SEED PRODUCTION STUDIES IN RUZI GRASS

(*Brachiaria ruziziensis* Germain and Everard)

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ABSTRACT

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(*Brachiaria ruziziensis* Germain and Everard).

The effect of photoperiod on seed production in Ruzi grass (*Brachiaria ruziziensis*) was investigated in order to gain a better understanding of reproductive behaviour. The experiment was conducted in a controlled temperature glasshouse which provided day and night temperatures of $25^0C \pm 5$. Plants were raised from seed obtained from Thailand. During the early stage of growth (10 days after germination) Ruzi grass plants were exposed to controlled photoperiods of 14, 13, 12 or 11 hours for a period of 30 days followed by exposure to the decreasing natural daylength occurring in the New Zealand autumn.

The results of this experiment showed there was no "trigger" daylength requirement for reproductive initiation, since Ruzi grass was able to produce flowers in all daylength treatments (14, 13, 12 and 11 h). However, the data did show that the shorter the daylength, the greater the seed yield (i.e. potential seed yield was greatest at 11 h). Accordingly, the results confirm the conclusion of Dirven *et al.*, (1979), that Ruzi grass is a quantitative short-day plant.

The results of this study appear to contradict the reproductive behaviour of Ruzi grass in Thailand, where a critical daylength of approximately 12½ h to trigger reproductive development seems necessary. These results suggest that it is

theoretically possible for Ruzi grass to produce seed over the entire range of daylengths which occur in Thailand, as recorded in this experiment. The fact that this does not occur is possibly due to a juvenility problem, as the plants are only 4-6 weeks old after the onset of the rainy season in late April/early May when the daylength is approximately 13 h, and due to drought conditions which occur in December under the shortest daylength of approximately 11½ h. However, this needs to be confirmed, possibly by conducting trials in Thailand under an 11½ h daylength with irrigation to overcome lack of water.

Daylength strongly affected inflorescence numbers and inflorescence components. As daylength declined the number of racemes/inflorescence arising from basal tillers tended to decrease. This was accompanied by a corresponding increase in aerial tiller numbers. Floret number/raceme was a more important factor influencing seed yield than raceme number/inflorescence.

Basal reproductive and vegetative tiller numbers were not significantly affected by daylength, although aerial reproductive tillers did increase as daylength declined. Total tiller numbers were low, even although they continued to show a steady increase through to harvest, when approximately 30% were reproductive and 70% vegetative. Ruzi grass produced more inflorescences from aerial tillers than from basal tillers.

The morphological changes occurring during the changeover from vegetative to reproductive development were divided into five stages. The time required from

early raceme initiation in the "double ridge" stage to inflorescence exsertion was 22 days.

It appears that Ruzi grass does not have a characteristically prolonged anthesis within an individual inflorescence, as all anthers were exserted within 1-2 weeks. Despite this, approximately 80% of florets within an inflorescence completed anthesis in 7 days. Within individual inflorescences, anthesis began in the middle region of the uppermost raceme and subsequently extended to the upper and lower raceme(s). Ruzi grass exhibited a prolonged head emergence period of about 3 months which was highly variable both within and between individual plants.

Seed development studies suggested that although some variation in the extent and timing of seed shedding may occur between plants, harvesting should not be carried out before 20-25 days after anthesis (maximum viability), and should not be delayed longer than 30 days after anthesis (maximum dry weight).

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New Zealand, April 1994

Mr. Narongrit Wongsuwan

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GENERAL INTRODUCTION

Ruzi grass (*Brachiaria ruziziensis*) is now the dominant pasture grass used in Thailand for beef, and especially for dairy production. The area sown has increased rapidly over the past decade, due to its ease of establishment, its high quality forage and its relatively high seed yield. However, although seed production has increased from 18 tonnes in 1983 to 466 tonnes in 1991 (Phaikeaw *et al.*, 1993), demand continues to outstrip supply, and it is therefore important to promote and encourage Thai farmers to produce adequate quantities of high quality seed. Currently, around 2700 farmers are involved in seed production programmes under government schemes with each contracted to grow 0.32 ha of Ruzi grass for seed at a guaranteed price of US$ 2/kg (Phaikaew *et al.*, 1993). Accordingly, it is essential that growers improve their understanding of this species in terms of development, crop management, environmental response, and factors affecting seed yield, in order to increase and improve the yield and quality of Ruzi seed in the future.

The present study on Ruzi grass was divided into four sections, as follows:

1. The effect of photoperiod on seed production
2. Morphological changes from the vegetative to the reproductive stage
3. Flowering pattern, floret fertility and seed shedding
4. Seed development

GENERAL DESCRIPTION OF THE SPECIES

Brachiaria ruziziensis Germain and Everard is synonymous with *Brachiaria eminii* Mez (Skerman and Riveros, 1990) and has been renamed *Brachiaria decumbens* var. ruziziensis comb. nov. by Ndabaneze (1989). However, *Brachiaria ruziziensis* is still the most widely used botanical name.

The species is known under various common names depending on the countries in which it is grown. These include: Kennedy ruzi grass (Australia), Congo Signal grass (Africa), prostrate Signal grass (Kenya), and Ruzi grass (Thailand). The names 'Ruzi grass' and *Brachiaria ruziziensis* are used in this study.

Ruzi grass originated from the Lake Edward and Lake Kivu districts of Rwanda, Burundi and the Ruzizi plains in Zaire but is now widely distributed throughout the tropics (Skerman and Riveros, 1990). It is regarded as a pioneer species of cleared rain forests in Africa, providing an important grazing species in the wetter tropics (Skerman and Riveros, 1990).

Descriptions of the morphological features of Ruzi grass have been made by Bogdan (1977), Whiteman (1980) and Skerman and Riveros (1990). It is a spreading perennial with short rhizomes, similar in habit to Para grass (*Brachiaria mutica* (Fork.) Stapf). The inflorescence consists of dense and spike-like racemes. The spikelets are all sessile and close together, the rachis of the racemes winged, broad and over 3 mm wide. The lower glume is under half the length of the spikelet

which is hairy (Harker and Napper, 1960).It has softer leaves than *Brachiaria brizantha* and is leafier than *Setaria sphacelata* (Schum) Stapf and Hubbard (Deinum and Dirven, 1976).

Many characteristics of Ruzi grass are similar to *B. decumbens* (Humphreys and Riveros, 1986). However, one of the obvious differences between the two species is the mode of reproduction, *B. decumbens* being tetraploid and an obligate apomictic (Humphreys and Riveros, 1986) while *B. ruziziensis* is a diploid with 18 chromosomes and cross-fertilization occurs at a very high frequency under conditions of natural open pollination (Ferguson and Crowder, 1974). Seed-set averages 21 and 0.4% for open-pollination and self-pollination respectively, suggesting the operation of a self-incompatibility mechanism. Similarly, CIAT (1972) reports that *B. ruziziensis* clones when cross-pollinated, averaged 30% of spikelets with a caryopsis, but only 0.5% when selfed. Skerman and Riveros (1990) and Bogdan (1959a, 1965a), state that Ruzi appears to be apomictic.

Being a tropical species, its season of growth is during the rainy season in the so-called summer or warmer period of the year, with an optimum temperature of 33^0C day and 28^0C night (Deinum and Dirven, 1972). Plant growth is stimulated by increasing temperature which leads to lower protein content and lower digestibility of organic matter in leaves and stems. Temperature also has a direct negative effect on stem digestibility, apart from its effect on stem development (Deinum and Dirven, 1976). Low temperatures, as found by Ludlow (1976), adversely affect the growth rate of this species.

Rattray (1973) reported that Ruzi grew successfully in an altitude range of 1000 - 2000 m above sea level in Kenya and up to 1200 m in Panama. It is most productive under an average annual rainfall of about 1000 mm and can endure hot dry spells (Skerman and Riveros, 1990), and hence is described as drought resistant. It will not, however, withstand flooding or frost.

To achieve high dry matter production Ruzi grass requires a soil of relatively high fertility and good drainage, but performs well on a wide range of soil types. It will tolerate acid soils and also responds well to nitrogen, either from fertiliser or legumes, but has a higher requirement than Guinea grass (Mellor *et al.*, 1973b). Yonken *et al.*, (1986) showed that application of phosphorus did not lead to improved yield of Ruzi grass on feratic soils but suggested that about 50 units of P_2O_5/ha would seem necessary to maintain production on non-feratic soils. On the Adamawa plateau of the Cameroons, Pamo and Pieper (1989) showed that nitrogen fertilization in combination with phosphorus and potassium increased the productivity of Ruzi grass and recommended that a fertilizer rate of between 60 and 90 units of nitrogen/ha be applied after each cutting, with a single application of 100 units of triple superphosphate and potassium sulphate/ha at the beginning of each rainy season. This response was obtained under a 30 day cutting frequency.

Associations with legumes such as *Centrosema pubescens* (Mellor *et al.*, 1973a), *Stylosanthes humilis* (Falvey, 1976), *C. pubescens* and *Macroptilium atropurpureum* (Nitis *et al.*, 1976) have all proved successful. However, Ruzi was grouped into the least shade-tolerant species along with *Setaria sphacelata*

cv.Kazungula, *B. decumbens* cv.Transvala. These compare with *P. maximum* cv.Common, *P. maximum* cv.Tanganyika, *Digitaria setivalva* and *B. decumbens* which are regarded as more shade-tolerant grasses (Wong *et al.*, 1985).

Seed dormancy is generally high in freshly harvested seed, because of an impermeable seed coat (Davidson, 1966; McLean and Grof, 1968). The period of seed dormancy is normally 4 to 6 months, but can extend to 18 months if the seed is stored at low temperature (Devahuti and Sirisomparn, 1985). Seed dormancy can be broken by treating the seed with concentrated sulphuric acid for 15 minutes (Barnard, 1969) which can increase germination from 17 to 40% (Mclean and Grof, 1968), or by mechanical scarification (Jones, 1973).

Ruzi grass is the most widely used grass species grown in Thailand because of its ease of establishment and its relatively good forage quality (Phaikaew and Pholsen, 1993) and is normally sown at 15-25 kg/ha. Bogdan (1964) recommended that the seed be sown at a depth of 2 cm and in rows 60 cm apart, but it can be broadcast over the land after scarification of the soil with a disc harrow or brushcutter, without burning the native pastures (Risopoulos, 1966). However a well prepared seed bed is highly recommended for best results (Skerman and Riveros, 1990).

Ruzi grass is suitable not only as a green forage but also as hay and silage, and is very palatable to stock. In Thailand, Ruzi grass is commonly used for both forage and seed production, with the success of seed production being dependent on

the cessation of grazing and closing of the pasture for seed production at the correct time, normally towards the end of August (Phaikaew, *et al.*, 1985).

Dry matter production of Ruzi grass can vary considerably, depending on rainfall, fertility conditions and management. In Tanzania, dry matter yields of 21,159 kg/ha have been recorded (Naveh and Anderson, 1967), and at South Johnstone,(North Queensland) Grof and Harding (1970) recorded dry matter yields of 19,500 kg/ha under a six week cutting interval and an input of 220 kgN/ha/annum. In Sri Lanka dry matter yields of 16,807, 22,031 and 25,585 kg/ha/year were obtained with nitrogen applications of 112, 224 and 366 kg N/ha (Appadurai, 1975).

For seed production, farmer yields of 400 kg/ha are commonly achieved in Thailand where repeated hand harvesting is practised (Phaikaew, *et al.*, 1993), compared with a yield of 125 kg/ha in Queensland and 200 kg/ha in Zaire (Risopoulos, 1966) where harvesting is a single machine operation. Like other tropical grasses, Ruzi exhibits a prolonged flowering period with uneven ripening of seed on individual inflorescences and quick shedding of seed when ripe (Boonman, 1971a; Hare and Waranyuwat, 1980; Phaikaew and Pholsen, 1993).

In Thailand, Devahuti *et al.*, (1986) showed that the flowering period of Ruzi grass occurred from October to November, with peak flowering near the end of October. At 10 days after flowering, about 5% of seed was shed and this had increased to 60% when the seed reached physiological maturity, approximately 3

weeks after flowering.

As shown in work on harvesting times by Phaikaew *et al.*, (1986), 15-20 days after 50% of seedheads had emerged was the best compromise to achieve both high seed yield and good quality. Seed can be harvested either by hand shaking for small scale production or cutting of seedheads for large scale production (Phaikaew, 1989). In Queensland, a tractor-mounted buffel type seed harvester is often used but yields are lower than by hand harvesting (Davidson, 1966).

Phaikaew *et al.*, (1987) found that a single cut of seedheads followed by 2-3 days "sweating" produced lower quality seed, compared with the daily shaking of seedheads.

In Queensland, Australia 15% germinable seed and 40% purity are required for commercial sale (Skerman and Riveros, 1990).

Ruzi grass is relatively free of disease and pest problems (Skerman and Riveros, 1990).

CHAPTER ONE

EFFECTS OF PHOTOPERIOD ON SEED PRODUCTION IN RUZI GRASS (*Brachiaria ruziziensis* Germain and Everard)

1.1. INTRODUCTION

In many plants photoperiod is of immense ecological significance, since it "triggers" flowering well in advance of the occurrence of unfavourable climatic conditions and facilitates outcrossing by synchronizing flowering in neighbouring plants (Humphreys and Riveros, 1986).

Although photoperiodic variation in the tropics is small, several tropical grasses are still strongly affected by these small changes. Grasses occurring in tropical areas tend to be short-day plants and flower only when the days are less than a certain critical length (Langer, 1972). This allows maximum vegetative growth during the rainy season and is followed by reproductive growth before winter cold or drought becomes adverse (Humphreys and Riveros,1986).

Daylength in Thailand ranges from approximately 13-h in June to approximately 11-h in December (Figure 1.1) and from observations in Thailand, Ruzi grass tends to act as a qualitative short-day plant, because it appears that flowering requires a critical daylength of approximately 12½-h in August (Figure 1.1), with the seed being harvested in October/November. However Dirven *et al.*, (1979) showed that Ruzi grass was a quantitative short-day plant.

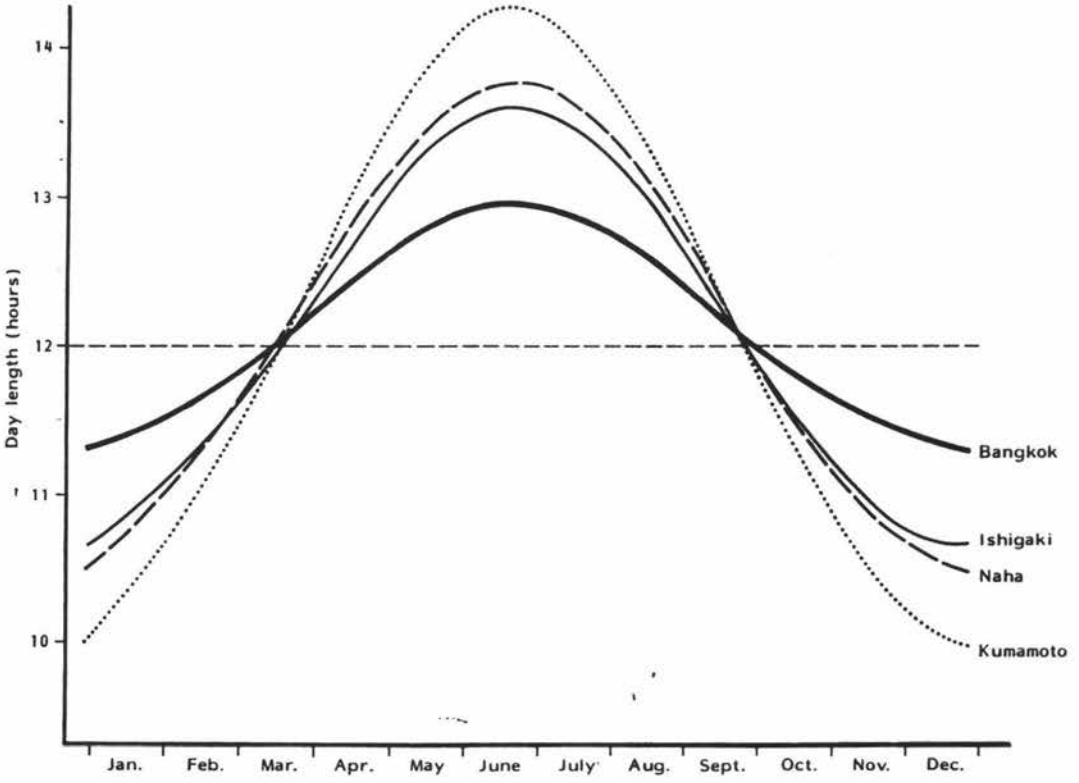


Figure 1.1. Mean daylength range in Thailand (Yoshiyama, 1984)

To investigate these apparently conflicting daylength observations, an experiment was undertaken with the following three objectives:

1. To examine the reproductive sensitivity of Ruzi grass to daylength
2. To study tillering activity and development by assessing the number of tillers produced, and tiller fertility
3. To investigate potential seed yield and components of potential seed yield

1.2. LITERATURE REVIEW

According to Zeevaart, (1976) flowering or reproductive development is not the result of a series of autonomous processes, determined solely by genetic constitution, but is controlled by environmental factors which interact with genetic constitution in a specific manner. The two climatic factors which play by far the most important role in controlling this are temperature and daylength, with the effect of daylength on inflorescence initiation capable of being modified considerably by temperature (Evans, 1964; Cooper, 1960). In the view of Evans (1964) other factors such as defoliation, nutrition and light intensity, do not seem to play a specific role in the control of inflorescence initiation in grasses.

