clones are shy flowering at Mount Edgecombe (30°S). For these reasons, parental clones used in the crossing programme of the South African Sugar Association Experiment Station are propagated in large bins filled with river sand and induced to flower inside photoperiod facilities. Flower initiation in these facilities is fairly good (above 80% of stalks initiate). However, some problems with panicle emergence and survival are being experienced and are presumed to be the result of incorrect nutrition of the sugarcane plants.

Sugarcane produces flowers under a wide range of nutritional conditions; however, this does not mean that specific nutrients do not affect flowering (Arceneaux, 1967). Despite this, little research has been conducted on the nutrition of flowering sugarcane plants used for breeding new varieties. A preliminary investigation revealed that nutrients applied during cane growth might influence the extent of flowering (Brunkhorst, 2001). This prompted further investigation into the impact of nutrition on panicle and seed production in the crossing programme at Mount Edgecombe. The aim of this study was to investigate the effect of withdrawing nitrogen, phosphorus and potassium from the fertiliser applied during the first four months of plant growth, on the panicle formation and seed production of variety N29 in the photoperiod facilities. As calcium, magnesium and micronutrients are not routinely applied to plants in the photoperiod facilities, a further treatment was included to test whether repeated application of these nutrients would have an effect on flowering. It is hoped that the knowledge gained will enable future improvements to flowering and seed set in the crossing programme through manipulation of the fertiliser regime.

## **Materials and Method**

### *Plant establishment*

The sugarcane variety N29 was chosen for this experiment. When used as a parent in the photoperiod house, this variety had a mean panicle initiation rate of 88%, a mean panicle emergence rate of 68% and a mean of 60% stalks flowering. This meant that there was scope for improving the flowering of N29 in the facilities. In September 2001, single budded setts of N29 were germinated in vermiculite in plastic seedling trays (280 x 310 x 100 mm) placed on trestle tables inside a heated glasshouse (thermostat set at a minimum of 30°C). The trays were watered daily. After one week, the transplants were replanted into river sand in 25 L pots; three plants per pot. The pots were placed outside on trolleys on rails and were watered twice a day using drip irrigation. Each pot was fertilised weekly using the appropriate nutrient solution. All tillers were removed to ensure only three primary stalks per pot.

### *Fertiliser treatments*

Ten fertiliser treatments were used. These involved five nitrogen (N), phosphorus (P) and potassium (K) treatment combinations by two calcium, magnesium and micronutrient treatments (Table 1). For clarity, the calcium, magnesium and micronutrients were applied as one treatment with two levels, and will hereafter be referred to as CaMgMicro. During the first four months of growth, the plants received weekly applications of complete liquid fertiliser containing N, P and K; half of the pots were also fertilised with CaMgMicro. Thus, the CaMgMicro treatments were applied from the start of the experiment (September 2001). Macronutrient treatments began on 3 January 2002 when the cane was 120 days old. Four of the macronutrient treatments involved the removal of N, NK, NP or NPK from the fertiliser applied during the first four months of growth, whereas in the fifth treatment plants received NPK until anthesis (Table 1). All treatments were applied weekly until anthesis. Three pots were used for each fertiliser treatment (three replications of one pot per replication; nine plants in total per treatment).

The following analytical grade chemicals were used to prepare the fertiliser solutions: orthophosphoric acid, potassium sulphate, potassium hydroxide, calcium sulphate, magnesium sulphate heptahydrate, boric acid, cupric sulphate, ferrous sulphate, manganous sulphate monohydrate, sodium molybdate, and zinc sulphate. Urea was applied to supply nitrogen. According to the specific treatment being applied, each pot received all or a combination of the elements in the following amounts per application: 2.13 g N, 0.42 g P, 2.13 g K, 75 mg Ca, 24 mg Mg, 0.25 g B, 0.024 mg Cu, 2.01 mg Fe, 0.25 mg Mn, 0.009 mg Mo and 0.05 mg Zn.

The amount of N, P and K calculated to be placed in each pot was determined from the amount supplied by the standard fertiliser used in the photoperiod facilities, namely 5:1:5 (46). The rest of the nutrients were applied according to those used in standard hydroponic mixtures (Harris, 1994).

