Original Research Article

SUGAR CANE WHIP SMUT (*Sporisorium scitamineum* Syd) CAUSED FIELD SUCROSE AND JUICE QUALITY LOSSES OF TWO SUGAR CANE VARIETIES IN NIGERIA`

ABSTRACT

Two sugar cane varieties were evaluated in a split plot design experiment at Badeggi (lat.9^{o}045'N; long 6^{o}07'E at an altitude of 70.57 m.a.s.l. ~~above sea level~~) with four whip smut (*Sporisorium scitamineum*) inoculum concentrations 0 x 10^{6}, 2 x 10^{6}, 4 x 10^{6} and 6 x 10^{6} teliospores/ml in four replicates between 1998 and 2000. The field sucrose production (% brix) was measured with a hand refractometer by using the stalks of five tagged healthy and smutted canes which were individually punched and a drop of the juice from each of them placed on the hand refractometer and covered. This was then held against the sun and viewed for the brix reading, which was recorded in percent. For the juice quality laboratory yield loss assessment, 2 healthy stalks were randomly cut from each plot and five smutted stalks were crushed using the Jeffco cutter to obtain at least 2 kg of crushed material for quality analysis. Six hundred grams ~~grammes~~ of the crushed material were taken and pressed using the hydraulic hand press. The resulting juice was collected in 250ml conical beakers. The first and last expressed brix of the juice were recorded. The temperature and hydrometer readings of the juice were also recorded. The weight of the wet bagasse was taken and again recorded after oven drying to a constant weight. These readings were used in the calculation of % reducing sugars, % Pol., Corrected brix, % Purity and % Fibre. Results showed that *S. scitamineum* reduced field sucrose (% Brix), % Pol., % Purity and % Fibre but increased % reducing sugars of the two test infected cane varieties.

Key words: Field sucrose, % Pol, % Purity, % Fibre, Juice quality loss, Expressed brix.

INTRODUCTION

Sugar cane whip smut caused by the dimorphic basidiomycete fungus *S. scitamineum* Sydow [M. Piepenbr., M. Stoll & Oberw. 2002 (Syn: *Ustilago scitamiea* H. & P.Sydow)] incites considerable losses in sugar cane yield and quality in almost all cane growing countries of the world. In Florida, USA, Valladares and Gonzáles (1986) and Rott and Comstock (2002) investigated the quality and juice lowering effect of *S. scitamineum* and found that the disease caused a highly significant decrease in the height and diameter of the stalk, plant weight and juice in plant and ratoon crops. In Louisiana, Irvine (1982) reported drop in sucrose; purity and viscosity of cane juice and 20% loss in sugar recovery of smut infected cane. Also, other workers reported reduced number of healthy stalks of sugar cane infected by smut in Louisiana (Hoy *et al*., 1986).

Peros (1984) reported sucrose inversion effect of *S. scitamineum* in France. Also Peros *et al*., (1986) studied carbohydrate metabolism of *S. scitamineum* from Florida and indicated that glucose, fructose or sucrose could be used interchangeably as C sources and noted the rapid inversion of sucrose. This result demonstrates the negative effect of *S. scitamineum* on sucrose, the actual yield of sugar cane. The negative effect of *S. scitamineum* on sucrose concentration in sugar cane leaves had earlier been reported (Taneja *et al*., 1987).

From the West Indies, report by Whittle (1982) shows that *S. scitamineum* caused low yield of infected cane. Elsewhere, Gomez *et al*., (1989) conducted studies on exudate effects of *S. scitamineum* on cells of sugar cane. They observed that addition of the exudate of the pathogen unto media containing suspensions of known sugar cane varieties increased cell size and caused cell death, particularly in the more susceptible variety.

Msechu and Keswani (1982) conducted yield loss studies by smut in Tanzania and reported its effect to be poor juice quality. Glaz *et al*., (1989) studied the effect of *S. scitamineum* on 4 cane varieties of variable resistances from plant to 2nd ratoon crop and reported reduced cane and sucrose yields. Indi *et al*., (2012) and Sundar *et* al., 2012) reported that whip smut of sugar cane caused by the dimorphic basidiomycete fungus *S. scitamineum* incited considerable losses in sugar cane yield and quality. Similarly, studies on quality parameters by Indi *et al*., (2012) showed that field sucrose, brix and purity of sugar are adversely affected in smutted canes.