In some young plants, the transition of a stimulus to the vegetative apex which initiates morphogenetic changes leading to flowering cannot take place, despite apparently favourable climatic conditions. This condition, called "juvenility", is considered by Humphreys and Riveros (1986) to be short-lived and

unimportant in many species which come from semi-arid areas, and whose adaptation necessitates rapid flowering soon after effective rainfall has occurred, so that seed formation may be completed before the onset of drought conditions. Juvenility in *Panicum maximum* can apply to the single tiller, as well as to the whole seedling plant (Felippe, 1979). However, Humphreys and Riveros (1986) and Humphreys (1981) state that the mechanisms controlling juvenility are not clear, as plant growth and hormonal balance are, among other factors, temperature dependent and are known to affect juvenility.

Boonman and van Wijk (1973) noted that variation in flowering behaviour within a population occurs more conspicuously in sexually reproducing, cross-pollinated species like *Chloris gayana* and *Setaria sphacelata* than in predominantly apomictic species like *Panicum maximum*. In addition, in some instances expressions of this variation in flowering time may be the result of variability in plant vigour, and hence rate of passage of a juvenile phase, as they suggested for *Chloris gayana* and *Setaria sphacelata*.

Following the early work of Garner and Allard (1923), plants have been classified into three groups: short-day plants which will flower if days are shorter than a critical length, long-day plants which will flower if days are longer than a critical length, and indeterminate or day neutral plants which will flower in days of any length.

This phenomenon has been reviewed by Chailakhyan (1968) and provides

four additional groups: long-short day (requiring long before short days), short-long day (requiring short before long days), stenophotoperiodic (requiring medium daylength) and amphiphotoperiodic (requiring long or short days but not medium days).

Possibly the most widely accepted classification is that presented by Whiteman (1980) who divided plants into a number of major groups: Short-day plants (SDP) - (that will flower in response to a range of relatively short days, i.e. require relatively long dark periods to build up the floral stimulus); Long-day plants (LDP) - (that will flower in response to a range of relatively long days, i.e. require relatively short dark periods for floral initiation, and will not flower in night lengths longer than some critical maximum), Intermediate plants (IP) - (that will only flower within certain limits of daylength, i.e. they have both a critical maximum and a critical minimum night length); Indeterminate or day-neutral plants (DNP) - (that are not sensitive to photoperiod and hence will flower over a wide range of daylengths).

The photoperiodic responses may be "qualitative" (obligate) - plants will not flower unless daylength is less than a certain critical photoperiod, or "quantitative" - plants flower in a wide range of photoperiods but are more productive at certain daylengths (Humphreys and Riveros, 1986).

Dirven *et al.*, (1979) noted that very little detailed work has been done on photoperiodism in tropical pasture grasses, and that many tropical grasses were

capable of producing seed heads the whole year round in tropical environments. However, reviews by Evans (1964) and Humphreys (1975) report that many tropical grasses are short-day plants, with only a few grasses flowering when the photoperiod is less than a critical length [qualitative (obligate) short-day plants], while other species flower more rapidly in short days (quantitative short-day plants).

Although a requirement for vernalization has not been found for tropical plants, temperature conditions may modify the critical daylength for flowering (Humphreys and Riveros, 1986). For example, the heading date of *Chloris gayana* cv. Fords Katambora was delayed 45 days at 23^0/16^{0}C and 15 days at 28^0/21^{0}C relative to heading date at 33^0/26^{0}C (Sato *et al.*, 1981). Moreover, head density of *Panicum maximum* var. trichoglume and of *Setaria sphacelata* var. sericea cv. Kazungula was higher at 25^{0}C than at 30^{0}C or 20^{0}C (Nishihira and Nishimura, 1982).

According to Schwabe and Wimble (1976) cool night temperatures limit the formation of an inhibitor to flowering in short-day plants, which agrees with Summerfield and Wien (1980) who suggested that flowering of short-day plants is usually promoted by high night temperatures.

The sensitivity of tropical grasses to daylength was studied by Dirven *et al.*, (1979), using *Brachiaria* species [*B. brizantha* (Hochst.) Stapf, *B. decumbens* Stapf, *B. mutica* (Forsk.), and *B. ruziziensis* Germain et Everard]. They exposed these species to photoperiods of 10, 12 or 14 h in 1971 and 9, 10¼ and 12½-h in 1973,

using 9 hours natural daylength in the summer, supplemented with low intensity light from incandescent lamps (40 W).

From the first experiment, they found that *B. brizantha* and *B. decumbens* did not response to photoperiod, *B. mutica* did not produce heads in the 14-h treatment but showed earlier and accelerated heading in the 12-h to 10-h treatments while *B. ruziziensis* was also stimulated to produce heads earlier and quicker by the 10-h treatment than by the longer daylength treatments. However, the number of heads per unit area was approximately the same for all treatments by the respective termination dates.

In order to confirm the above results, a second experiment was conducted with an average day and night temperature of 33 and 25^{0}C respectively, using plants at a younger growing stage than in the former experiment. Similar reactions were found in *B. brizantha* and *B. decumbens* viz. that in all photoperiod treatments the first heads were observed 14 days after the start of the experiment. However, the 9-h photoperiod tended to accelerate heading. Shortening the daylength resulted in an increase in the number of heads in *B. decumbens*, whereas in *B. brizantha* the same number of heading tillers was present in all photoperiodic treatments by the termination date.

In *B. mutica*, no heading tillers were recorded at a daylength of 12½-h, but the number of heading tillers produced was considerably higher in the 9-h treatment at the termination date than in the 10¼-h treatment. *B. mutica* was therefore

regarded as a qualitative (obligate) short-day plant.

In *B. ruziziensis*, Dirven *et al.*, (1979) found that the emergence of the first heads occurred later in the 12½ h treatment than in the two shorter photoperiods. Moreover, the shorter the daylength the greater the number of heading tillers produced (181 heads per pot in the 9 h treatment compared with 100 heads per pot in the 12½ h treatment). Therefore, they concluded that *B. ruziziensis* was a quantitative short-day plant.

1.3. MATERIALS AND METHODS

Ruzi grass seed obtained from Thailand was used in this study. It had been harvested by hand, cleaned and quality tested approximately one year before commencing this experiment.

Seed germination was tested in New Zealand using the top of paper method (ISTA, 1993) in a controlled environment chamber which provided a temperature of 30/20^{0}C (day/night), 70% R.H. and 12 hours of light. To break possible dormancy, the seed was treated with 0.2% KNO_3 solution and prechilled at 5^{0}C for 2 days before moving seed to the required conditions in the germination cabinet.

The experiment included four treatments of differing daylength i.e.

- Treatment 1 : 14 hours daylength, sown on 31 December 1992
- Treatment 2 : 13 hours daylength, sown on 22 January 1993

- Treatment 3 : 12 hours daylength, sown on 12 February 1993

- Treatment 4 : 11 hours daylength, sown on 4 March 1993

Sowing dates were calculated from local daylength data (Figure 1.2) to allow 10 days for germination yet provide 30 days under the desired daylength.

Ten days after sowing in the controlled environment chamber, seedlings were transplanted into individual plastic pots (15 cm diameter and 30 cm deep) containing a uniform potting mixture (Ponga fibre, pumice, 3-4 months Osmocote plus N:P:K - 15:4.8:10.8 and trace elements).

Forty plants for each of the four daylength treatments (twenty plants for dissection and twenty plants for seed production) were grown in a controlled temperature glasshouse which provided day and night temperatures of $25^{0}C \pm 5$.

The particular daylength duration of each treatment was achieved by covering the plants with black plastic supported on iron frames (width: 70 cm x length: 100 cm x height: 100 cm), with each frame covering 20 plants.

The plants received natural sunlight from sunrise, but after receiving the required hours of daylength, they were then covered until the next morning. The schedule of covering and removing the black plastic sheets is presented in Table 1.1.

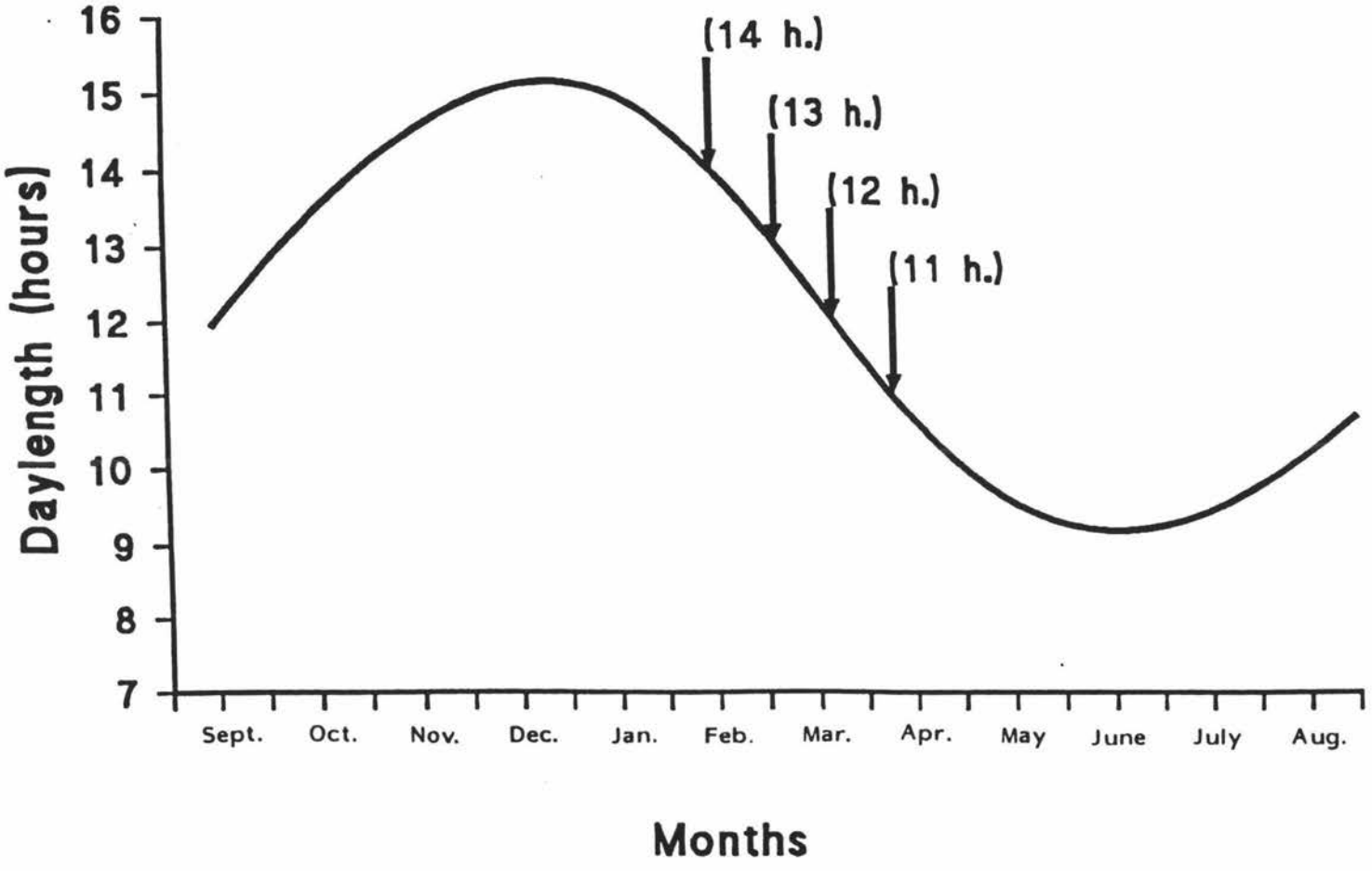


Figure 1.2. The date of Ruzi grass exposure to natural photoperiod (Wellington NZ. 1992)

14 h. Daylength

	JANUARY																						FEBRUARY							
Date	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8
S-rise (h.m)	5.59	6.00	6.01	6.02	6.03	6.04	6.06	6.07	6.08	6.09	6.10	6.12	6.13	6.14	6.15	6.16	6.18	6.19	6.20	6.21	6.22	6.24	6.25	6.26	6.27	6.29	6.30	6.31	6.33	6.34
C-time (h.m)	19.59	20.00	20.01	20.02	20.03	20.04	20.06	20.07	20.08	20.09	20.10	20.12	20.13	20.14	20.15	20.16	20.18	20.19	20.20	20.21	20.22	20.24	20.25	20.26	20.27	20.29	20.30	20.31	20.33	20.34

13 h. Daylength

	FEBRUARY																												MARCH	
Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	1	2
S-rise (h.m)	6.25	6.26	6.27	6.29	6.30	6.31	6.33	6.34	6.35	6.37	6.38	6.39	6.40	6.42	6.43	6.44	6.46	6.47	6.48	6.50	6.51	6.52	6.53	6.54	5.56	6.57	6.58	6.59	7.02	7.03
C-time (h.m)	19.25	19.26	19.27	19.29	19.30	19.31	19.33	19.34	19.35	19.37	19.38	19.39	19.40	19.42	19.43	19.44	19.46	19.47	19.48	19.50	19.51	19.52	19.53	19.54	19.56	19.57	19.58	19.59	20.02	20.03

12 h. Daylength

	FEBRUARY							MARCH																						
Date	22	23	24	25	26	27	28	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
S-rise (h.m)	6.52	6.53	6.54	6.56	6.57	6.58	6.59	7.02	7.03	7.04	7.05	7.06	7.07	7.08	7.09	7.10	7.11	7.13	7.14	7.15	7.16	7.17	7.18	7.19	7.20	7.21	7.22	6.24	6.25	6.26
C-time (h.m)	18.52	18.53	18.54	18.56	18.57	18.58	18.59	19.02	19.03	19.04	19.05	19.06	19.07	19.08	19.09	19.10	19.11	19.13	19.14	19.15	19.16	19.17	19.18	19.19	19.20	19.21	19.22	18.24	18.25	18.26

11 h. Daylength

	MARCH																		APRIL											
Date	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12
S-rise (h.m)	7.16	7.17	7.18	7.19	7.20	7.21	7.22	6.24	6.25	6.26	6.27	6.28	6.29	6.30	6.31	6.32	6.33	6.35	6.36	6.37	6.38	6.39	6.40	6.41	6.42	6.43	6.44	6.46	6.47	6.48
C-time (h.m)	18.16	18.17	18.18	18.19	18.20	18.21	18.22	17.24	17.25	17.26	17.27	17.28	17.29	17.30	17.31	17.32	17.33	17.35	17.36	17.37	17.38	17.39	17.40	17.41	17.42	17.43	17.44	17.46	17.47	17.48

Table 1.1. Timing to control daylight for Ruzi grass as required for each treatment for a 30 day period before exposure to natural photoperiod (data from Wellington NZ. 1992 with times corrected for daylight saving). S-rise = Sun rise; C-time = time plants were covered to exclude light.

