SHORT NON-REFEREED PAPER

EFFECT OF PHOTOPERIOD TREATMENTS ON POLLEN VIABILITY AND FLOWERING AT THE SOUTH AFRICAN SUGARCANE RESEARCH INSTITUTE

HORSLEY T AND ZHOU M

South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa.
Tasmien.Horsley@sugar.org.za

Abstract

Sugarcane does not produce viable pollen in South Africa because flowering occurs in winter when temperatures fluctuate below 20°C, the minimum threshold for pollen survival. Photoperiod treatments are required to enhance pollen survival and induce, synchronise and distribute flowering across the pollination season. At the South African Sugarcane Research Institute (SASRI) six photoperiod treatments are deployed: G1, G2 and G3 in the glasshouse and P1, P2 and P3 in the photoperiod house. In the glasshouse, natural day-length is extended by reducing the rate at which it declines naturally. In the photoperiod house, day-length is reduced by 30 seconds per day and the treatments are started at different times. The aim is to synchronise flowering between G1 and P1, G2 and P3, and G3 and P2. The objective of this study was to evaluate the effect of photoperiod treatment on 'pollen viability' and 'days until flowering'. Analysis over a five-year period showed that genotypes from the photoperiod house produced significantly ($P<0.001$) more fertile pollen than genotypes from the glasshouse. None of the photoperiod treatments showed synchronised flowering. Genotypes from G1 flowered two days earlier than those from P1 ($P<0.001$); G2 genotypes flowered 12 days earlier than P3 genotypes ($P<0.001$); and G3 genotypes flowered three days later than P2 genotypes ($P<0.001$). Since sugarcane pollen is generally viable for only 20 minutes, female flowers from G1 and G2 could lose receptivity before male flowers from P1 and P3 are fertile. Likewise, males from P2 could shed pollen before females from G3 are receptive. These results emphasise the need for further optimisation of photoperiod treatments at SASRI.

Keywords: plant breeding, flowering, photoperiod treatments, glasshouse, pollen viability

Introduction

Flowering of sugarcane is a subject of considerable significance to the entire sugarcane industry for very different reasons. From a production viewpoint, flowering is looked upon with disfavour since it results in reduced sugar yields (Coleman, 1960). On the other hand, flowering is essential for plant breeders to develop new varieties. Its importance has led most sugarcane growing countries to conduct research on factors involved in the flowering process so that they might be controlled. While the flowering of sugarcane occurs naturally in many countries, the production of fertile pollen occurs only rarely under field conditions in South Africa, thus seriously hindering the breeding programme. Since flowering is regulated by day-length, photoperiod treatments are used to enhance pollen survival and induce, synchronise and distribute flowering across the pollination season (Brett, 1951; Moore and Nuss, 1987).

Flower induction in sugarcane is generally effective only after the juvenile period has been completed, when at least two to four internodes have matured (Clements and Awada, 1967; Coleman, 1969; Julien, 1973). Adequate water and temperatures above 18°C are also necessary (Coleman, 1969). The optimal temperatures for panicle development and pollen fertility are 28°C during the day and 23°C at night. Temperatures below 23°C delay panicle development and reduce pollen fertility (Brett and Harding, 1974; Berding, 1981), while daytime temperatures above 31°C and night-time temperatures below 18°C are detrimental (Clements and Awada, 1967; Moore and Nuss, 1987).

Photoperiod treatments at SASRI have centred around inducing flowering in non-flowering genotypes, synchronising flowering between early and late flowering genotypes, and increasing the amount of fertile pollen produced. The purpose of the present investigation was to determine the effectiveness of the photoperiod treatments. Bearing in mind that there are genotypic differences in response to photoperiod, this report deals only with the broad trends observed over a five-year period. Throughout the text, 'flower initiation' refers to the visible transition of the apex from a vegetative stage to a reproductive stage (arrows just starting to emerge), and 'flower emergence' refers to a fully differentiated inflorescence (tassels fully emerged).

Materials and Methods

Photoperiod treatments

Six photoperiod treatments are deployed at SASRI, viz. G1, G2 and G3 in the glasshouse and P1, P2 and P3 in the photoperiod house (Table 1). In the glasshouse, natural day-length is extended by reducing the rate at which it declines naturally. In the photoperiod house, day-length is reduced by 30 seconds per day and the treatments are started at different times. The aim is to synchronise flowering between G1 and P1, G2 and P3, and G3 and P2. The photoperiod house, because of greater day-length control, is used to promote flowering in shy-flowering varieties and to increase the incidence of males. Each parent variety is allocated to a particular photoperiod treatment, which determines the approximate flowering date of the variety and whether it will be male or female.

Table 1. Photoperiod treatments deployed in the breeding facilities at the South African Sugarcane Research Institute.