The effects of *S. scitamineum* are aggravated when susceptible varieties are cultivated. Barnabas *et al*., (2012) reported significant tonnage loss and reduced juice quality as the result of *S. scitamineum* infected cane which they said could devastate large areas when cultivated with susceptible varieties. Sahu and Kumar (2012 in their report asserted that besides heavy quantitative losses, *S. scitamineum* also reduces cane quality parameters like Brix, sucrose and purity of affected canes.

On quality parameters like reducing sugars in juice, apart from the effect of *S. scitamineum*, factors such as harvesting time, storage duration, pH value, presence of bacteria and temperature affect reducing sugars in juice (Tan *et al*., 2011 and Muangmontri, *et al*., 2014).

In Nigeria *S. scitamineum* is reported to be the most important sugar cane disease (Obakin, 1978 and Wada, 1997). The seeming yield or quality effect of *S. scitamineum* on cane in Nigeria is on the report of it being responsible for the discontinued cultivation of the commercial variety

D141/46 by the then Nigerian Sugar Company (NISUCO), Bacita in 1978 (Ogunwolu, 1986). There have been no detailed studies carried out to investigate the qualitative losses caused on sugar cane in terms of total solids and juice quality parameters like sucrose, temp. corrected brix, %Pol, %purity and %reducing sugars in Nigeria by *S. scitamineum*. In order to bridge this gap in knowledge and provide sugar cane growers with information on the qualitative losses incited by whip smut, the present study was, therefore, set up to investigate the effects of varying concentrations of *S. scitamineum* on the yields of two cane varieties and to ascertain their losses in juice quality terms.

MATERIALS AND METHODS

Preparation of Smut teliospores

Fresh smut whips were collected from the field of a Bida local cane in the early hours of each morning for three days as described by Nasr (1977). These were dried under shade for one hour, scrubbed with hands covered with sterilized gloves to obtain smut teliospores, and then sieved using 53µm mesh. The sieved teliospores were weighed out in three categories of 10g, 20g and 30g and sealed in cellophane bags and stored in the refrigerator in the laboratory for inoculation process at a later date.

Preparation of smut teliospores suspension

The 10, 20 and 30g smut teliospores earlier weighed out and stored in cellophane bags were each emptied into separate 50l ~~litres~~ of sterile water in three different inoculating containers as described by Nasr (!977). These were vigorously stirred to obtain a homogenous suspension of the teliospores corresponing to 2, 4 and 6 g teliospores l~~itre~~$^{-1}$ which gave three haemocytometer values of 2 x 10^{6}, 4 x 10^{6} and 6 x 10^{6} teliospores/ml concentrations.

Preparation of planting setts and inoculation

Cuttings of the two test cane varieties Co 957 and Bida local were made from 7 old canes. The stalks of the test canes were detrashed to expose the buds. The detrashed stalks were then cut into 3-budded setts and subjected to hot water treatment at 52^{o}C for 30 min~~utes~~ in separate batches until the whole planting setts were heat treated. One thousand, nine hundred and twenty (1920) 7-budded sett sod each of the two varieties were hot water treated in all. The heat treated cane setts were then separated into groups of 120 -3 budded setts each representing the four treatments. The cane cuttings were then immersed in each of the three teliospore concentrations for 1 h~~our~~ and incubated overnight in wet sterile gunny jute bags under the shade according to Nasr (1977). They were removed and planted in 5m x 5m plots in the field the following day. There was uninoculated control for each of the two varieties.

Determination of qualitative losses

Brix or field sucrose production measurement

The field sucrose production (% brix) was measured with a hand refractometer as described by Meade and Chen (1977). The stalks of five tagged healthy and smutted canes were individually punched and a drop of the juice from each of them was placed on the hand refractometer and covered. The hand refractometer was held against the sun, viewed for the brix reading, which was recorded in percent.

Juice quality analysis

For the qualitative laboratory yield loss assessment, 2 healthy stalks were randomly cut from each plot and five smutted stalks were crushed using the Jeffco cutter to obtain at least 2 kg of crushed material for quality analysis. Six hundred grams ~~grammes~~ of the crushed material was taken and pressed using the hydraulic hand press. The resulting juice was collected in 250ml conical beakers. The first and last expressed brix of the juice were recorded. The temperature and hydrometer readings of the juice were also recorded.