The exposure of plants to the required daylength in each treatment continued for 30 days by the process of covering and removing the plastic sheets.
After 30 days the plants were then exposed to natural daylength (Astronomical Handbook, 1992) which continued to decline as the season advanced (Figure 1.2).

The plants were "supported" with bamboo canes as they grew taller, were watered daily to field capacity and received nutrient solution (Liquid Lush - 10% N, 3% P, 6% K plus trace elements) every 2-3 weeks.

Tillers were counted every 30 days until the reproductive stage occurred, when they were classified into vegetative tillers (shoots that developed from axillary or adventitious buds at the base of a stem, and did not produce an inflorescence), reproductive basal tillers (shoots that developed from axillary or adventitious buds at the base of a stem, and produced inflorescences), and reproductive aerial tillers (shoots that developed from nodes on the stem of a basal tiller, and produced inflorescences).

The transition from the vegetative to the reproductive stage was determined by looking for the first visible sign of the onset of reproductive growth, the so-called double ridge (See CHAPTER TWO). One or two plants were dissected every 2-3 weeks and examined under the binocular microscope. However, as the plants approached the transition stage, dissections were made every few days thereby enabling the transition date to be more accurately determined. The emergence of the inflorescence through the flag-leaf was used as the criterion for heading. The

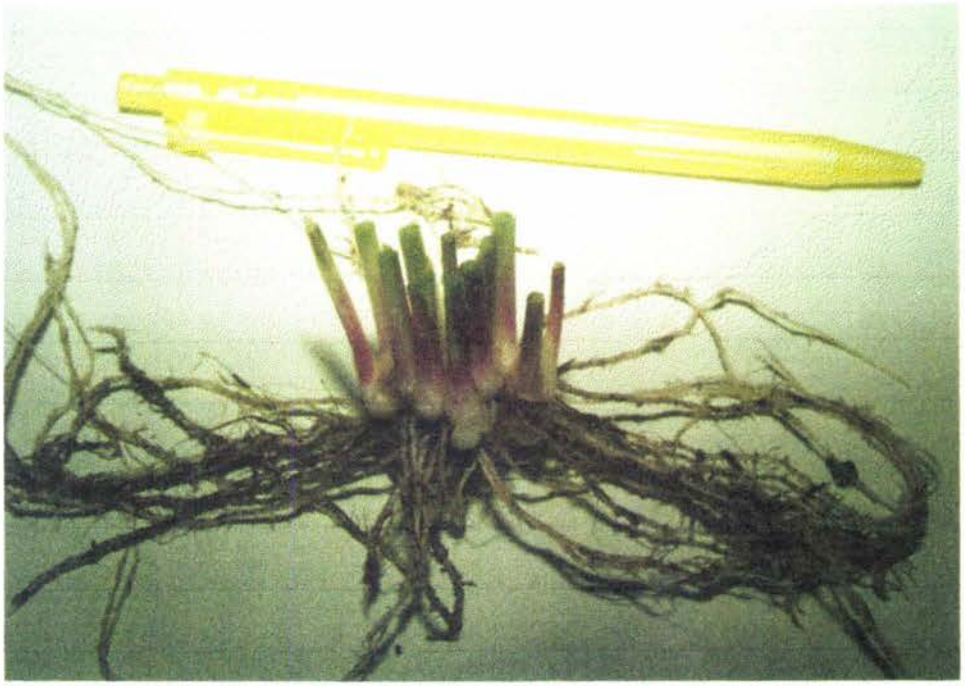
date of first anthesis in each treatment was also recorded, being the date at which the first spikelet exerted anthers and stigmas (Humphreys and Riveros, 1986).

Each plant was harvested at 60 days after first anthesis. The number of seed heads was recorded and divided into those from basal tillers (Plate 1.1.- shoots that developed from axillary or adventitious buds at the base of a stem) and those from aerial tillers (Plate 1.1.- shoots that developed from nodes on the stem of a basal tiller).

Inflorescences were tagged on the day anthesis was first observed. Forty days after tagging, five complete inflorescences (i.e. without florets which had been shed) per treatment were hand harvested. Each inflorescence was then dissected and florets divided into two categories : (1) Seed (Fertile floret) - where the floret contained a caryopsis (Ferguson and Crowder, 1974) at the firm or "hard" stage (Humphreys and Riveros, 1986); (2) Sterile floret - a floret which did not contain a caryopsis. From these data the percentage seed set was calculated. However, in some treatments it was not possible to harvest five complete inflorescences because of shedding. In such cases it was assumed the shed floret contained a caryopsis, and therefore the missing florets were defined as category (1).

Seed fresh weight was determined directly after finishing the separation between categories (1) and (2). The number of seeds was counted and weighed for each individual inflorescence. Fresh weight per seed was calculated by dividing

(a)



(b)

Plate 1.1. Tiller origin (a) Basal tillers, and (b) Aerial tillers

total seed weight by the total number of seeds. For these data, each inflorescence was taken as a replicate.

Potential seed yield was calculated from : (1) the number of seedheads {inflorescence (raceme number/inflorescence and floret number/raceme)} per plant from aerial tillers and from basal tillers, (2) the percentage seed set, and (3) seed fresh weight.

1.4. RESULTS

1.4.1. Time to Floral Initiation and Onset of Anthesis

The first floral apex (double ridge) appeared 87, 94, 128 and 125 days from sowing in daylengths of 14, 13, 12 and 11 hours respectively (Table 1.2), i.e. as daylength was reduced from 14 to 12 hours, the time from sowing to floral initiation increased, but with no significant difference between daylengths of 12 and 11 hours.

Time from sowing to first anthesis showed a similar trend (Table 1.2). Days to first anthesis did not differ at 14 or 13 hour daylengths, but took 47 and 35 days longer at 12 and 11 hour daylengths. By comparison days from floral initiation to first anthesis were relatively similar for all daylengths (Table 1.2).

Table 1.2. Effect of daylength on days from sowing to floral initiation and date of first anthesis in Ruzi grass

Daylength (hours)	Sowing date	Floral initiation		First anthesis		
		date	days after sowing	date	days from sowing to first anthesis	days from floral initiation to first anthesis
14	31/12 1992	28/3 1993	87	26/4 1993	116	29
13	22/1 1993	26/4 1993	94	20/5 1993	118	24
12	12/2 1993	20/6 1993	128	26/7 1993	164	36
11	4/3 1993	7/7 1993	125	3/8 1993	152	27

1.4.2. Tiller Numbers

The number of basal vegetative tillers per plant was not significantly affected by daylength and, apart from the anomalous result in the 13 hour daylength treatment, this also applied to the number of basal reproductive tillers produced, the number of aerial reproductive tillers produced, and to total basal tiller production (Table 1.3). A large number of aerial tillers occurred in this study. Presumably the absence of cutting allowed uninterrupted development of the plant throughout the experimental period. This resulted in tall plants, makimg it very difficult to count all tillers accurately, because of the intertwined stems. Therefore, only aerial tillers which bore inflorescences were recorded. The number of vegetative tillers was considerably greater than the number of reproductive tillers at the time of harvest (Table 1.3).

The reason for the seemingly anomalous result recorded in the 13 hour treatment is not known, but may have been due to the effect of a chemical spray (Malathion - 500 g/l Maldison and Coopers mite killer - 80 g/l dicofol) applied to an adjacent experiment, which subsequently caused deleterious effects (i.e. damage to young leaves) on the plants in that treatment.

Tiller number per plant increased from just only one at 30 days after sowing to 15-20 by 180 days after sowing, but, with the exception of the 13 hour treatment, tiller number was not affected by daylength (Table 1.4).

Table 1.3. The effect of daylength on the number of vegetative and reproductive (basal) tillers produced per plant at final harvest (60 days after anthesis)

Daylength (hours)	Vegetative basal tillers	Reproductive basal tillers	Total basal tillers	Reproductive aerial tillers
14	13.00a (±1.29)	7.83a (±0.87)	20.83ab (±1.84)	24.58a (±4.87)
13	12.57a (±4.71)	2.14b (±0.34)	14.71b (±2.21)	8.26b (±2.09)
12	15.37a (±2.86)	8.63a (±1.02)	24.00a (±3.40)	16.63ab (±4.17)
11	11.67a (±3.36	9.33a (±1.82)	21.00ab (±4.41)	17.33ab (±6.26)
Mean	13.24	7.09	20.33	17.88
c.v.	48.9%	42.4%	39.6%	76.8%

{Means with the same letter are not significantly different at $P \geq 0.05$ (Duncan's multiple range test)}

Table 1.4. Tillers per plant every 30 days from sowing to harvest in Ruzi grass grown under different daylengths

Daylength (hours)	Number of tillers per plant							
	30 DAS[1]	60 DAS	90 DAS	120 DAS	150 DAS	165 DAS	180 DAS	210 DAS
14	1.64 ±0.67	5.46 ±1.75	8.46 ±3.33	12.00 ±4.60	18.27 ±6.28	20.55 ±6.59	-	-
13	1.14 ±0.38	4.14 ±1.77	4.71 ±2.43	4.71 ±2.43	7.86 ±1.95	-	14.71 ±5.85	-
12	1.38 ±0.52	3.50 ±0.93	7.13 ±2.59	10.25 ±2.92	11.75 ±3.69	-	15.63 ±4.93	24.00 ±9.61
11	1.17 ±0.41	2.00 ±1.10	4.17 ±1.94	7.83 ±3.13	13.67 ±5.16	-	21.00 ±10.79	-

DAS[1] = days after sowing

- = no data recorded

1.4.3. Raceme Number per Inflorescence and Floret Number per Raceme

Inflorescences were classified into two categories based on the origin of the tillers viz. basal tillers, arising from the base of the plant, and aerial tillers arising from the elevated nodes on the parental culm. Raceme number per inflorescence tended to be greater in basal tillers than in aerial tillers (Table 1.5).

In terms of daylength effects, raceme number per inflorescence on basal tillers was significantly greater under the longest day of 14 hours than under shorter days. However raceme number on the inflorescence of aerial tillers showed a very different effect, being significantly greater under shorter daylengths of 11 and 12 hours than under longer daylengths of 13 and 14 hours.

Floret number per raceme was similar in both basal and aerial tillers, and increased significantly as daylength decreased (Table 1.5).

1.4.4. Potential Seed Yield

Inflorescence numbers per plant arising from aerial tillers was consistently greater than that from basal tillers in all daylength treatments (Table 1.6). Apart from the 13 hour treatment there was no significant effect of daylength on the number of inflorescences arising from basal or aerial tillers.

Table 1.5. The effect of daylength on raceme numbers per inflorescence, and floret numbers per raceme at final harvest (60 days after anthesis)

Daylength (hours)	Raceme numbers per inflorescence		Floret numbers per raceme	
	from basal tillers	from aerial tillers	from basal tillers	from aerial tillers
14	3.97a (±0.18)	1.86b (±0.07)	23.04c (±0.41)	16.41b (±0.29)
13	2.80b (±0.33)	1.67b (±0.11)	17.98d (±1.41)	15.54b (±0.59)
12	3.26b (±0.16)	2.61a (±0.12)	36.29a (±0.55)	32.56a (±0.49)
11	3.27b (±0.12)	2.63a (±0.10)	31.87b (±0.72)	32.17a (±0.51)
Mean	3.52	2.14	28.34	24.17
c.v.	40.3%	53.4%	30.0%	31.9%

{Means with the same letter are not significantly different at $P>0.05$ (Duncan's multiple range test)}

Table 1.6. Effects of daylength on Ruzi grass seed yield and its components at final harvest (60 days after anthesis)

Daylength (hours)	Inflorescence no.per plant		Floret no. per inflorescence		% seed set per plant	Seed weight (g)			Potential seed yield per plant (g)		
	from basal tillers	from aerial tillers	from basal tillers	from aerial tillers		from basal tillers	from aerial tillers	Average	from basal tillers	from aerial tillers	g/plant (total tillers)
14	7.83a (±0.87)	24.58a (±4.87)	91.64b (±5.28)	30.48b (±1.32)	27.00a (±3.35)	0.0066	0.0060	0.0063b (±0.0003)	1.21ab (±0.13)	1.24b (±0.18)	2.46b (±0.16)
13	2.14b (±0.34)	8.26b (±2.09)	50.33c (±7.41)	26.16b (±1.96)	17.80a	0.0056	0.0051	0.0055b (±0.0003)	0.11c (±0.02)	0.22c (±0.06)	0.32c (±0.07)
12	8.63a (±1.02)	16.63ab (±4.17)	117.59a (±6.59)	84.95a (±4.27)	10.31a (±3.51)	0.0085	0.0082	0.0080a (±0.0004)	0.81b (±0.08)	1.10bc (±0.23)	1.91b (±0.24)
11	9.33a (±1.82)	17.33ab (±6.26)	106.32ab (±6.43)	84.45a (±3.55)	16.56a (±2.43)	0.0092	0.0090	0.0090a (±0.0003)	1.49a (±0.25)	2.20a (±0.68)	3.69a (±0.55)
Mean	7.09	17.88	100.16	51.58	15.93			0.0079	0.93	1.16	2.10
c.v.	42.4%	76.8%	50.4%	62.7%	84.7%			11.9%	42.3%	73.3%	35.2%

{Means with the same letter are not significantly different at $P \geq 0.05$ (Duncan's multiple range test)}.

Floret number per inflorescence was considerably greater from basal tillers than from aerial tillers and was also greater in plants grown under shorter daylengths (12 and 11 hours) than under longer daylengths (14 and 13 hours). This effect was particularly apparent in inflorescences arising from aerial tillers.

Daylength did not significantly affect percentage seed set per plant, although it tended to be higher in the longest daylength treatment of 14 hours than in the remainder. Unfortunately the percentage seed set in inflorescences from basal and aerial tillers could not be determined owing to difficulty in separating and identifying the intertwined stems and the associated danger of excessive error through seed loss.

Shorter daylengths of 12 and 11 hours produced seeds of significantly greater weight than those from longer daylengths of 14 and 13 hours. No difference was recorded in the weight of individual seeds from basal and aerial tillers.

Basal tillers and aerial tillers made a very similar contribution to potential yield under the longer daylength of 14 hours. However as daylength was reduced, aerial tillers made an increasing contribution to yield, mainly because of the greater number of florets per inflorescence.

1.5. DISCUSSION

Results from this study were somewhat different from those obtained by Dirven *et al.*, (1979) whose statement that *B. ruziziensis* is a quantitative short-day plant was based on data from the time of first head appearance, and the number of heads or inflorescences among daylength treatments. For example, Dirven *et al.*, (1979) reported that the emergence of the first heads occurred later in a 12½-h daylength treatment than in two shorter photoperiods of 10¼-h and 9-h, whereas in the present study the shorter the daylength treatment the longer the time from sowing to the appearance of the first seedheads. Furthermore, Dirven *et al.*, (1979) reported that the shorter the daylength the greater the number of inflorescences produced, whereas in the present study there was no significant effect of daylength (apart from the 13-h treatment) on the number of inflorescences arising from either basal or aerial tillers.

Although the present results do not agree with some of the findings of Dirven *et al.*, (1979), care must be taken in making such a direct comparison, as the present study used seed material which resulted in a wide genetic variation between plants - as shown by the large coefficients of variation recorded - whereas Dirven *et al.*, (1979) used clonal material which provided a very narrow genetic base and hence relatively little statistical variation. In addition, the environmental conditions of the present study were much less controlled in terms of temperature and light intensity than in Dirven *et al*'s experiment (1979), and was conducted under decreasing natural daylength following the initial 30 days. By comparison Dirven *et al.*, (1979)

maintained constant daylength and light intensity conditions throughout their study.