**Table 1. Fertiliser treatments applied to potted plants of N29 subjected to a standard photoperiod treatment.**

Treatment No.	Macronutrient treatment			Ca, Mg and Micronutrient treatment
	N	P	K	
1	+	+	+	-
2	+	+	+	+
3	-	+	+	-
4	-	+	+	+
5	-	-	+	-
6	-	-	+	+
7	-	+	-	-
8	-	+	-	+
9	-	-	-	-
10	-	-	-	+

#### *Flower induction and handling*

The heated glasshouse and photoperiod facilities used in this work have been described previously (Brett and Harding, 1974). All plants were subjected to the same photoperiod regime in the photoperiod house, which started in February 2002, with a daylength of 12h30 on 18 February. The standard photoperiod treatment is a 30 s/d decline, with the daylength of 12h30 being regarded as the start of the initiation period. Once the photoperiod treatment was imposed, the cane was moved into the photoperiod house each night, and out again in the morning. When panicle tips emerged, the stalks were cut from the pots, marcotted (air layered) and placed on racks inside a heated glasshouse. When florets from each panicle had opened, a small sample containing mature anthers was removed for pollen stain tests. These sampled panicles were placed in separate crossing compartments within the glasshouse, where they were left to self-pollinate and mature.

When the panicles were ripe (as indicated by fluffing up of the topmost portion of the panicle) they were harvested and placed overnight in a drying oven at 30°C to remove excess moisture. The fuzz containing all spikelet material, including seed, was removed from each dried panicle and weighed. Two 5% samples were removed from the fuzz of each panicle for duplicate germination tests. The fuzz was then stored in sealed plastic bags in a cold room at -20°C. The germination test procedure described previously (Brunkhorst *et al.*, 2000) was used to determine the number of viable seeds per panicle and the number of viable seeds per gram of fuzz.

#### *Data collection and analysis*

Eight flowering traits were investigated in this study. All flowering observations were determined on a stalk basis. At the completion of anthesis, each stalk that did not flower was bisected with a knife to determine the presence or absence of flower initials. The percentage stalks initiating and flowering were then determined for each pot. These percentages were transformed to degrees using the angular transformation (Gomez and Gomez, 1984) before statistical analysis.

The number of days to flowering was calculated from the start of the initiation regime (18 February 2002) to panicle emergence. Pollen stain was determined by calculating the percentage stained pollen grains from a starch iodide test. Panicle length (cm) was measured immediately after harvesting, before drying. The length was measured from the base of the panicle (where the first

rachilla joined the rachis) to the tip. Fuzz (containing all spikelet material, including seed) weight (g) was determined after it was removed from the dried panicle. The number of viable seeds per panicle or per gram of fuzz was determined for each panicle by calculating a mean from the two germination tests. After all measurements had been obtained, the results for all the flowered stalks in one pot were then combined to calculate pot means.

A factorial analysis was performed using GenStat (Release 4.2, fifth edition, 2000) testing four main effects (nitrogen, phosphorus, potassium and CaMgMicro) and their interactions. The Least Significant Difference test ( $P=0.05$ ) was used to compare treatment means.

## Results and Discussion

### *Initiation and flowering*

The initiation of stalks of sugarcane variety N29 was comparable, regardless of the fertiliser treatment they received (Table 2). Other authors pointed out that too little nitrogen might affect the intensity of flowering (Nuss and Berding, 1999). In this case, the application of nitrogen or CaMgMicro weekly until anthesis significantly increased the number of panicles obtained ( $P=0.041$  and  $P=0.020$  respectively; Table 2). Previously, it was found that phosphate application appeared to reduce flowering of sugarcane (Gosnell, 1973). In the current study, a highly significant phosphorus by CaMgMicro interaction ( $P=0.006$ ) was observed for the number of stalks flowering (Figure 1a). The application of phosphorus when CaMgMicro was not applied significantly reduced the number of stalks flowering. However, when phosphorus was withdrawn from the fertiliser, the CaMgMicro treatment had no effect. The best flowering was observed where phosphorus was applied with CaMgMicro.