Facility	Treatment	Day-length (hours)	Rate of decline
Glasshouse	G1	12.30	constant dawn at 05h45
	G2	12.30	constant dawn at 05h30
	G3	13.00	constant day-length of 13 hours for 60 days; then 60 sec decline for 10 days; then 90 sec decline until end of treatment
		12.40	
		12.30	
Photoperiod house	P1	12.35	30 sec decline
	P2	12.30	30 sec decline
	P3	12.30	30 sec decline

Setts from the selected parent varieties are planted into canisters on a rail track outside the glasshouse and photoperiod house, where they are left until flower induction treatments commence. Plants are fertilised with weekly applications of NPK (5:1:5) fertiliser during the main period of growth. Several months before inductive treatments, the nitrogen (N) fertiliser is very considerably reduced, as N is known to inhibit flowering (Brett and Harding, 1974).

At the time of treatment (when stalks have 2-4 internodes), the trains are moved into the heated breeding facilities every night, where manipulation of day-length stimulates the plants to flower. Both fluorescent and incandescent lights are used and heating is provided by means of hot water pipes, with temperatures prevented from falling below 20°C and relative humidity kept above 70%.

Pollen viability

For determining pollen viability, a 1% iodine solution is used to stain pollen. Slides are viewed under a light microscope where the amount of darkly stained pollen is recorded as a percentage (representing fertile pollen). Genotypes with less than 30% staining are used as females during crossing, while those with staining above 30% are used as males.

Statistical analysis

To determine the effectiveness of the photoperiod treatments, 'time to flowering' and 'percentage pollen stain' data from 2008-2012 were analysed using the statistical linear mixed model of SAS (2009). Graphs were generated using Microsoft Excel.

Results and Discussion

Analysis over a five-year period showed that genotypes from the SASRI photoperiod house produced significantly ($P<0.001$) more fertile pollen when compared to genotypes from the glasshouse. This is similar to the finding of Brett and Harding (1974), who also reported that glasshouse treatments at Mount Edgecombe had lower male fertility than photoperiod house treatments. They attributed this to high temperatures within the glasshouse at the start of the induction period leading to overheating and consequent pollen damage. Previous studies have shown that after 35 min at 26.5°C and 67% relative air humidity, sugarcane pollen loses all viability (Moore, 1976; Venkatraman, 1922). In addition, even under ideal conditions, some sugarcane varieties show poor pollen fertility or even male sterility due to the cytogenetic abnormalities that occur during meiosis that are associated with sugarcane's high polyploid multispecies genetic complex (Ethirajan, 1987). In the present study, average pollen fertility in both facilities ranged from 33-41%.

Of the total number of stalks treated in the South African Sugarcane Research Institute (SASRI) plant breeding facilities from 2008 to 2012, only 24-57% produced flowers available for crossing (Figure 1a). For all treatments, there was a drastic reduction in the number of stalks that showed flower emergence, compared to the number of stalks that showed flower initiation. Failure to emerge may be attributed to several reasons. In some stalks, the young primordium fails to develop due to sub-optimal environmental conditions and the apex reverts to the vegetative stage. At other times, a fully differentiated inflorescence may be present, but for unknown reasons fails to reach its maximum size and therefore does not emerge. There are also instances when mature inflorescences fail to emerge because the leaf sheath acts as a mechanical barrier (Julien, 1969). This highlights the importance of studying the effect of photoperiod treatments on different stages of flowering, since not all individuals that show flower initiation progress to flower emergence.

When comparing time to flower (where day one is the first day of the year), none of the photoperiod treatments showed synchronised flowering (Figure 1b). Genotypes from G1 flowered two days earlier than those from P1 ($P<0.001$); G2 genotypes flowered 12 days earlier than P3 genotypes ($P<0.001$); G3 genotypes flowered three days later than P2 genotypes ($P<0.001$). Since pollen viability generally lasts for only 20-24 minutes (Moore

(1976) predicted a half-life of 12 minutes), males from P2 could shed pollen before females from G3 are receptive. Likewise, female flowers from G1 and G2 could lose receptivity before male flowers from P1 and P3 are fertile. These results emphasise the need for further optimisation of photoperiod treatments at SASRI.