Humphreys and Riveros (1986) stated that temperature conditions may affect a wide range of plant responses such as vegetative growth of the crop, floral initiation, growth and differentiation of the inflorescences, blooming, pollen germination, seed setting and seed formation. Moreover, the optimum temperature and the shape of the temperature response is different for each phase, and also varies between and within species.

In Dirven *et al*'s experiments (1979), day and night temperatures of 25^{0}C and 20^{0}C produced different results from day and night temperatures of 33^{0}C and 25^{0}C, with the former combination showing no significant difference between daylength treatments in terms of number of seedheads per unit area, while in the latter experiment, the shorter the daylength the greater the number of seedheads produced.

The results of the present study, with day and night temperature of 25^{0}C, were actually similar to and hence support the findings of Dirven *et al*'s first experiment (1979). Furthermore these relatively low temperatures - compared with those in Dirven *et al*'s second experiment (1979) - help to explain the relatively slow rate of reproductive development recorded. However, Humphreys and Riveros (1986), considered that temperature usually acts as a modifier of the basic control exerted by photoperiod, which possibly explains why the lower night temperatures experienced in March and April (Appendix 1.1) during the 12 and 11-h daylength treatments - causing an uncontrollable drop in glasshouse temperature - prolonged

the time from sowing to floral initiation. This agrees with the view of Schwabe and Wimble (1976) that cool night temperatures limit the formation of an inhibitor to flowering in short-day plants. Moreover, Summerfield and Wien (1980) reported that flowering in short-day plants is usually promoted by high night temperatures.

According to Evans (1964) light intensity does not appear to play a specific role in the control of inflorescence initiation in grasses. Similarly, Warrington and Norton (1991) who studied the effects of light under various daily quantum integrals on plant growth and development in chrysanthemum, radish, corn and cucumber reported there were no effects of light intensity on floral initiation. However Oliveira and Humphreys (1986) found that reduction of natural light intensities reduced seed yield of *Panicum maximum* cv. Gatton because head density was reduced, particularly when plants were shaded at the early stage of floral initiation.

Unfortunately, light intensity was not measured inside the glasshouse during this study, but the experiment was certainly conducted under decreasing light intensity as shown in Appendix 1.1. This may have affected the components of seed yield but not the timing of floral initiation.

Patterns of tiller development in field experiments have been reported by many workers. Loch (1989), who studied tiller development in relation to seed development of tropical grasses, found that maximum basal tillering occurred during early vegetative growth but Boonman (1971b) reported a later peak at the start of inflorescence emergence. In the present study, however, the number of basal tillers

continued to show a steady increase right through to harvest (Table 1.4), by which time approximately 30% were reproductive and 70% still vegetative.

Unfortunately it was not possible, because of the mass of intertwined stems, to accurately determine progressive number of reproductive tillers on each plant or their contribution to potential seed yield at harvest. However, as shown by other workers (Haggar, 1966; Banisch and Humphreys, 1977), it is probable that early reproductive tillers were the major contributor to potential seed yield.

Aerial tillers arising from the elevated nodes on the parental culm were major contributors to the total number of tillers at harvest, and also to potential seed yield per plant. Although other workers have noticed and reported the occurrence of aerial tillers, (Dirven *et al.*, 1979; Boonman, 1971b) they have not been examined in detail. Dirven *et al.*, (1979) considered that aerial tillers were probably caused by high relative humidities and an optimal supply of nutrients.

Although the present results highlight the importance of aerial tillers to seed production it is important to appreciate that this occurred under conditions allowing uninterrupted growth from sowing to harvest. This would seldom occur in practice, as farmers would normally defoliate their Ruzi stands probably several times during the vegetative stage prior to closing for seed production, and hence the opportunity for the development of aerial tillers would be significantly reduced. As a result they would probably make only a minor contribution to seed yield compared with that from basal tillers.

In terms of potential seed yield, the present study showed that the shorter the daylength (11-h) the greater the seed yield and this tends to support the view that Ruzi is a quantitative short-day plant. However, there was no evidence to suggest that there was a critical daylength requirement, as flowering occurred at all the daylengths compared.

When one endeavours to interpret the results of this experiment in relation to the pattern of seed production in Thailand it is possible to suggest why Ruzi appears to require a critical daylength (of approximately 12½ h) to trigger reproductive development. Under the longest daylength of 13 h (Figure 1.1), which occurs in Thailand in June, the plants do not flower as they are only 4 - 6 weeks old, following the onset of the rainy season in late April/early May (Appendix 1.2), and hence are still in a juvenile stage. Under the shortest daylength of approximately 11½ hours (Figure 1.1), which occurs in December, the plants, although quite capable of becoming reproductive, apparently do not flower because of drought conditions following the onset of the dry season in early November (Appendix 1.2.), which inhibits any plant response. This suggests that although it is theoretically possible for Ruzi grass to produce seed over the entire range of daylengths which occur in Thailand, as recorded in this experiment, this does not occur possibly due to juvenility problems, in the 13 hour daylength and due to drought conditions in the 11½ hour daylength. This therefore perhaps explains why the daylength of approximately 12½ hour appears to be critical. However, this explanation will need to be confirmed, possibly by conducting trials in Thailand under an 11½ hour daylength with irrigation to overcome the lack of water.

CHAPTER TWO

REPRODUCTIVE MORPHOLOGICAL CHANGES IN RUZI GRASS

(*Brachiaria ruziziensis* Germain and Everard)

2.1. INTRODUCTION

As mentioned by many workers, photoperiod and temperature are the most important factors influencing reproductive development. Once initiation has occurred, these two factors continue to play a part in determining the rate of inflorescence development and the rate of stem elongation (Langer, 1972). This suggests that while the sequence of reproductive development within the species should be similar, the time duration between the stages may vary under different environments.

The objective of the present study, was to describe the morphological changes occurring from vegetative to reproductive development and to follow this sequence of development until the inflorescence emerged from the flag leaf.

2.2. LITERATURE REVIEW

Seed production is the culmination of a number of developmental phases (Humphreys and Riveros, 1986) which are influenced largely by daylength and temperature. According to Cooper (1960; cited by Hill, 1971) the initiation and development of grass inflorescences can be divided into three stages:

1) Floral induction, during which apical meristems become capable of responding to flowering stimuli.

2) Floral initiation, in which visible changes in the inflorescence occur in response to environmental conditions.

3) Floral development of the meristem tissue with accompanying elongation of the stem internodes.

FLORAL INDUCTION

Wareing and Phillips (1986) noted that among plants which are sensitive to daylength or chilling there are some species which will not respond until they have reached a certain minimum size ("ripeness-to-flower"). Before they have reached this stage seedling plants may be described as being in a "juvenile phase". In the view of Humphreys and Riveros (1986) the period of such "juvenility" is short-lived and less important in many species which come from semi-arid areas, and whose adaptation necessitates rapid flowering soon after effective rainfall has occurred, so that seed formation may be completed before drought conditions occur. In others crops the duration of the juvenile phase is longer and is a more significant factor influencing decisions about sowing time and controlling the potential number of sites on the plant which may subsequently develop inflorescences, especially in plants with a determinate habit such as some grasses and legumes. According to Langer (1972) the length of the vegetative phase can be measured on a time scale or by the number of leaves produced by the stem apex before floral organs are initiated.

Many temperate perennial grasses must pass through winter conditions of low temperature and/or short daylength, if they are to flower subsequently (Holmes, 1989). However, this so-called vernalization requirement has not been reported for tropical pasture plants, although temperature conditions may modify the critical daylength for flowering (Humphreys and Riveros, 1986).

FLORAL INITIATION and DEVELOPMENT

A flowering stimulus arises in the leaves of both SDP and LDP under favourable daylength conditions and is transported to the meristems where it causes a vegetative apex to change to the flowering condition (Wareing and Phillips, 1986).

The transition from a vegetative to a floral apex is marked by an increase in length, a cessation of leaf primordia initiation, and the development of floral primordia in the axils of the leaves.

The visible onset of this reproductive stage is usually determined by using the "double ridge" identification in many grasses. These so-called "double ridges" arise from a double structure composed of leaf and bud primordia which grows rapidly. However, in physiological terms, this event reflects a complete change in the relationship of leaf and bud. Whereas in the vegetative condition the bud remains dormant, often for prolonged periods, the reverse takes place in the reproductive stage. The developed of leaves is now inhibited and the reproductive buds develop further (Langer,1972). The formation of seeds follows the

differentiation of inflorescences on the terminal apices of the shoots or tillers, which formerly budded off leaf initials. In addition, axillary buds located within the leaf bases expand and provide a further reservoir of sites for inflorescence differentiation.

Morphological changes in the apex of temperate grasses and development and visible changes occurring during reproductive development have been studied by a number of workers, for example, Sharman (1947) with *Agropyron repens*; Evans and Grover (1940) with perennial ryegrass, timothy and cocksfoot; Evans and Allard (1934) and Griffiths *et al.*, (1967) with cocksfoot; Jeater (1956) with perennial ryegrass, cockfoot, timothy and meadow fescue; Cooper (1950) with a range of ryegrasses; and Hill (1971) with ryegrass, timothy and prairie grass. By comparison, only limited studies of these changes have been made in tropical grasses, possibly the best of which is by Humphreys and Riveros (1986), who divided them into six stages of morphological change in *Brachiaria decumbens* viz. stage 1: Vegetative stage, stage 2: Transitional stage, stage 3: Raceme Initiation (RI) stage, stage 4: Spikelet Initiation (SI) stage, stage 5: Spikelet Differentiation (SD) stage and stage 6: Inflorescence Exsertion (IE) stage.

2.3. MATERIALS AND METHODS

Samples were collected from plants which were used to determine the changeover from the vegetative phase to the reproductive phase in the first experiment (CHAPTER ONE).

Tillers for dissection were removed at approximately ground level. The leaves and leaf sheaths were then removed, using a razor blade and needle, working under a binocular microscope. The investigation started with the first basal tiller and dissection from the top node to the lowest node in each tiller.

Selected samples of apices were kept in FAA solution (Formalin 5%, Acetic acid 5% and Alcohol 90%) until photographs were taken by Scanning Electron Microscope (SEM) and by a regular camera for larger specimens to pictorially present the sequence of floral development.

2.4. RESULTS

The developmental stages are shown in Plates 2.1-2.5. The series of floral developments used to describe the sequence of the change-over from vegetative to reproductive development, are based on those used by Humphreys and Riveros (1986) who studied these changes in *Brachiaria decumbens*. However, in the present study, it was considered more appropriate to divide *Brachiaria ruziziensis* reproductive development into 5 stages as follows :

Stage 1 : Vegetative stage (Plate 2.1)

This refers to seedlings or new tillers from established pastures which have been previously defoliated and pass through a vegetative phase in which leaf differentiation and basal shoot production increase the density of growing points.

The vegetative apex is characterized by a simple dome-like structure which has leaf primordia initiating on alternate sides. At the beginning of this stage, the vegetative apex is usually situated at ground level before the node is developed and is enclosed within the leaf sheath.

Stage 2 : The appearance of early raceme initiation in the "Double ridge" stage (Plate 2.2)

The transition from the vegetative to the reproductive phase is commonly observed in the structure of the shoot apical meristems, described as the "double ridge" stage. According to Humphreys and Riveros (1986), this visible onset of reproduction takes place rapidly in *Brachiaria decumbens* and it is common to discover apices that are either vegetative or exhibit raceme initials (RI) even at some later development stage. Similarly, in the present study, as shown in plate 2.2, early raceme initiation had already appeared in the transition stage. Hence, it was decided to combine these two stages, described by Humphreys and Riveros (1986), as the double ridge stage and the raceme initiation stage into a single stage 2. In *B. decumbens*, the first raceme initiates in the axil of the most recently initiated leaf primordium, and the initiation of further racemes continues basipetally (Humphreys and Riveros, 1986). The most advanced raceme is always uppermost. This was also observed in the present study.

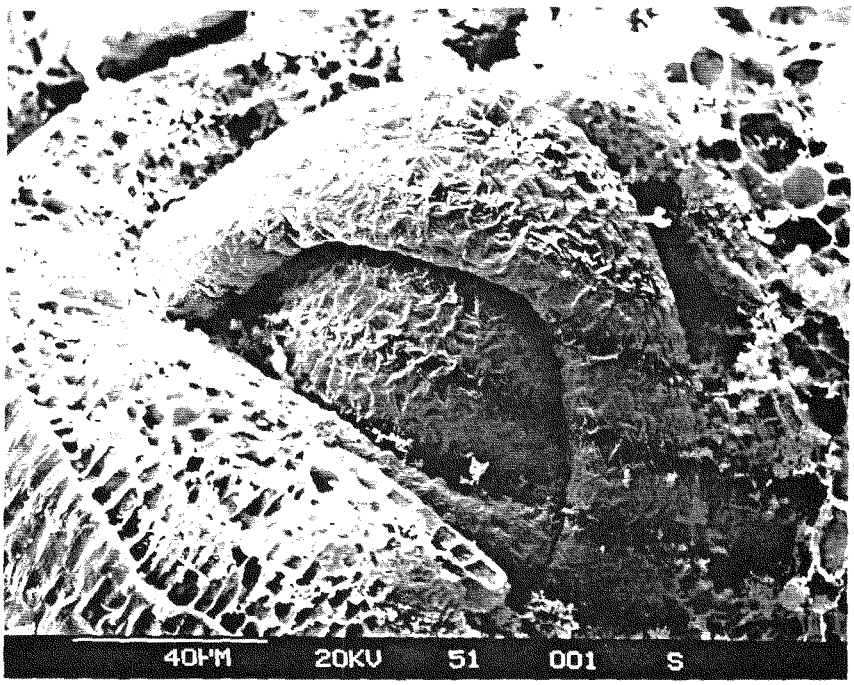


Plate 2.1. *Brachiaria ruziziensis* Vegetative apex with leaf primordia, stage 1, SEM x 650

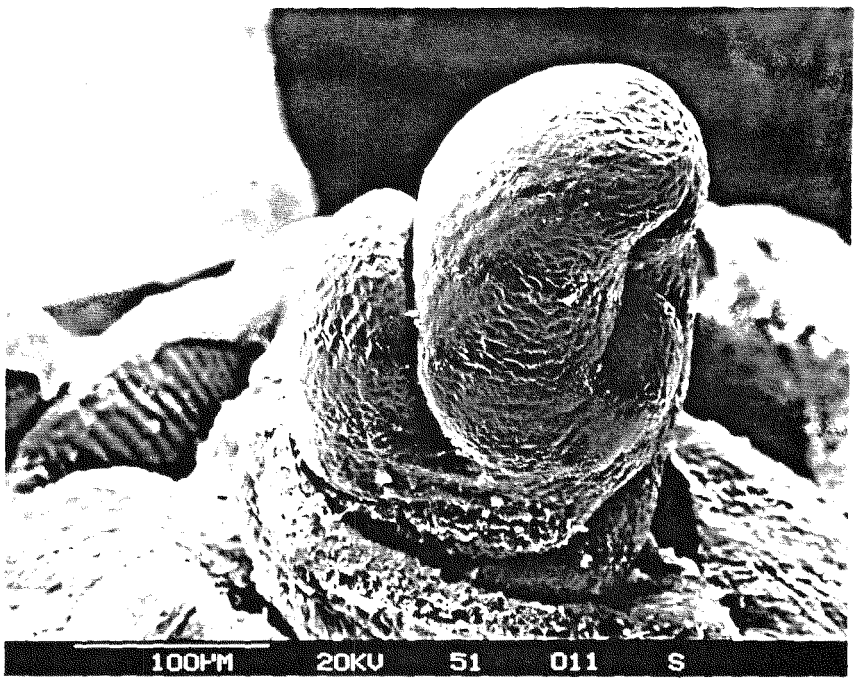


Plate 2.2. *Brachiaria ruziziensis* The transition from a vegetative to a floral apex showing the double ridge at the base and early raceme initiation, stage 2, SEM x 250

Stage 3 : Floret Initiation (FI) stage

Florets emerge first as ridges in the middle region of each raceme, and develop both acropetally and basipetally. This pattern of emergence helps to explain why the pattern of anthesis usually commences in the middle region of the uppermost raceme. The number of racemes is always completed during this stage, and varies from 1 to 8. The original apex then degenerates.