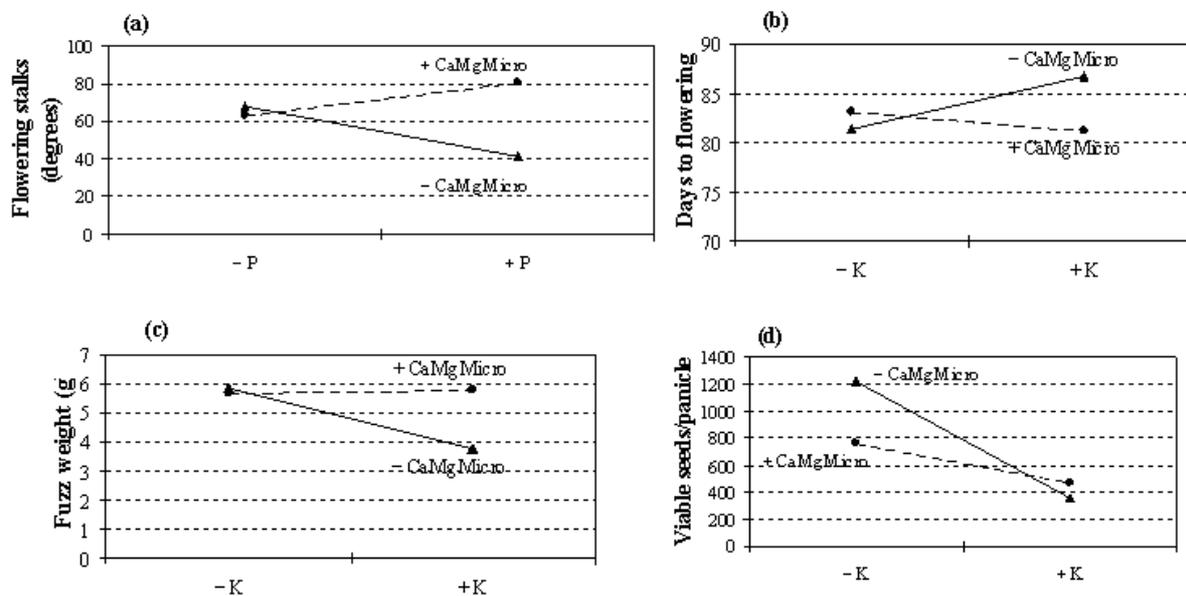
**Table 2. Means for eight flowering traits at two levels of nitrogen (N), phosphorus (P), potassium (K), or calcium, magnesium and micronutrients (CaMgMicro) for N29 subjected to a standard photoperiod treatment.**

Nutrient	Level	Flowering trait							
		Initiated stalks (degrees)	Flowered stalks (degrees)	Days to flowering	Pollen stain	Panicle length (cm)	Fuzz weight (g)	Viable seeds per panicle	Viable seeds per g fuzz
N	–	78.3	63.2	83.1	76.2*	45.9	5.3	701	133*
	+	85.0	80.0*	79.8	70.6	52.8*	8.8*	529	61
	LSD( $\alpha=0.05$ )	11.2	16.0	3.9	5.5	3.1	1.2	283	55
P	–	80.3	68.9	83.9	74.3	47.8	5.9	750	131
	+	79.2	65.1	81.4	75.6	47.0	6.0	612	110
	LSD( $\alpha=0.05$ )	10.0	14.3	3.5	4.9	2.8	1.0	254	49
K	–	83.2	64.4	81.4	76.9	48.7	6.6	1013*	169*
	+	77.2	68.1	83.1	73.9	46.4	5.6	436	85
	LSD( $\alpha=0.05$ )	10.0	14.3	3.5	4.9	2.8	1.0	254	49
CaMg Micro	–	76.2	58.8	83.8	74.8	46.3	5.4	791*	145*
	+	83.1	74.4*	81.1	75.4	48.3	6.6*	542	92
	LSD( $\alpha=0.05$ )	9.0	12.8	3.1	4.4	2.5	0.9	227	44

\*Treatment means are significantly larger than those for the alternate level.

High levels of nitrogen, especially during initiation, may reduce or delay flowering of sugarcane (Van Dillewijn, 1952; Clements and Awada, 1967; Nuss and Berding, 1999). However, in this experiment, there was no significant difference observed in the number of days to flowering for any of the main effects studied, and, as already mentioned, the application of nitrogen significantly increased the number of panicles obtained (Table 2). It is possible that the amount of nitrogen applied was not sufficient to induce the effects observed by other researchers. However, a

significant interaction ( $P=0.045$ ) for the number of days to flowering was observed between potassium and CaMgMicro (Figure 1b). The application of potassium when CaMgMicro was not applied significantly delayed flowering. However, when potassium was withdrawn from the fertiliser, the application of CaMgMicro had no effect. Therefore, in this instance, the delay in flowering could only be prevented by CaMgMicro being applied with potassium.