The weight of the wet bagasse (that is the chaff left after juice had been pressed) was taken and again recorded after oven drying to a constant weight. These readings were used in the calculation of % reducing sugars, % Pol., Corrected brix, % Purity and % Fibre as follows:

Determination of reducing sugars

Five millilitres each of Fehlings solution A which contains 7% anhydrous copper (II) sulphate ($CuSO_4 . 5H_2O$) and Fehlings solution B which contains 25% Potassium hydroxide (KOH) and 35% sodium potassium tartarate (alcoholic sodium/potassium tartarate) were pipetted into each clean conical flask depending on the number of samples following the methods of David (1976), AOAC (1984) and CAC (1989). To each of these were added 10ml of distilled water and 5ml of the juice. This mixture was heated to boiling on a hot plate for 2 min~~utes~~. Five drops of methyl blue indicator were added to it and titrated with the addition of fresh juice to the boiling mixture till a brick red colour resulted. The amount of juice added plus the quantity (ml) used for titration was the reducing sugar titre. This was checked from the tables (Payne, 1968), and the corresponding figure gave the % reducing sugars.

Determination of Polarimeter Reading for % Pol calculation

One hundred millilitres of the juice was pipetted into conical flasks and 1 g of lead acetate was added to it, covered with a rubber bung and shaken vigorously. The mixture was filtered using Whatman paper No. 1. The first 10 ml of the filtrate was discarded, while the next was used to read the Polarimeter (Pol R).

Determination of % pol

Percent Pol was calculated by checking up the temperature corrected brix against the hydrometer brix from tables (Payne, 1968).

Temperature corrected brix %

The resulting value was added or subtracted to, or from the hydrometer reading to obtain the corrected brix. To calculate % Pol, the temperature corrected brix was checked against the hydrometer brix from the tables (Payne, 1968) to give hydrometer reading. This was used to check the Pol factor; the resulting value gave the % Pol. In cases where the juice did not give the hydrometer reading and temperature, % Pol was calculated using the first expressed brix:

% Pol = brix x 2.5 x Pol R (Payne, 1968; Barnes 1974).

Determination of % Purity

$$\% \text{ Purity} = \frac{\% \text{ Pol} \times 100}{\text{First expressed brix}}$$

Wet weight of bagasse - Oven dried weight = Moisture.

$$\frac{\text{Moisture x last expressed brix}}{100} = \text{Sugar left in bagasse}$$

$$\% \text{ Fibre} = \frac{\text{Dried weight of bagasse - sugar left in bagasse}}{600}$$

Where 600 was the weight of crushed cane used for the quality analysis.

RESULTS AND DISCUSSION

Qualitative Assessments

Effects of inoculum concentration and sugar cane variety on sucrose production, 1998 - 2000

Table 1 shows that there was significant (P = 0.01) difference between the % brix or sucrose of Bida local and Co 957 at 6, 9 and 12 MAP and MAR. In other words sucrose was significantly and consistently greater in Co 957 than in Bida local at all the three stages of the cane sampled from 1998 - 2000. Also Table 1 indicates that there was no significant difference between the sucrose of Co 957 and Bida local in 1998. On the other hand, there were significant differences of effects of variety and inoculum concentration on field sucrose in 1998. Significantly (P=0.01, 0.05) less sucrose was obtained at both 9 and 12 MAP in canes from treatments with high smut inoculum loads than the significantly higher sucrose obtained from the lower inoculum concentration and the check. The same table shows that smutted canes of Co 957 consistently contained significantly (P =0.01) higher sucrose than those of Bida local. Variation in inoculum concentration did not influence sucrose accumulation of ratoon crop at 6, 9 and 12 MAR in 1999 at the three sampling periods.

Table 2 shows that significantly (P=0.05) less sucrose was contained in smutted canes sampled from treatments with the highest inoculum load in 1998. Bida local, however, recorded significant (P=0.01) interactions of variety and inoculum concentration on field sucrose of smutted cane stalks that were not linear. The highest inoculum concentration treatments, recorded significantly the least amount of sucrose compared to the less inoculum concentration treatments, which recorded significantly higher amounts in 1999 and 2000 ratoon canes. These were similar to those recorded with the uninoculated control treatments in Co 957 and Bida local at 6 MAR in 2000.