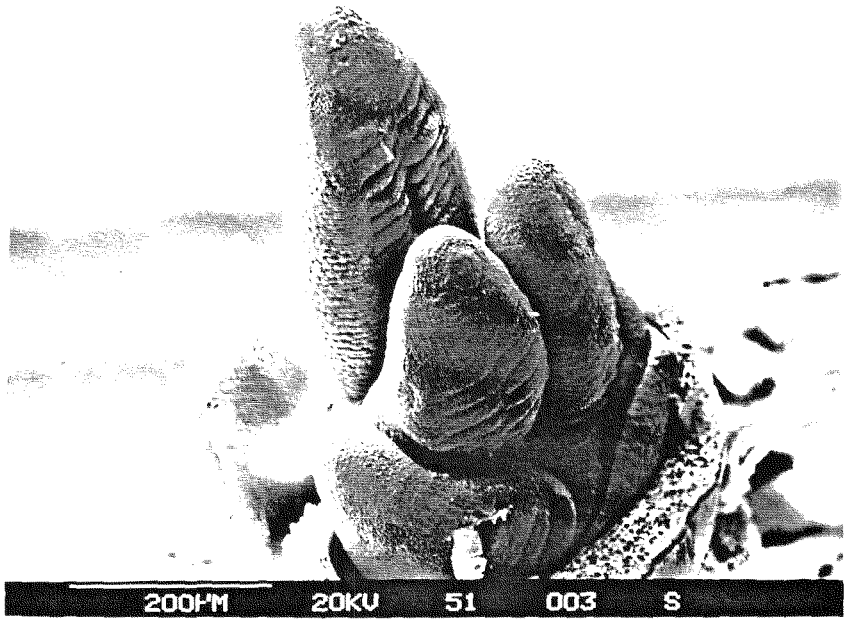
This stage, could be divided into 2 sub-stages according to floret appearance as follows:

3.1. Early Floret Initiation

This sub-stage starts when a raceme shows a clear location of florets in the middle part of the raceme which appear as two parallel vertical lines, as shown in Plate 2.3.1.

3.2. Advanced Floret Initiation

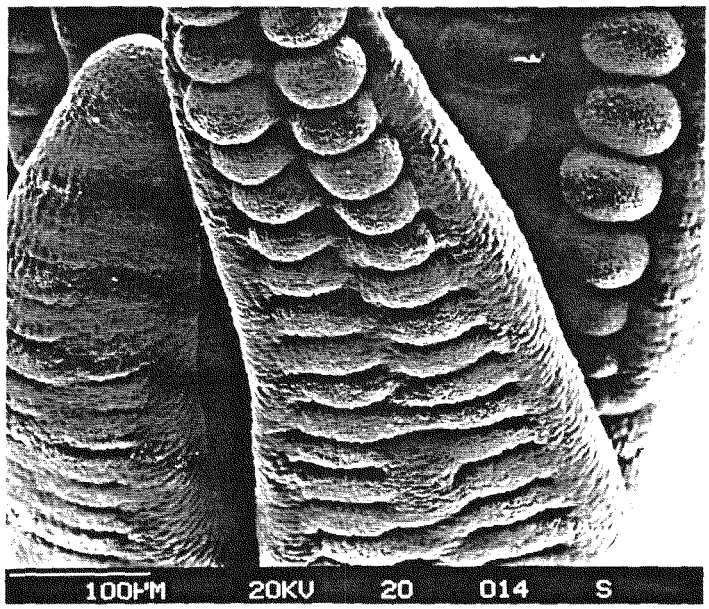
This sub-stage is apparent when the florets increase in size (Plates 2.3.2a and b) and fully occupy the whole raceme (Plate 2.3.2c).



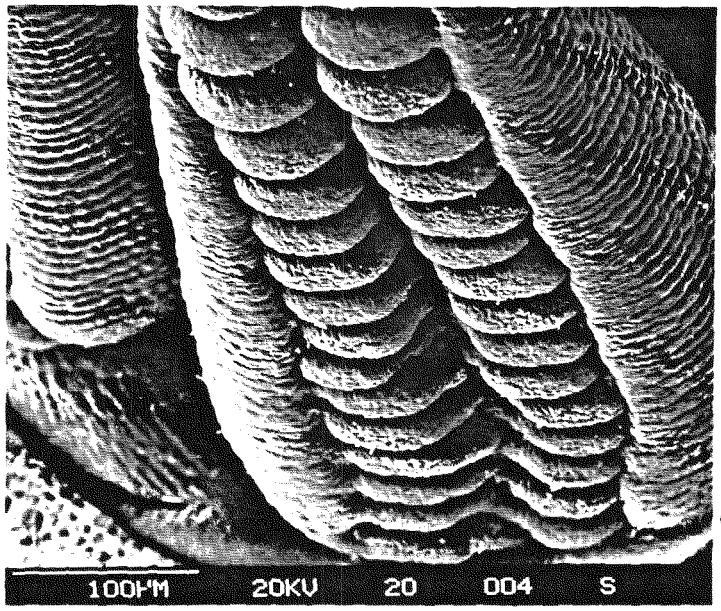
(a) early FI, 4 racemes, SEM x 137

(b) early FI, 5 racemes, SEM x 137

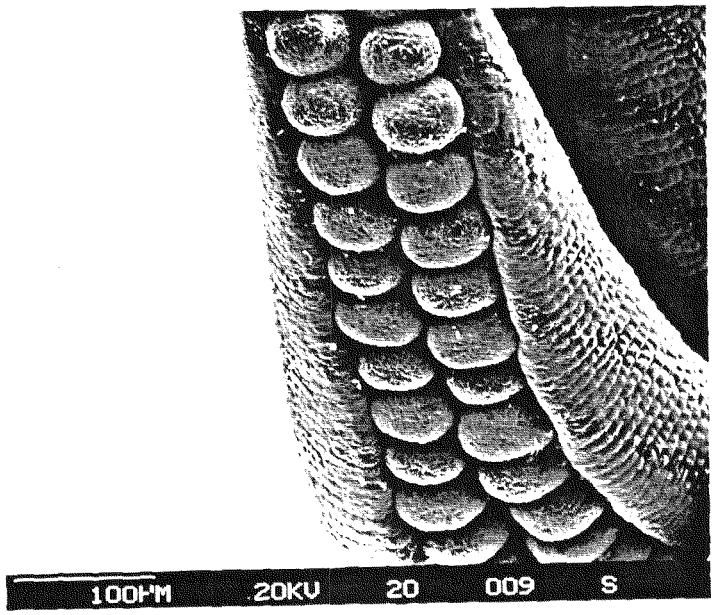
Plate 2.3.1. *Brachiaria ruziziensis* Floret initiation (FI), stage 3, (a) and (b) early floret initiation



(a) advanced FI, SEM x 180



(b) advanced FI, SEM x 240



(c) advanced FI, SEM x 180

Plate 2.3.2. *Brachiaria ruziziensis* Floret initiation (FI), stage 3, (a), (b) and (c) advanced floret initiation

Stage 4 : Floret Differentiation (FD) stage

This event occurs when ridges appear on the most advanced florets in the middle region of the uppermost raceme. Floret numbers range from 7-50 per raceme and the average is about 33 floret per raceme (CHAPTER THREE). This stage could also be classified into 2 sub-stages as follows:

4.1. Early Floret Differentiation

The first appearance which identifies the starting point of early floret differentiation is the development of glumes as shown in Plate 2.4 and a close-up of a floret in Plate 2.4.1. Lemma (l) also develops at the latest phase of early floret differentiation (Plate 2.4.1f).

4.2. Advanced Floret Differentiation

This sub-stage presented in Plate 2.4.2 (the whole onset) and Plate 2.4.2.1 (close-up of florets) shows the further development of the floret, the appearance of the palea (P), the differentiation of the top of the floret into stamens and an ovary in the upper fertile floret, and stamens only in the lower fertile floret. In the final phase the ovary and stamens are enclosed by the lemma and glume. This sub-stage reflects a similar pattern to that described by Humphreys and Riveros, 1986).

Stage 5 : Inflorescence Exsertion (IE) stage (as shown in Plate 2.5)

This stage appears when an inflorescence is fully exserted above the flag leaf.

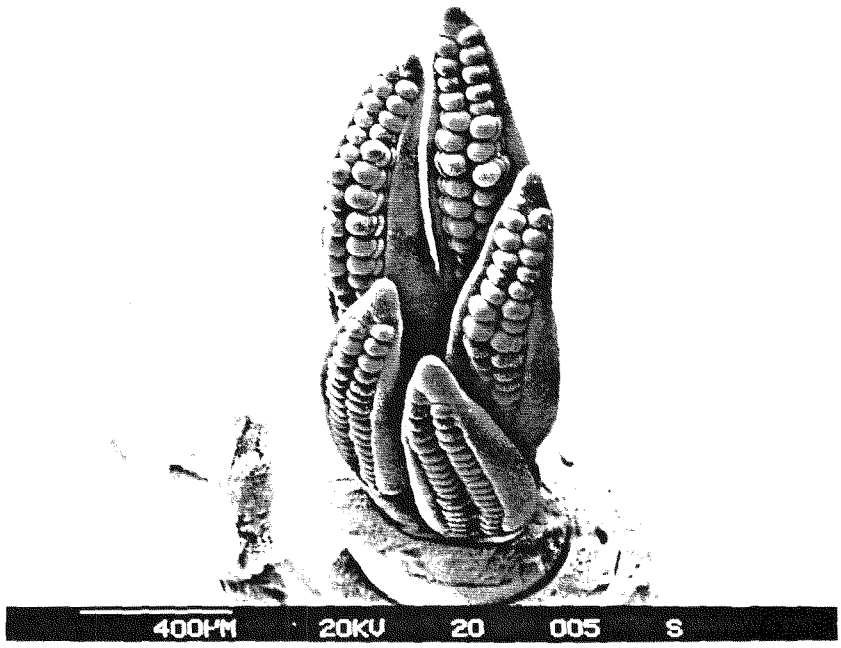
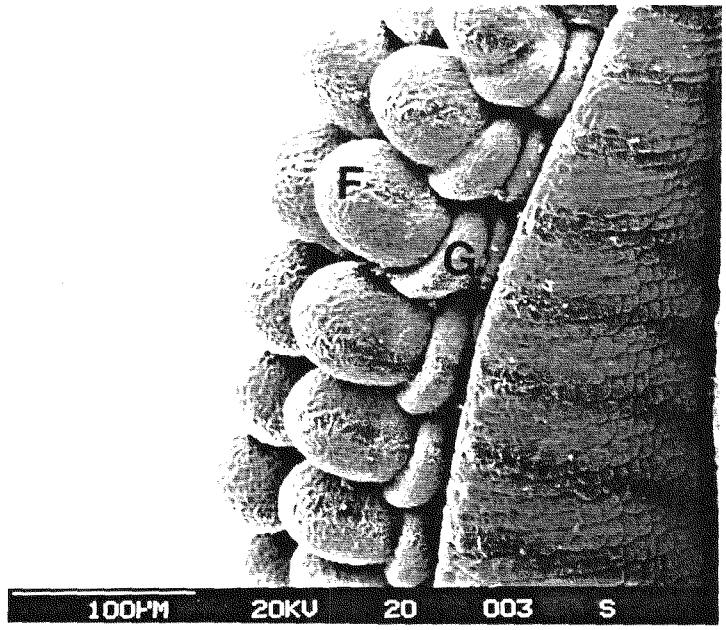
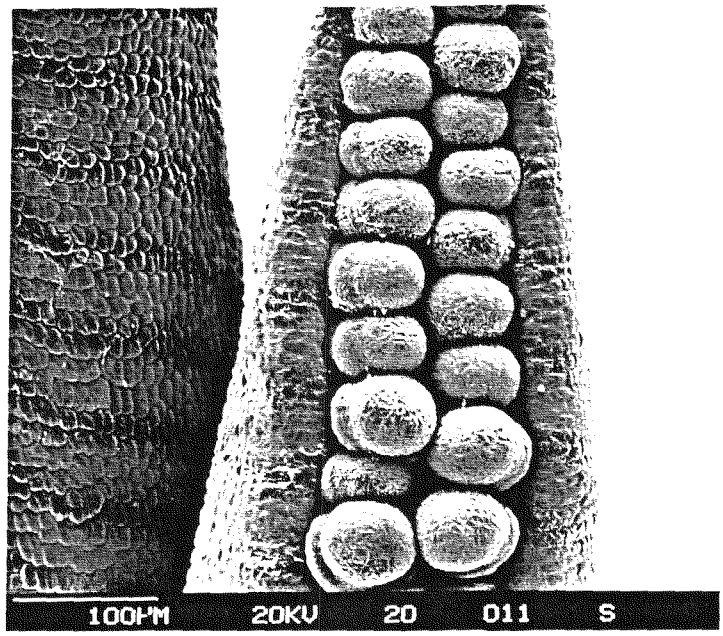


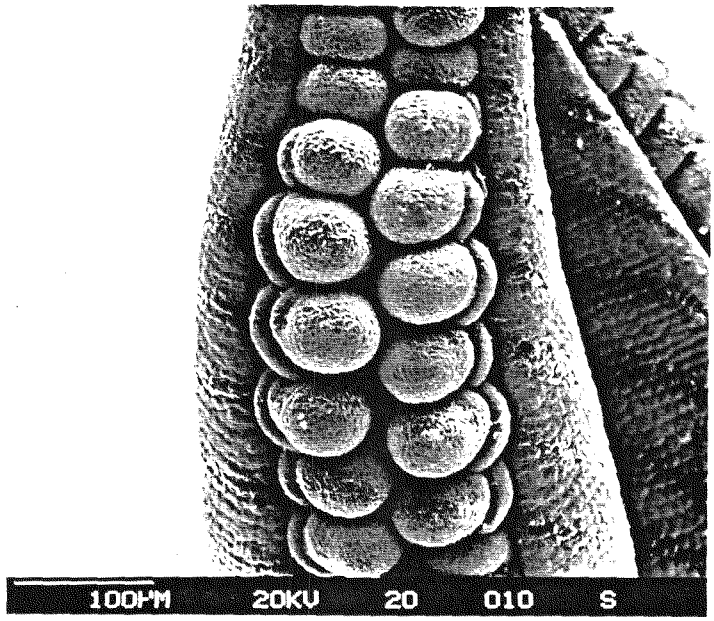
Plate 2.4. *Brachiaria ruziziensis* Floret differentiation (FD), stage 4, early floret differentiation, 5 racemes, SEM x 50