**Figure 1. Significant interactions observed between phosphorus (P) or potassium (K) and calcium, magnesium, micronutrient (CaMgMicro) treatments applied to potted plants of N29 subjected to a standard photoperiod treatment, for (a) the number of flowering stalks, (b) the number of days to flowering, (c) fuzz weight and (d) the number of viable seeds per panicle.**

#### *Panicle characteristics and seed set*

The application of nitrogen weekly until anthesis significantly reduced the percentage of stained pollen ( $P=0.044$ ; Table 2). However, stalks in these treatments produced significantly larger panicles, i.e. the panicles were significantly longer ( $P<0.001$ ) with significantly heavier fuzz weights ( $P<0.001$ ) (Table 2). Similarly, other researchers found that too little nitrogen may affect flower size (Nuss and Berding, 1999). Conversely, the number of viable seeds per gram of fuzz was significantly higher ( $P=0.014$ ) where nitrogen was withdrawn from the fertiliser treatment (Table 2). As the panicles were self-pollinated, this effect may have been linked to the significantly higher percentage of stained pollen.

As for nitrogen, the weekly application of CaMgMicro until anthesis played a role in the production of panicles with significantly heavier fuzz weights ( $P=0.017$ ; Table 2) but lower number of viable seeds per panicle or per gram of fuzz ( $P=0.033$  and  $P=0.022$  respectively; Table 2). Significant potassium by CaMgMicro ( $P=0.029$ ) and phosphorus by potassium by CaMgMicro ( $P=0.026$ ) interactions were observed for fuzz weight. The application of potassium significantly decreased fuzz weight when CaMgMicro was not applied (Figure 1c). When potassium was withdrawn from the fertiliser, the CaMgMicro treatment had no effect. Similarly, when potassium was withdrawn from the fertiliser, the effect of either phosphorus or CaMgMicro was not significant (Table 3). However, when potassium was applied, the effect of phosphorus was observed only when CaMgMicro was not applied, i.e. application of phosphorus increased fuzz weight if potassium was applied without CaMgMicro. Similarly, the effect of CaMgMicro was observed only when phosphorus was withdrawn from the fertilizer, i.e. application of CaMgMicro increased fuzz weight

if potassium was applied, but not phosphorus. Therefore, application of potassium without phosphorus or CaMgMicro seems to lower fuzz weight; however, application of phosphorus or CaMgMicro, or both, negates this negative effect. Interestingly, if potassium is applied, the addition of phosphorus and CaMgMicro together is equivalent to the addition of either treatment on its own.

**Table 3. Three-way table of means for fuzz weight (g) of panicles of N29 (subjected to a standard photoperiod treatment after being grown with 10 fertiliser treatments), showing differences between the two levels of phosphorus (P), potassium (K) and calcium, magnesium and micronutrients (CaMgMicro) used.**

		Mean fuzz weight (g)					
		- K			+ K		
		- CaMgMicro	+ CaMgMicro	Difference	- CaMgMicro	+ CaMgMicro	Difference
P	-	6.67	5.47	1.20 <sup>ns</sup>	2.78	6.00	3.22*
	+	4.99	5.90	0.91 <sup>ns</sup>	4.73	5.60	0.87 <sup>ns</sup>
	Difference	1.68 <sup>ns</sup>	0.43 <sup>ns</sup>		1.95*	0.40 <sup>ns</sup>	

\* = Significant difference ( $LSD_{(\alpha=0.05)} = 1.92$ ).

<sup>ns</sup> = Not significantly different.

Significantly more viable seeds were obtained per panicle or per gram of fuzz when weekly treatments of potassium ( $P < 0.001$  and  $P = 0.002$  respectively) or CaMgMicro (Table 2) were not applied until anthesis. A significant potassium by CaMgMicro ( $P = 0.049$ ) interaction was also observed for the number of viable seeds obtained per panicle (Figure 1d). The application of potassium without CaMgMicro significantly reduced the number of viable seeds per panicle. Similarly, the application of CaMgMicro when potassium was withdrawn from the fertiliser also significantly reduced the number of viable seeds per panicle. Therefore, when no potassium or CaMgMicro were applied, the highest number of viable seeds per panicle was obtained. Contrary to this, in plants potassium is found in areas of high physiological activity (Harris, 1994) and deficiencies may lead to poor flowering, fruiting and berrying (Greenwood, 1996). It is possible that in this experiment the plants received too much potassium, while plants in treatments where potassium was withdrawn from the fertiliser had sufficient reserves within their tissues to ensure good seed set.

### Conclusion

The results of this study indicate that the flowering and seed set of sugarcane variety N29 was significantly influenced by the fertiliser treatments applied. The role of nitrogen, potassium, calcium, magnesium and micronutrients needs to be studied further. In addition, the effect of fertiliser treatments would also have to be examined using other sugarcane varieties. Some progress can be made in the flowering and seed set of parents used in the crossing programme through manipulation of the fertiliser regime.

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