Table 3 shows that Co 957 and Bida local recorded significant ((P=0.05, 0.01) differences on the % brix, % reducing sugar and % fibre out of the five parameters assessed with 1998 plant cane. Significantly more brix and fibre were obtained in Co 957 than in Bida local, but less reducing sugar was obtained from Co 957 than Bida local cane. On the other hand, effects of variety and inoculum concentration on juice quality of canes harvested from sugar cane with varying levels of smut inoculum load and check did not differ significantly among themselves in 1998. There were, however, no significant interactions of variety and inoculum concentration on the five-juice quality parameters assessed at harvest.

All the five quality parameters assessed were significantly higher in Co 957 than in Bida local cane, except % reducing sugar which was significantly less in Co 957 than in Bida local in 1999. On the other hand, no significant differences in temperature corrected brix, % polarity and purity were observed among the different treatments as the result of varying inoculum concentration levels in 1999. Interaction of variety and inoculum concentration on the temperature corrected brix, % pol, purity; reducing sugar and fibre of cane juice were also not significant in 1999.

Effects of sugar cane variety and inoculum concentration on juice quality, 1999 - 2000

Table 4 shows that of the five parameters assessed, significant difference was observed on the brix and on % pol as well as on % fibre between the two varieties. In other words, % brix, polarity and fibre of Co 957 were significantly higher than those in Bida local. On the other hand, increases in inoculum concentration did not result in significant differences in the juice quality parameters of temperature-corrected brix, percent reducing sugar, and percent fibre.

Significant (P=0.05, 0.01) differences on percent polarity and % purity of the juice were observed with increase in inoculum concentration. Though non-significant differences were observed, temperature corrected brix, % reducing sugar and % fibre were least in cane harvested from treatments with the highest inoculum concentration than the higher values recorded in the other treatments which were again lower than the highest values in the check. Percent polarity and purity were significantly least in canes harvested from treatments with the highest inoculum concentration, while higher values in these parameters were recorded in the other treatments. There was, however, no significant interaction of variety and inoculum concentration observed on any of the five quality parameters assessed at harvest in 1999.

Table 4 also shows that there was significant (P=0.05, 0.01) difference between the juice quality parameters of brix, % purity and fibre of ratoon crops of Co 957 and those of Bida local at harvest in 2000. These quality parameters were significantly higher in Co 957 than in the juice of Bida local in 2000. Similarly, there were significant (P=0.05, 0.01) differences observed on % pol and % purity of the juice assessed at harvest with increase in inoculum concentrations in 2000. That is to say, significantly, the least pol and purity were recorded with the 6 x 10^6 teliospores/ml inoculum concentration treatments which were significantly lower than those recorded with the other treatments. The juice quality parameters recorded from the 2 x 10^6 and 4 x 10^6 teliospores/ml inoculum concentration did not differ significantly from each other. However, no significant interaction of variety and inoculum concentration was observed on any of the juice quality parameters assessed at harvest in 2000.

The qualitative assessment of loss caused to sugar cane by *S. scitamineum* was investigated on field sucrose (% brix) of healthy and smutted canes and on juice quality from 1998 – 2000. Generally, effects of variety and inoculum concentration on juice quality parameters were significant with increase in inoculum concentration. However, significantly lower sucrose accumulated in smutted canes from the high inoculum concentration treatments compared to higher sucrose content in lower inoculum concentration treatments in the two cane cycles from 1998 - 2000. All the control treatments did not record smutted stalks and consequently the sucrose values for these treatments were zero.

The observed significant reduction in the brix of smutted Co 957 and Bida local by 4-7 units in the present study conforms to the findings by several workers (Taneja *et al*., 1987; Padmanaban *et al*., 1988a; 1988b and Glaz *et al*., 1989). Smut reduced the field sucrose of affected stalks by at least half, compared to those of healthy stalks in the present study.

The quality parameter of the two test varieties was generally significantly different. On the other hand, effects of inoculum concentration and variety generally did not significantly affect the quality parameters of the assessed juice with increase in inoculum concentration. Interactions of variety and inoculum concentration were also generally not significant.

Generally, increased disease level resulted in decreased quality parameters of % brix; % pol, % purity and % reducing sugar and increase in % fibre in the present study. Other workers also observed similar reduction in the juice quality of infected cane (de Ramallo, 1980; Irvine, 1982; Kumar *et al*., 1989; Padmanaban *et al*., 1989b; Taneja *et al*., 1987, Tai *et al*., 1996, Singh, 1998 and Pushpavalli *et al.*, 2014). On the contrary, report by Martinez *et al*., (2000) indicated variation in some juice quality parameters among three sugar cane varieties studied. They showed that infection of cane with whip smut resulted in decrease in the content of reducing sugars of juices, most markedly for Mayari plants, and increase in the value of % pol. They also observed that the value of brix remained unchanged for Jaromi and Barbados varieties following infection but increased for Mayari plants.