(a) early FD, floret (F) with glume (G), SEM x 240

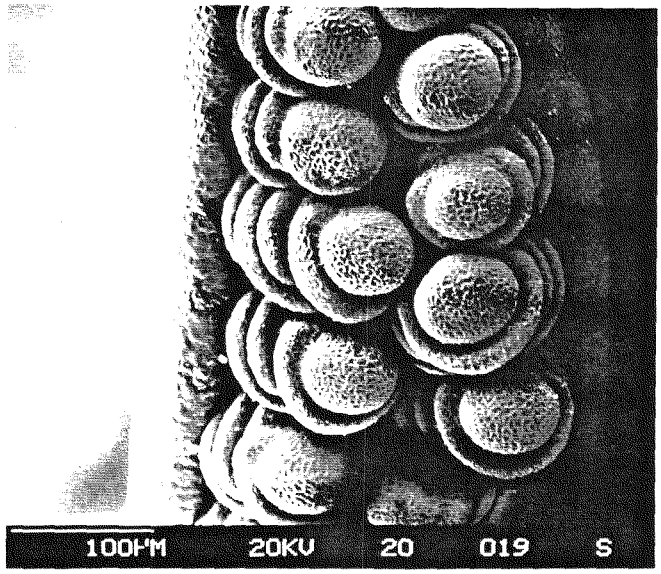


(b) early FD, SEM x 190

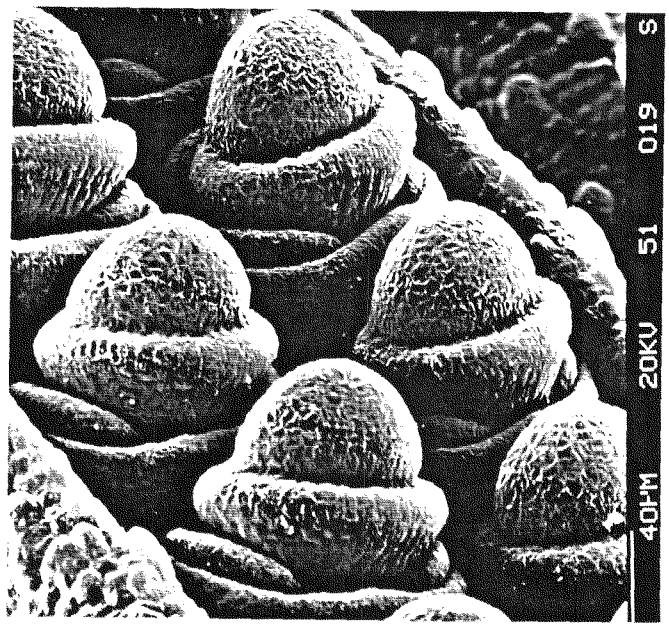


(c) early FD, SEM x 180

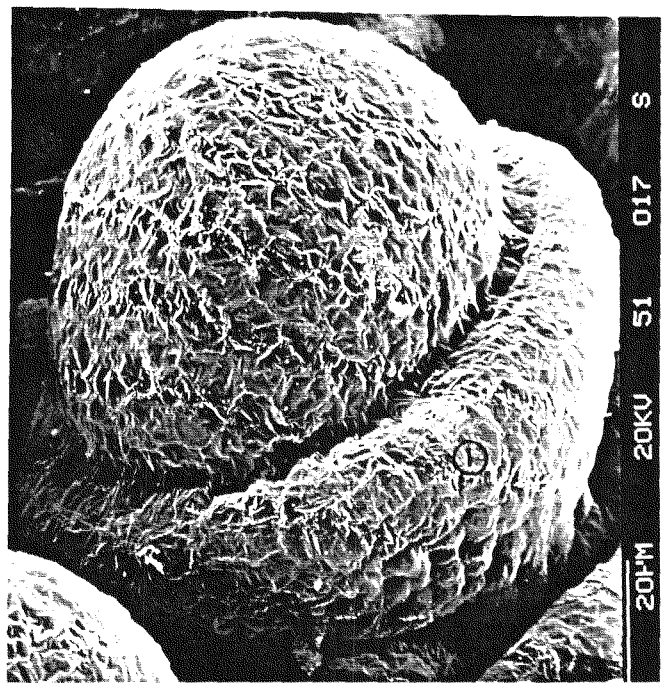
Plate 2.4.1. *Brachiaria ruziziensis* Floret differentiation (FD), stage 4, (a), (b) and (c) early floret differentiation (close-up of floret with glumes)



(d) early FD, SEM x 180



(e) early FD, SEM x 330



(f) early FD, floret with lemma (l), SEM x 950

Plate 2.4.1.(cont.) *Brachiaria ruziziensis* Floret differentiation (FD), stage 4, (d), (e) and (f) early floret differentiation (close-up of floret with glumes and lemma(l) development)

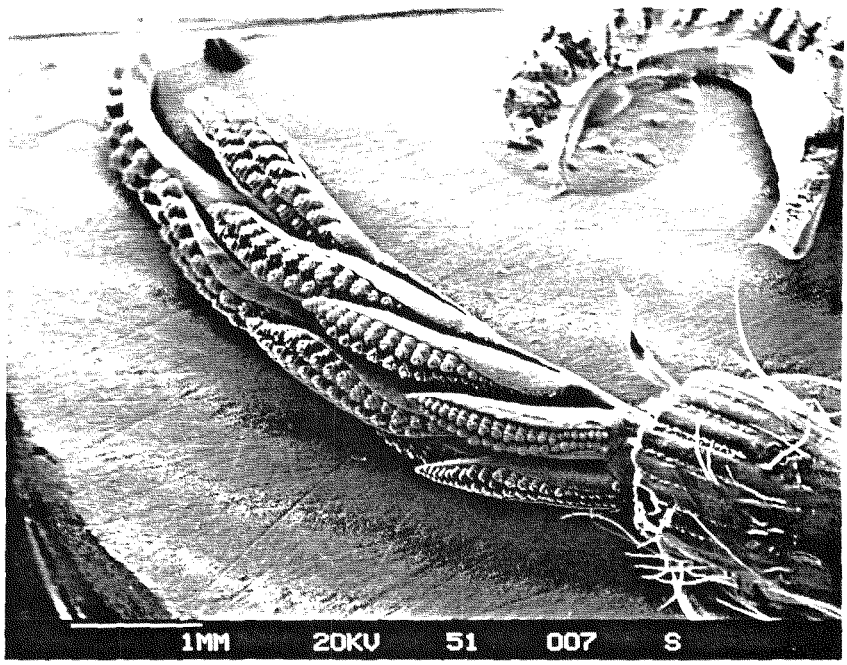
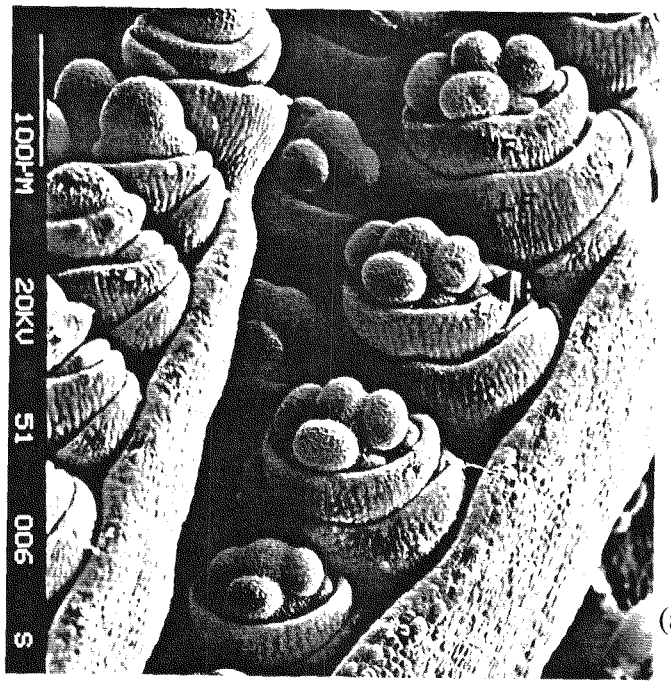
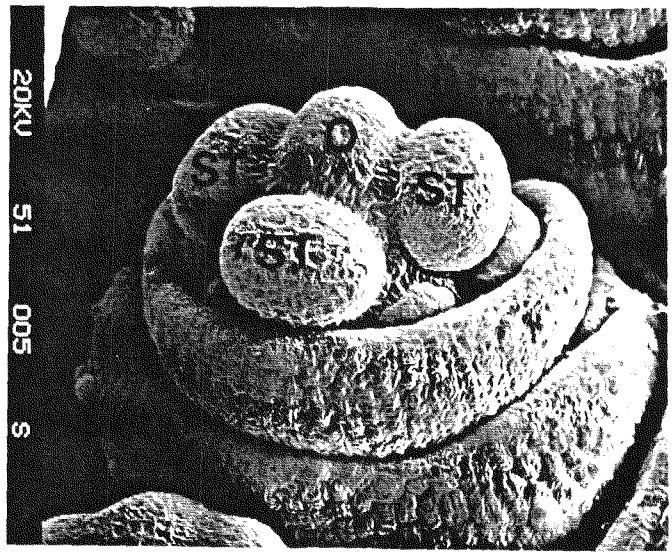


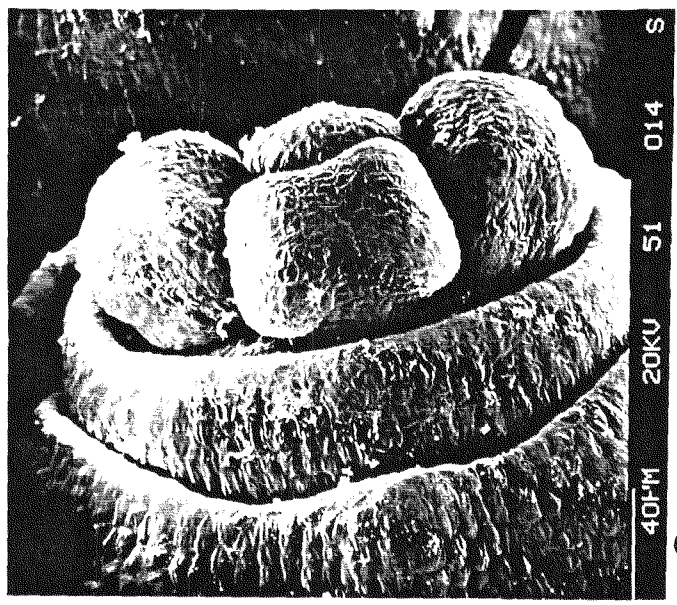
Plate 2.4.2. *Brachiaria ruziziensis* Floret differentiation (FD), stage 4, advanced floret differentiation, 8 racemes, SEM x 16



(a) advanced FD, SEM x 160



(b) advanced FD, SEM x 430



(c) advanced FD, SEM x 460

Plate 2.4.2.1 *Brachiaria ruziziensis* Floret differentiation (FD), stage 4, (a), (b) and (c) advanced floret differentiation showing (a) palea (P), upper floret (UF) and lower floret (LF), (b) ovary (O) and stamens (ST)

Plate 2.5. *Brachiaria ruziziensis* Inflorescence Exsertion (IE), stage 5

2.5. DISCUSSION

Langer (1972) emphasised that one or many individual flowers or florets may make up a spikelet as shown in Figure 2.1 (Gill and Vear, 1969; cited by Langer, 1972). Therefore, in this study it is preferable to use the term "floret" instead of "spikelet" which Humphreys and Riveros (1986) used to define the individual flower within the raceme.

Although the stages of morphological change in this study are based on those described by Humphreys and Riveros, 1986 for *Brachiaria decumbens,* it was considered more appropriate to combine their stages 2 and 3, and thereby reduce the total number to 5 rather than 6 stages. However, stages 3 and 4 in this study were each divided into two sub-stages which appeared to differentiate the developmental changes more precisely.

Unfortunately the time between each morphological stage could not be determined in this study. However, the time from floral initiation to first anthesis was approximately 29 days (CHAPTER ONE). From observation, it appears that the duration from first raceme emergence above the flag-leaf to first anthesis is approximately 7 days. Therefore, it can be assumed that it takes approximately 22 days for the morphological changes commencing at stage 2 to be completed to stage 5, the first day of emergence.

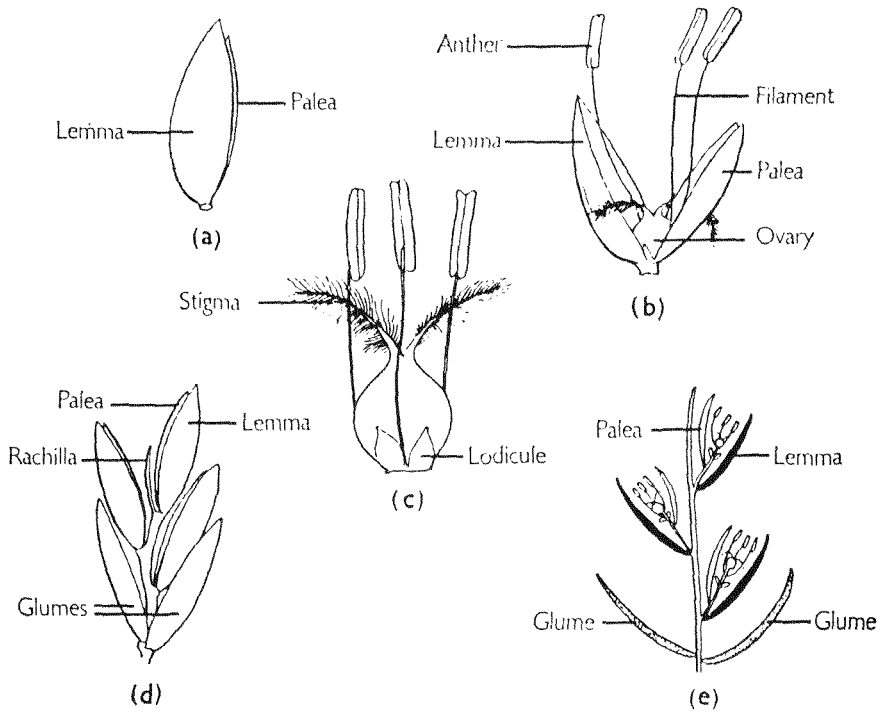


Figure 2.1. Floral biology of Ruzi grass (a) single floret closed; (b) single floret open; (c) floral parts; (d) spikelet; (e) diagram of spikelet. (From Gill and Vear, 1969; cited by Langer, 1972)

CHAPTER THREE

FLOWERING PATTERN, FLORET FERTILITY AND SEED SHEDDING IN RUZI GRASS (*Brachiaria ruziziensis* Germain and Everard)

3.1. INTRODUCTION

Many tropical grasses have the potential to produce high seed yields, only a small proportion of which is actually harvested by normal harvesting methods. The problem of low seed yields and quality of tropical grasses has been reviewed by many workers e.g. Boonman, 1971a; Langer, 1972; Humphreys and Riveros, 1986. However, in *Brachiaria ruziziensis* little information on the factors affecting seed yield and quality is available at the individual inflorescence level.

The objectives of this study therefore were:

1. To determine the position of the first floret to exert anthers in the first raceme (the raceme which first reached anthesis)
2. To record the duration from first anthesis until all florets per raceme had anthers exerted
3. To compare the percentage of fertile florets, sterile florets and florets shed in inflorescences bearing different numbers of racemes

3.2. LITERATURE REVIEW

Bogdan (1977) described the process of flowering in tropical grasses and

stated that flowering or anthesis, usually begins with the opening of the floral glumes (lemma and palea) helped by swelling of the lodicules. Filaments of the stamens elongate and the anthers exert and hang outside the spikelet and dehisce and release the pollen. Stigmas either bend and protrude sideways from the floret or appear from its top. After the release of pollen the lemma and palea close up again. In most grasses, anthesis or opening of the floret occurs 1-2 weeks after the ear has emerged, although there are some in which it occurs sooner (Langer, 1972). Humphreys and Riveros (1986) noted that the anthesis of *Brachiaria decumbens* begins coincidentally for about 10 days.

In a study of *Brachiaria decumbens*, Humphreys and Riveros (1986) found that flowering generally occurs in the early morning from shortly after sunrise to c.0900 hours. However, the time of flowering and the process of flowering can vary, depending mainly on the species and also on the weather (Bogdan, 1977). Humphreys and Riveros (1986) also reported that anthesis in *Brachiaria decumbens* begins in the middle of the uppermost raceme, and that raceme numbers vary from 1-7 per inflorescence.

The number of seeds produced by an individual inflorescence will depend on the number of racemes and florets formed, which can be affected by photoperiod, temperature and time of initiation. However, not all spikelets can be fertilized and form a seed. Langer (1972) noted that the percentage of fertile florets can vary, from as low as 25% to more than 90% in field-grown cocksfoot and timothy.

Burton (1943) reported low seed setting in a number of tropical grasses. Boonman (1971) noted this low average percentage of seed setting is affected by the following factors : (1) prolonged head emergence within plants, (2) prolonged anthesis and stigma exsertion within single heads and (3) the decreasing duration of flowering and decreasing head length in progressively later emerging heads. All of these may determine the actual potential of individual florets setting seeds.

The degree of seed setting can be affected by three main factors (Humphreys and Riveros, 1986) : firstly, the weather conditions - e.g. low seed set results during dull, cool, rainy days because plants require sunshine for flower opening; secondly, the internal plant controls - e.g. seed number, seed size; and lastly, genetic control - e.g. the wide variation in seed production of clones of *Setaria sphacelata* var. *sericea* reported by Boonman and van Wijk (1973).