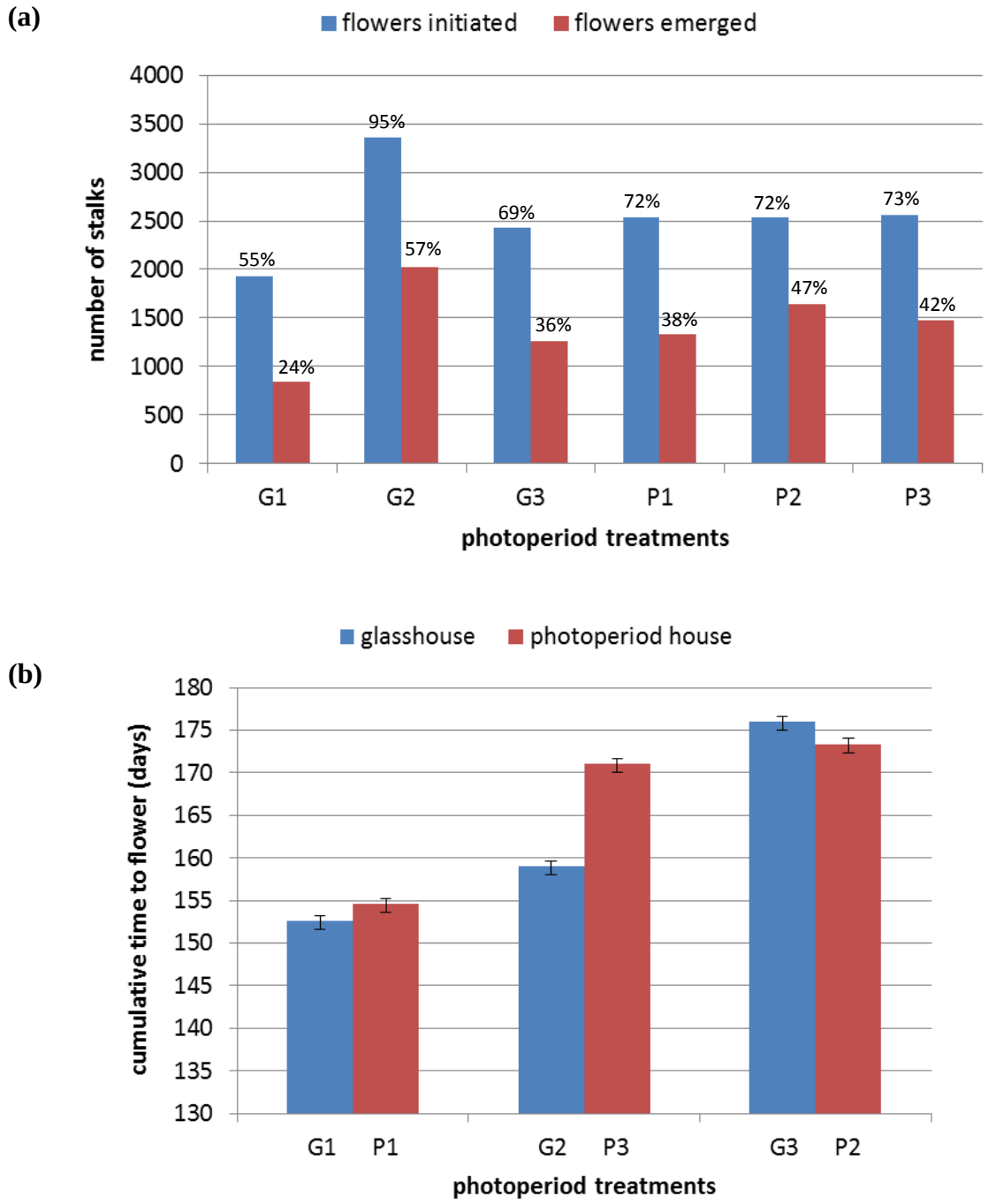

Figure 1. Flowering trends at the South African Sugarcane Research Institute plant breeding facilities over a 5-year period: (a) flower initiation and emergence (total number of stalks exposed to photoperiod treatment (3528) was used in calculating percentages); (b) cumulative time to flower, where day one represents 01 January and not the day on which photoperiod treatment started.

REFERENCES

Berding N (1981). Improved flowering and pollen fertility in sugarcane under increased night temperature. *Crop Sci* 21: 863-867.

Brett PGC (1951). Flowering and pollen fertility in relation to sugarcane breeding in Natal. *Proc Int Soc Sug Cane Technol* 7: 43-56.

Brett PGC and Harding RL (1974). Artificial induction of flowering in Natal. *Proc Int Soc Sug Cane Technol* 15: 55-66.

Clements HF and Awada M (1967). Experiments on the artificial induction of flowering in sugarcane. *Proc Int Soc Sug Cane Technol* 12: 795-812.

Coleman RE (1960). Factors involved in the flowering of sugarcane (*Saccharum* spp.). *Proc Int Soc Sug Cane Technol* 10: 805-814.

Coleman RE (1969). Physiology of flowering in sugarcane. *Proc Int Soc Sug Cane Technol* 13: 992-1000.

Ethirajan AS (1987) Sugarcane Hybridization Techniques. Copersucar International Sugarcane Breeding Workshop 1: 129-147.

Julien MHR (1969). Cane Breeding and Varieties. I: Investigations on the Physiology of Flowering. Mauritius Sugar Industry Research Institute Annual Report.

Julien MHR (1973). Physiology of flowering in *Saccharum*. I: Daylength control of floral initiation and development in *S. spontaneum* L. *J Exp Bot* 24: 549-557.

Moore PH (1976). Studies on sugarcane pollen. II. Pollen storage. *Phyton, Argentina* 34: 71-80.

Moore PH and Nuss KJ (1987). Flowering and flower synchronization. pp 273-311 In: Heinz DJ (Ed.) *Sugarcane Improvement Through Breeding.* Elsevier, Amsterdam, The Netherlands.

SAS Version 9.2 (2009). SAS for Windows, Version 9.2. Cary, NC, USA.

Venkatraman RSTS (1922). Germination and preservation of sugarcane pollen. *Agric J India* 17: 127-132.