The result of the present study, therefore, agrees with the majority observations by these workers and differs from the findings by Martinez *et al*., (2000) in some quality parameters. The high percent reducing sugar figures indicate that smut must have reduced the sucrose in the affected canes of Co 957 and Bida local.

CONCLUSION Conclusion must be without references, they should conclude with your own data

Studies on quality parameters by Indi *et al*., (2012) showed that the field sucrose, brix and purity of smutted cane juice were adversely affected by *S. scitamineum*. The markedly reduced percentage brix, pol, purity and fibre of *S. scitamineum* infected canes in the present study are in agreement with the report of Indi *et al*., (2012) and Tai *et al*., 1996 but differ from the report by Sandhu *et al*.,(2012) on fibre. On the result of increased reducing sugars, it could not be the effect of *S. scitamineum* alone but due to other factors like harvesting time, storage duration, pH value, presence of bacteria and temperature which affect reducing sugars in juice (Tan *et al*., 2011 and Muangmontri, 2014). In the present study, the test canes were harvested and crushed the same day, however, due to the large number of samples, the duration of the analysis must have increased and caused the sharp increase in reducing sugars other than the effect of *S. scitamineum* alone.

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Table 1. Effects of variety and inoculum concentration on field sucrose (% Brix) of smutted canes 1998, 1999 and 2000

	1998 plant crop			1999 ratoon crop			1999 plant crop			2000 ratoon crop		
Treatment	6	9	12 (Harvest)	6	9	12 (Harvest)	6	9	12 (Harvest)	6	9	12 (Harvest)
Variety (V)												
Co 957	0.0	10.8a	10.2a	14.2a	13.7b	13.7a	9.8a	13.1a	15.6a	14.4a	13.6a	16.5a
Bida local	0.0	8.1a	8.4a	4.0b	6.2b	6.1b	5.5a	7.8b	8.0b	9.0a	10.9a	10.9b
Mean	0.0	9.5	9.3	9.1	10.0	9.9	7.7	11.5	11.8	11.7	12.3	13.7
SE±	0.0	1.1	1.3	0.3	0.5	0.5	0.7	0.3	0.5	0.3	1.7	0.5
	NS	NS	NS	**	**	**	**	**	**	**	NS	**
Inoculum concentration (I) (teliospores/ml)												
0.0	0.0	0.0b	0.0c	8.9a	10.4a	10.1a	8.5a	12.0a	12.6a	12.2a	13.2a	14.4a
2×10^6	0.0	13.4a	13.6.a	9.3a	10.1a	9.7b	7.2a	11.6a	11.9a	12.0a	12.3a	14.0a
4×10^6	0.0	12.8a	12.4a	9.3a	9.8a	9.9b	7.8a	11.1a	11.5a	12.2a	11.9a	14.0a
6×10^6	0.0	11.7a	11.2b	9.0a	9.5a	9.7b	7.1a	11.0	11.5a	10.6b	11.7a	12.6a
Mean	0.0	9.3	9.3	9.2	10.0	9.9	7.7	11.4	11.8	11.8	12.3	13.8
SE±	0.0	1.3	1.3	0.5	0.5	0.4	0.5	0.7	0.8	0.5	1.1	1.1
	NS	**	*	NS	NS	NS	NS	NS	NS	*	NS	NS
Interaction												
V*I	NS	NS	*	NS	NS	*	NS	NS	NS	*	NS	NS

Means followed by similar letter(s) are not significantly different at P=0.01, P=0.05 according to Duncan's Multiple Range Test (DMRT)

NS = Not significant

Table 2. Interaction of variety and inoculum concentration on field sucrose (% Brix) of smutted canes, 1998, 1999 and 2000

Treatment	1998 plant crop variety		1999 ratoon crop variety		2000 ratoon crop vareity	
(teliospores/ml)	Co 957	Bida local	Co 957	Bida local	Co 957	Bida local
0.0	0.0d	0.0d	13.81b	6.1e	14.1c	9.9d
2×10^6	11.2b	11.3b	13.5b	6.8d	14.8a	9.5e
4×10^6	17.3a	9.9c	14.6a	6.2e	14.6a	9.8d
6×10^6	12.3b	12.5b	13.1c	5.3f	14.3b	6.9f
SE$\pm$	1.30		0.40		0.50	