Langer (1972) considered there are five factors which affect the proportion of florets capable of setting seed within individual spikelets or on the same inflorescence, i.e. : the genetic make-up; environmental influences especially extremes of temperature or reduced water supply; mineral supply such as nitrogen; competition for a limited supply of assimilates; and seed shedding.

Generally, seeds of tropical grasses shed rapidly as they are formed (Oliveira and Humphreys, 1986). The number of florets shedding usually increases with time and then becomes sigmoid on a weight basis, due to the failure of seed setting in early formed florets and their premature shedding. Mwakha (1970) suggested that

in *Entolasia imbricata* unfertilized spikelets absciss early, while fertilized ones only absciss on ripening. Boonman (1973) also reported that most of the early shedding in *Setaria sphacelata* cv. Nandi 1 and Nandi 3, *Chloris gayana* cv. Mbarara, Masaba and Pokot Rhodes and *Panicum coloratum* cv. Solai, was of empty spikelets. Boonman (1971), Langer (1972), and Hare and Waranyuwat (1980) noted that seed shedding or seed shattering usually occurs from ripe inflorescences. However, Devahuti *et al.*,(1986) indicated that although Ruzi grass seed (*B. ruziziensis*) reaches physiological maturity approximately 3 weeks after flowering, about 5% of seed is shed at 10 days after flowering and this increases to 60% when the seeds reach physiological maturity.

3.3. MATERIALS AND METHODS

Observations of flowering pattern, seed set and seed shedding of Ruzi grass were carried out during a preliminary study in a controlled temperature glasshouse using the same management as described for the first experiment (CHAPTER ONE). Seed had been sown on 10 September 1992, and the 10 plants reached first anthesis on 12 January 1993.

Onset of anthesis (i.e. first anthesis) was used as the starting point for the determination of flowering pattern, anthesis being recorded as the exsertion of the anthers and stigma (Humphreys and Riveros, 1986). Langer (1972) and Bogdan (1977) noted that anthesis, or opening of the floret, is the first outward sign that pollination is about to begin. The swelling of the lodicules is the most common

mechanism causing the lemma to part from the palea, allowing the feathery stigmas to spread out and the anther filaments to elongate.

Each inflorescence of the plant was tagged as it appeared and the position of the first floret to exsert anthers and the day of anthesis in other florets in each raceme was recorded daily until all florets in the inflorescence had completed anthesis. Anthesis state was determined between 9-11 a.m. every day.

Inflorescences were harvested thirty days after first anthesis. For each inflorescence, raceme numbers, floret numbers in each raceme, total fertile floret numbers, sterile floret numbers and shed floret numbers, were recorded.

Seed set was defined as the proportion of the total number of florets containing caryopses (Ferguson and Crowder, 1974) and caryopsis development stage was indicated by the onset of the hard dough stage (Humphreys and Riveros, 1986).

Sterile floret number was defined as the number of florets which were not occupied by caryopses, based on the number of florets originally present at the time of inflorescence emergence. Data did not include florets in which anthesis did not occur.

Shed florets number was defined as the number of missing florets at harvest. It seems likely that either fertile or sterile florets could have been shed, but from

experience, fertile florets which contained caryopses were more readily dropped from the raceme than sterile florets.

3.4. RESULTS

3.4.1. The pattern of first anthesis

First anthesis occurred at a wide range of floret positions within the raceme, irrespective of the number of racemes per plant (Figure 3.1.), but anthesis began more often in the uppermost raceme (Figure 3.2. and Plate 3.1.). Anthesis was completed more rapidly in the uppermost raceme than in the lowermost raceme. However, last anthesis generally occurred in both the upper and lowermost raceme.

3.4.2. Anthesis pattern

New flowers appeared over a period of 13-17 days from first anthesis, depending on raceme number (Figure 3.3.). The greatest percentage of first flowering occurred at 2,4,5,6 and 4 days for inflorescences bearing 1,2,3,4 or 5 racemes respectively.

3.4.3. The duration of flowering (days from first anthesis until all anthers per raceme were exserted)

The duration of flowering did not differ inflorescences bearing different

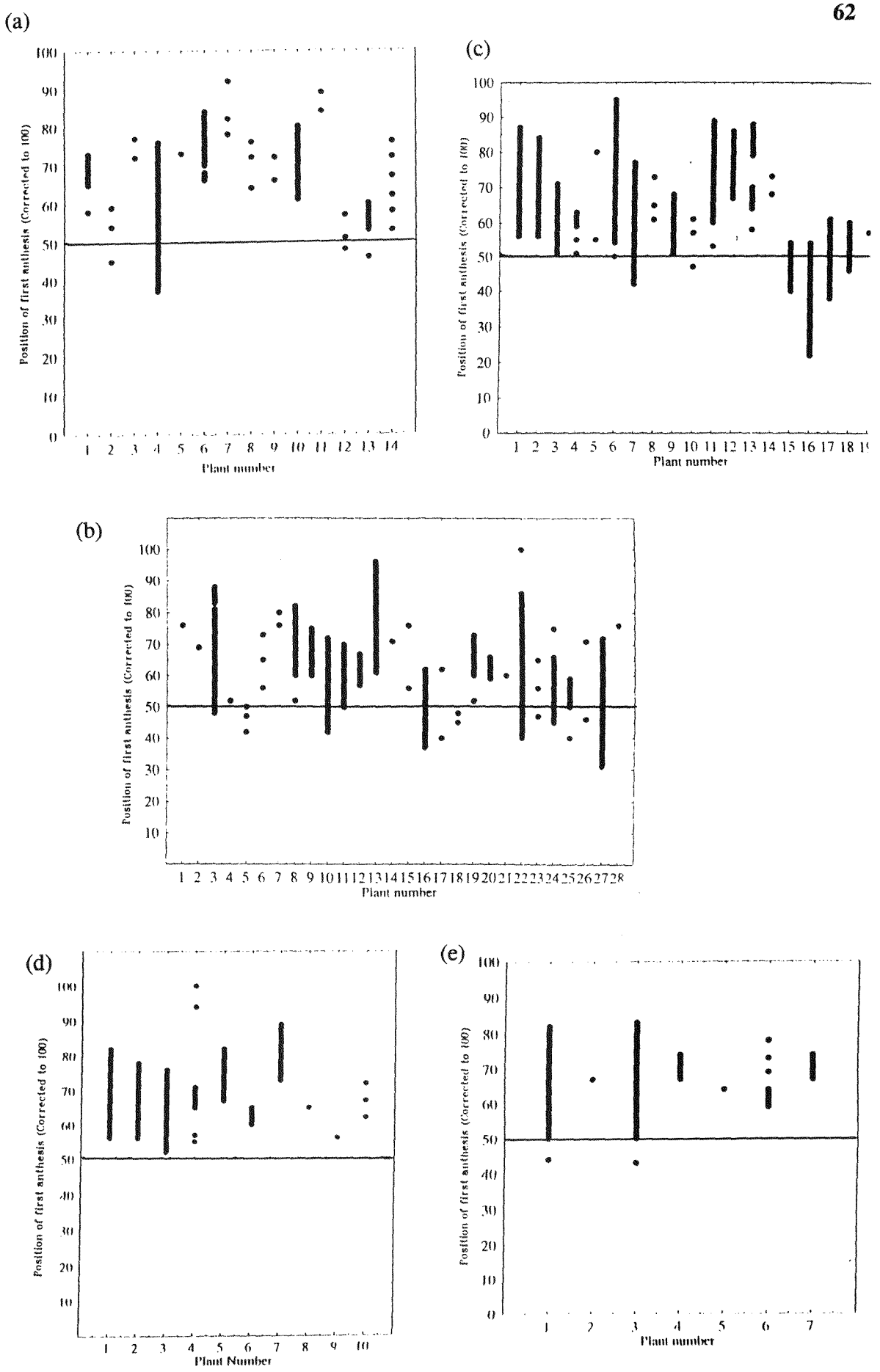


Figure 3.1. The position of the first floret which exserted anthers on the first raceme in different types of inflorescences (a) 1 raceme, (b) 2 racemes, (c) 3 racemes, (d) 4 racemes and (e) 5 racemes; (Line at 50 indicates half way between the uppermost and lowermost raceme)

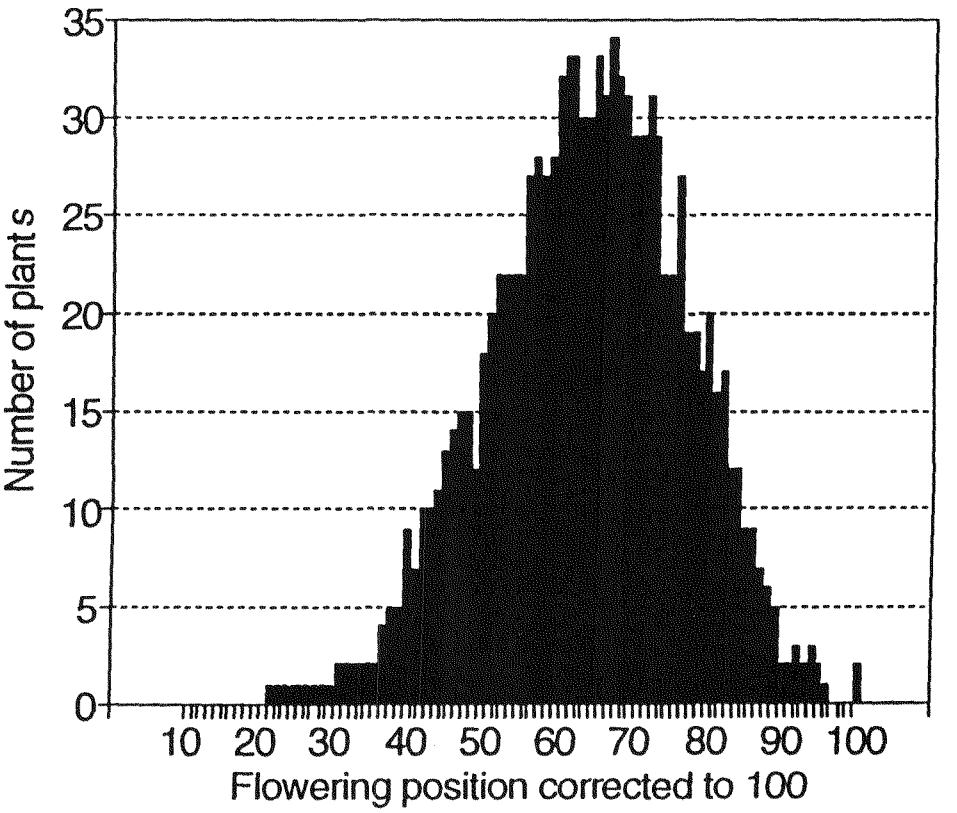
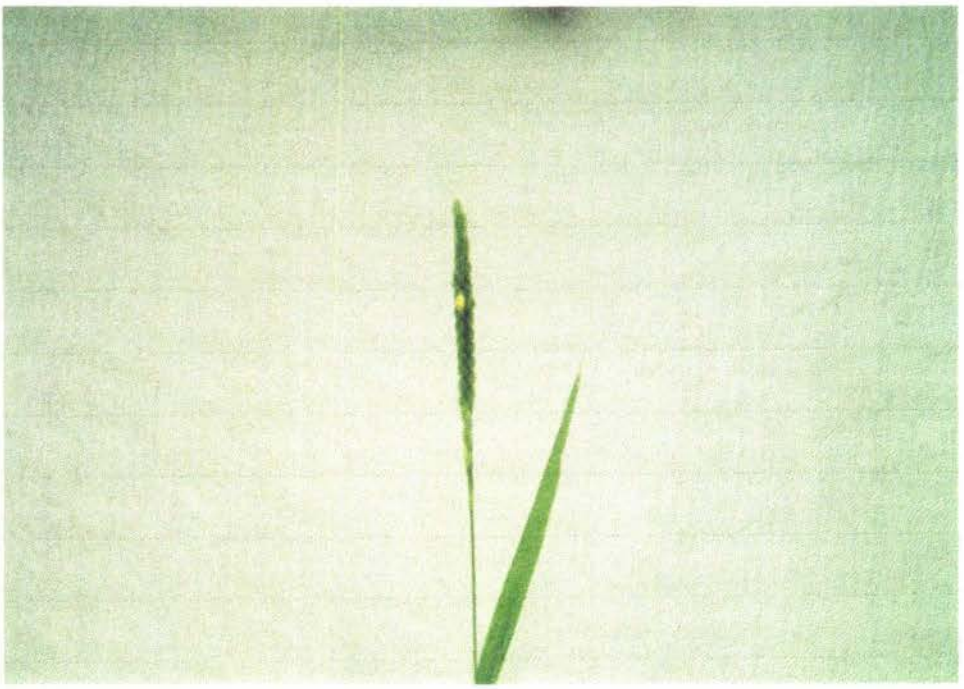


Figure 3.2. The position of first anthesis on the first raceme over all inflorescence types (1-5 racemes per inflorescence)

(a)



(b)

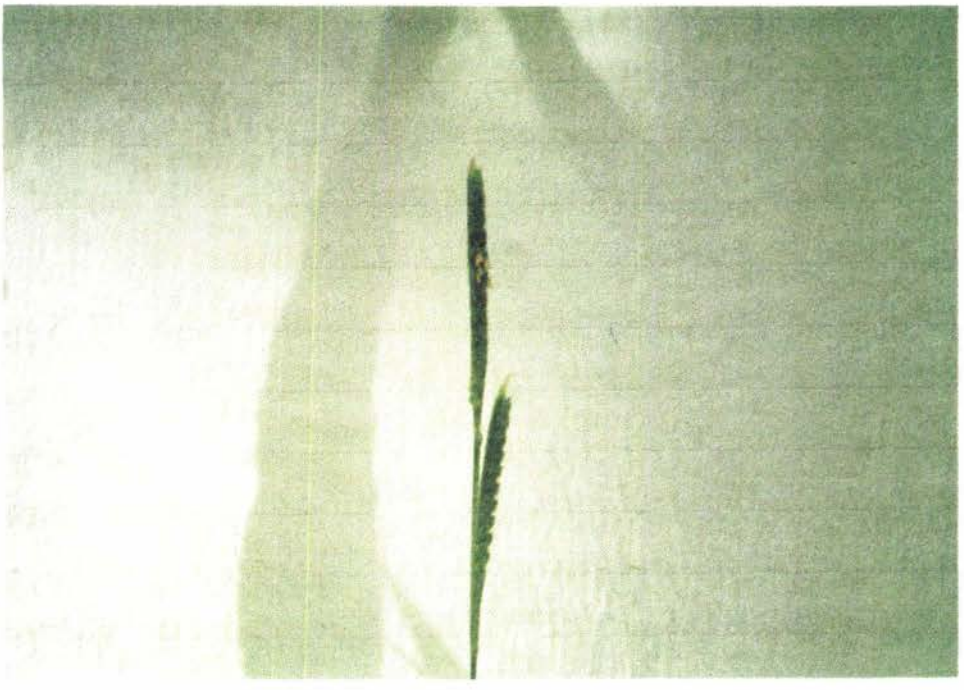


Plate 3.1. First anthesis in the middle region of the uppermost raceme, for (a) 1 raceme type, (b) 2 raceme type