Means followed by similar letters(s) are not significantly different at P=0.01, P=0.05 according to Duncan's Multiple Range Test (DMRT)

Table 3. Effects of variety and inoculum concentration on juice quality, 1998 and 1999

Treatment	Quality parameters									
	1998 plant					1999 ratoon crop				
	Tem. Corr. Brix (%)	% Pol	% Purity	% Reducing sugar	% Fibre	Tem. Corr. Brix (%)	% Pol	% Purity	% Reducing sugar	% Fibre
Variety (V)										
Co 957	20.6a	15.7a	80.5a	1.2a	16.8a	22.0a	18.0a	82.3a	0.3a	18.0a
Bida local	15.0b	11.1a	73.7a	2.7a	10.0a	15.0b	10.3b	71.9b	0.6a	10.0b
Mean	17.8	18.4	77.1	2.0	13.4	18.5	14.2	77.1	0.5	9.0
SE±	0.6	1.9	2.8	0.2	0.5	0.3	0.8	2.4	0.06	0.5
	*	NS	NS	**	**	**	**	**	*	**
Inoculum concentration (I) (teliospores/ml)										
0.0	18.3a	14.3a	77.9a	2.3a	14.6a	19.0a	15.5a	77.8a	0.5a	14.6a
2×10^6	17.9a	13.5a	77.7a	2.0a	13.6a	17.8a	14.3a	77.7a	0.4a	14.3a
4×10^6	17.3a	13.3a	77.0a	1.9a	13.2a	17.7a	13.9a	76.7a	0.4a	13.6a
6×10^6	17.1a	12.4a	75.9a	1.8a	12.4a	17.6a	12.9a	76.2a	0.4a	13.6a
Mean	17.5	13.4	77.1	1.6a	13.5	18.0	14.2	77.1	0.4	14.0
SE±	1.1	1.3	2.9	0.6	1.6	0.6	0.9	2.1	0.06	0.5
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction										
V*I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means followed by similar letters(s) are not significantly different at P=0.01, P=0.05 according to Duncan's Multiple Range Test (DMRT)

NS = Not significant.

Table 4. Effects of variety and inoculum concentration on juice quality, 1999 and 2000

Treatment	Juice Quality parameters									
	1999 plant crop					2000 ratoon crop				
	Tem. Corr. Brix (%)	% Pol	% Purity	% Reducing sugar	% Fibre	Tem. Corr. Brix (%)	% Pol	% Purity	% Reducing sugar	% Fibre
Variety (V)										
Co 957	22.1a	17.3a	78.6a	0.5a	17.6a	21.5a	17.7a	81.7a	0.6b	18.6a
Bida local	16.9b	13.0b	76.4a	0.6a	9.5b	14.7b	11.0b	75.1b	1.0a	10.0b
Mean	19.5	15.1	77.5	0.6	13.6	18.1	14.4	78.4	0.8	14.1
SE±	1.1	0.7	1.8	0.2	0.2	0.6	0.4	1.3	0.2	0.7
	**	**	NS	NS	**	**	**	**	NS	*
Inoculum concentration (I) (teliospores/ml)										
0.0	20.0a	16.6a	82.8a	0.6a	13.8a	18.7a	15.1a	80.2a	0.8a	14.4a
2×10^6	19.9a	15.7b	79.1ab	0.5a	13.6a	18.2a	14.9a	80.0a	0.8a	14.2a
4×10^6	19.1a	14.6c	76.2b	0.7a	13.2a	17.9a	14.4c	79.8a	0.7a	13.9a
6×10^6	18.9a	13.6a	71.9c	0.5a	13.8a	17.6a	13.1b	73.6	0.7a	13.7a
Mean	20.0	15.1	77.5	1.1	13.6	18.1	14.4	78.4	0.8	14.1
SE±	1.0	1.0	3.0	0.1	0.4	0.5	0.3	1.9	0.2	0.4
	NS	*	**	NS	NS	NS	*	**	NS	NS
Interaction										
V*I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means followed by similar letters(s) are not significantly different at P=0.01, P=0.05 according to Duncan's Multiple Range Test (DMRT)

NS = Not significant.