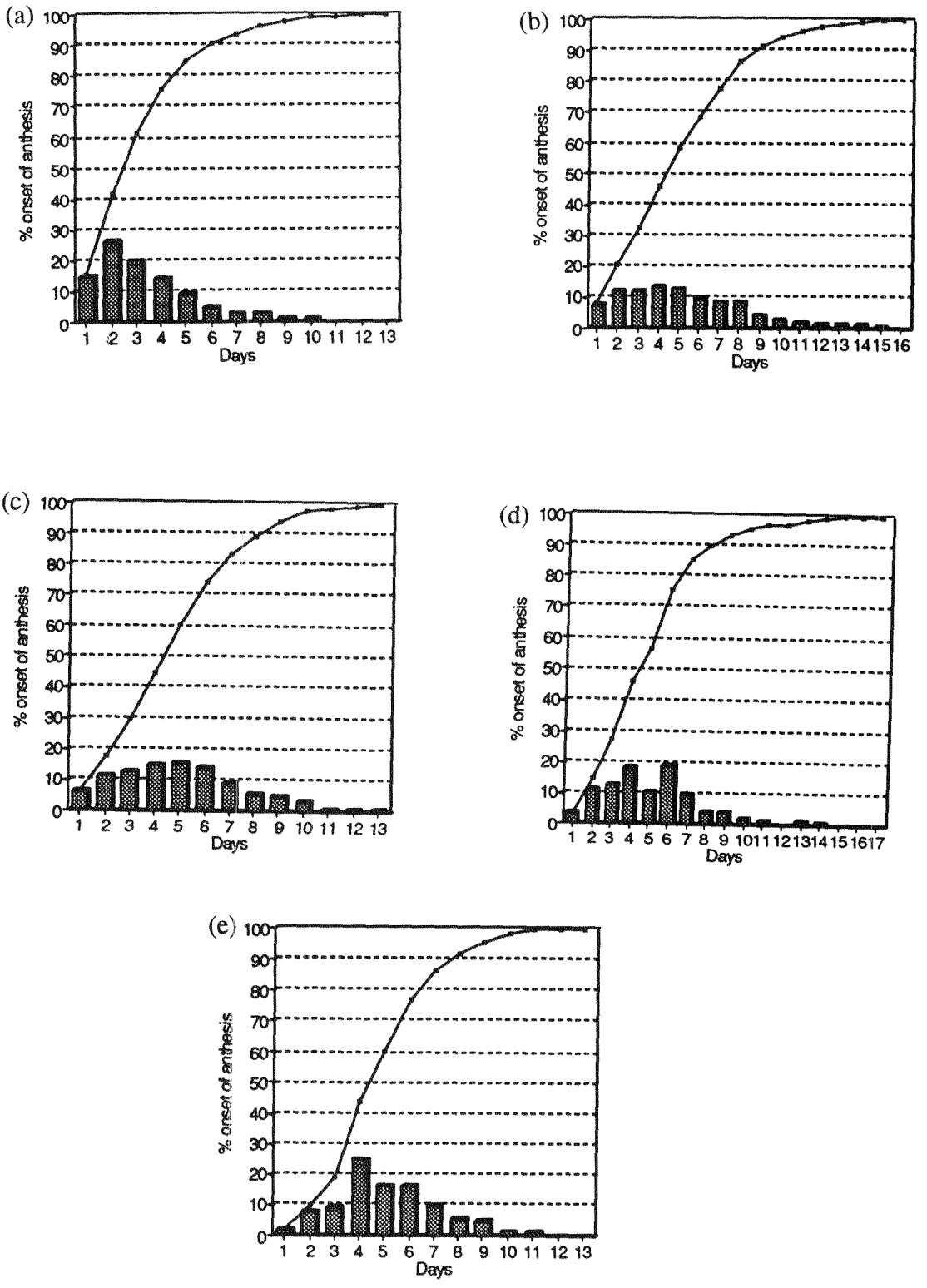


Figure 3.3. The percentage of florets with anthers exserted from first anthesis until all anthers per raceme were exserted in inflorescence containing different numbers of racemes (a) 1 raceme, (b) 2 racemes, (c) 3 racemes, (d) 4 racemes and (e) 5 racemes; ▩ % onset of anthesis/day, — % accumulation of anthesis/day

numbers of racemes (Table 3.1) averaging 10 days. It also did not differ between racemes within inflorescences bearing more than 1 raceme (Table 3.1).

Anthesis began earlier in plants with larger numbers of racemes per inflorescence than in those with smaller numbers of racemes per inflorescence (Table 3.2). However, it was possible to find racemes where anthesis began on the same day in inflorescences bearing different numbers of racemes (indicated by * in Appendix 3.1-3.4).

3.4.4. Fertile florets, sterile florets and florets shed

Floret number per raceme, % fertile florets, % sterile florets and % florets shed showed no significant difference between inflorescences bearing different numbers of racemes (Table 3.3), the mean being 33.20 florets per raceme with 20.3% fertile florets, 71.8% sterile florets and 7.9% of florets shed (Table 3.3). However, the data were highly variable, particularly for % fertile florets and % florets shed (Appendix 3.5-3.9).

Table 3.1. Days from first anthesis until all anthers per raceme were exerted over all inflorescence types (1-5 racemes per inflorescence)

Raceme per inflo.	Raceme number					Total (days)
	1st (top)	2nd	3rd	4th	5th (bottom)	
1	8.54 (±2.76)					8.54 (±2.76)
2	9.07 (±3.05)	7.64 (±2.09)				10.89 (±2.74)
3	7.29 (±1.69)	7.71 (±2.95)	7.06 (±2.16)			10.71 (±3.64)
4	7.56 (±3.64)	6.89 (±2.85)	7.00 (±3.61)	6.67 (±2.65)		9.56 (±4.07)
5	7.50 (±2.26)	7.33 (±1.86)	7.17 (±2.56)	6.67 (±1.75)	6.00 (±1.67)	10.00 (±2.19)

Table 3.2. Number of days from first anthesis in the first raceme to first anthesis in the second and subsequent racemes on individual inflorescences

Raceme per inflo.	Raceme number				
	1st	2nd	3rd	4th	5th
1	-				
2	-	3.44 (±1.98)			
3	-	2.00 (±0.78)	3.64 (±1.63)		
4	-	1.40 (±1.14)	2.00 (±1.23)	3.40 (±1.14)	
5	-	1.20 (±0.84)	1.80 (±0.84)	2.40 (±1.14)	3.80 (±1.30)

Table 3.3. Floret numbers, percentage fertile florets, percentage sterile florets and percentage florets shed at harvest 30 days after first anthesis in inflorescences bearing different numbers of racemes

	Racemes per inflorescence					Average
	1	2	3	4	5	
Floret numbers	32.38 (±8.28)	59.30 (±22.75)	93.55 (±27.46)	146.78 (±38.48)	180.60 (±54.10)	
Floret /raceme	32.38 (±8.28)	29.65 (±11.38)	31.18 (±9.15)	36.70 (±9.62)	36.12 (±10.82)	33.21 (±3.09)
% fertile florets	18.6 (±15.8)	16.7 (±19.2)	18.8 (±22.9)	25.7 (±32.6)	21.6 (±25.5)	20.3
% sterile florets	71.3 (±16.8)	70.3 (±22.3)	75.4 (±24.8)	71.6 (±33.0)	70.6 (±28.3)	71.8
% florets shed	10.1 (±15.5)	13.0 (±20.3)	5.8 (±10.7)	2.7 (±4.9)	7.8 (±15.4)	7.9

3.5. DISCUSSION

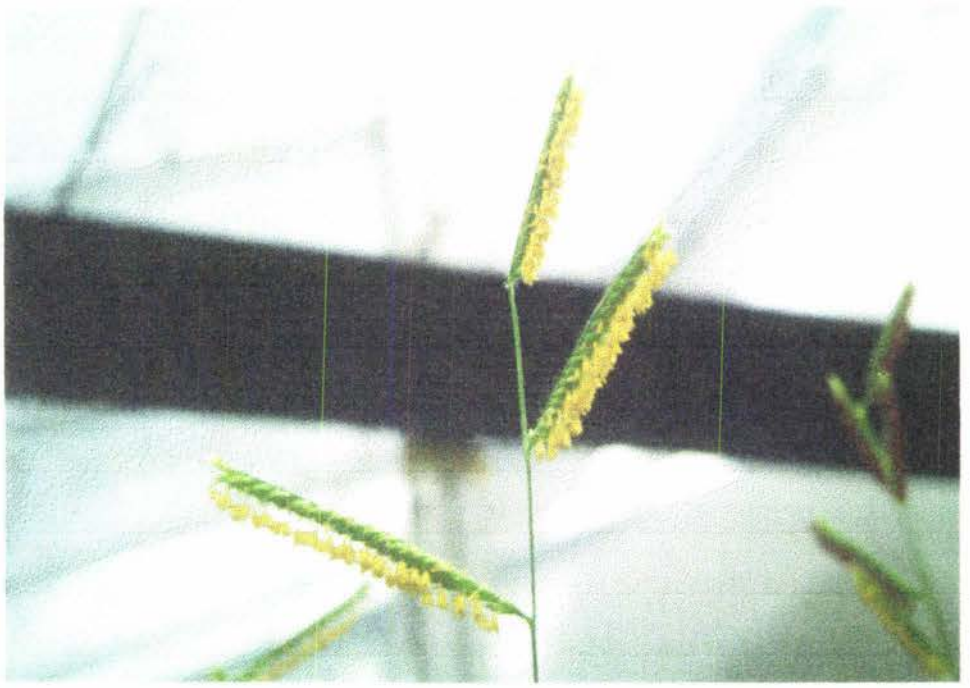
The number of racemes per inflorescence in *Brachiaria ruziziensis* ranged from 1-8, but in this study only inflorescences with from 1-5 racemes were used (Plate 3.2). This decision was taken because there were insufficient inflorescences with higher raceme numbers and also because of difficulties in handling them due the fact that they both lodged and shattered readily when handled.

Anthesis began in the middle region of the uppermost raceme, which is similar to the position described for *B. decumbens* (Humphreys and Riveros, 1986). The morphological study described in CHAPTER TWO clearly showed that differentiation was always initiated in the middle region of the raceme and expanded afterwards to the upper and lowermost raceme. This explains and confirms the pattern of first anthesis recorded in this study.

It was evident from Figures 3.1 and 3.2 that at some assessments only one floret had begun anthesis, whereas at other assessments, several florets had already exserted anthers. It is probable that on some days, racemes with florets in the latter category had actually achieved first anthesis before the assessment time i.e. before 9 am. In *B. decumbens* Humphreys and Riveros (1986) report that anthesis occurs in the early morning from after sunrise to c. 0900 hours.

Bennett (1959) noted that the daily timing of onset of anthesis varies with

(a)



(b)

Plate 3.2. Examples of inflorescences with (a) 3 racemes per inflorescence, and (b) 5 racemes per inflorescence in full bloom

species, and while it does not seem to be determined by atmospheric and soil moisture status, it does depend on temperature.

In the present study, an average individual raceme of *B. ruziziensis* consisted of 33 florets, and all completed anthesis within 1-2 weeks. When compared with some other tropical grasses, this is a relatively short duration of flowering. For example, in the digitate panicle of *Chloris gayana* cv."Mbarara", anthesis within individual heads takes 3 weeks (Boonman, 1971a), although flowering within racemes in the Kerio variety of *Chloris gayana* is often completed in 2 weeks Bogdan (1959b) .

Despite this, as shown in Figure 3.3, Table 3.1 and Table 3.2, approximately 80% of all florets within an inflorescence had completed anthesis in a week, suggesting that *B. ruziziensis* does not have a characteristically prolonged anthesis within an individual inflorescence.

In the present study, no distinction was made between inflorescences borne on aerial or basal tillers, or between heads emerging during a particular time. Therefore, it was not possible to determine differences between early formed inflorescences and later formed inflorescences.

Although head length was not recorded in the present study Boonman (1971a) reported that mean head length of *Setaria sphacelata* decreased from a maximum of 19 cm to only 13 cm for later emerged heads. Also, the earliest emerged heads

were shorter than those with maximum head length. In the view of Anslow (1963) seed setting efficiency in perennial ryegrass decreased progressively as head emergence continued. However, it was determined that *B. ruziziensis* has a prolonged head emergence within individual plants and a large variation in time of initial head emergence between plants, because the data collected in the present study were taken continuously over 4 months. This was similar to the observations of Boonman (1971a) who noted that individual plants of *Panicum coloratum* cv. "Solai"; *Setaria sphacelata* cv. "Nandi1" and *Panicum maximum* cv "Makueni" all continued to produce heads over a period of up to 3 months or longer.

In the present study, plants were allowed to grow uninterrupted from seedlings until seed head emergence (i.e. without cutting). As a result many aerial tillers appeared, a situation rarely found in the usual management for pasture seed production, when cutting is used to control lodging problems. This large number of aerial tillers which produced inflorescences contributed to the prolonged period of head emergence within individual plants. In addition, as plants were never short of water, the new basal tillers induced were also able to produce inflorescences, which again prolonged inflorescence production within plants.

There are many factors which can result in the generally low seed set of most tropical grasses (Burton ,1943; Boonman 1971a; Langer 1972; Humphreys and Riveros 1986). However, from this observation trial, no one factor could be identified. Most of the early inflorescences and those which emerged above the canopy had higher seed set. As this experiment was conducted in the glasshouse,

it was reasonably easy to control some environmental factors known to affect floret shedding, particularly wind. Therefore, the percentage shedding recorded (only 8%) was low. In their field experiment with Ruzi grass, Devahuti *et al.*, (1986) reported a 60% seed shedding loss by 21 days after anthesis. In *Brachiaria decumbens*, Humphreys and Riveros (1986) reported that seed shedding commenced 8-11 days after inflorescence exsertion, and continued at a daily rate of c. 6% of the total spikelet number. It can be assumed that *Brachiaria ruziziensis* has similar low seed retention to *B. decumbens*. However, it was evident from the statistical analysis that these results showed a very high variation in fertile florets and florets shed within inflorescences bearing the same number of racemes (Appendix 3.1-3.5). This is most probably because the species is still "wild" in many regards, not having undergone selection in a breeding programme. Dirven *et al.*, (1979) avoided similar problems by using clonal material in their experiment to achieve narrower genetic variation in the test plant population. The data recorded from the present trial do indicate however, a potential for selection from different genotypes to possibly improve floret fertility and reduce floret shedding.

CHAPTER FOUR

SEED DEVELOPMENT IN RUZI GRASS

(*Brachiaria ruziziensis* Germain and Everard)

4.1. INTRODUCTION

Seed production is the culmination of a number of developmental phases where the phenomena and the rates of the different processes are susceptible to the influence of different environmental factors (Humphreys and Riveros, 1986).

Seed development, which follows anthesis, pollination and seed set, is one of the most important phases, and at the end of this phase, the efficiency of recovery of ripe seeds at harvest, and the viability of these seeds are the final (and crucial) factors determining the success of seed production.

Normally, the decision on when to harvest a seed crop is a compromise which takes into account past and expected weather, as well as the stage of maturity of the crop (Roberts, 1972). However, judging the correct harvesting time of tropical pasture seed crops has special difficulties since many species flower over a long period and produce inflorescences with seed which ripens unevenly and falls as it ripens.

An understanding of seed development is important, in order to be able to more accurately predict best harvest timing. Although, Devahuti *et al.*, (1986) have

determined the time to physiological maturity in Ruzi grass (See Literature Review), the opportunity was taken to further investigate the sequence of seed development and changes in seed quality components, particularly in early emerged inflorescences, and also to determine and discuss the potential for seed production in this grass, using results obtained from plants grown in a controlled glasshouse environment.

4.2. LITERATURE REVIEW

To study seed development, it is necessary to describe the classical work done by Hyde (1950) and Hyde *et al.*, (1959) as this provides an important basic knowledge of the seed development processes which has since been used for other studies in both tropical and temperate grasses and legumes (Hill,1971; Kowithayakorn, 1978; Win Pe, 1978; Mullett, 1981; Barnes, 1990). Hyde (1950) considered two main aspects; the relationship between seed moisture content and seed dry weight, and the development of viable seed from anthesis to seed maturity.

Seed maturity was originally defined as the point at which maximum seed development is first obtained (Aldrich, 1943; quoted by Hill, 1971), but Shaw and Loomis (1950), and Anderson (1954), used the terms physiological maturity and morphological maturity respectively to describe the same development stage.

More recently, Harrington (1972) defined physiological maturity as the time when developing seeds achieved maximum viability and vigour. Seeds subsequently

begin to age, with a consequential decline in both viability and vigour. Many experiments have since been conducted to prove Harrington's hypothesis, including those by Rasyad *et al.*, (1990); Dell' Aquila and Tritto (1991); and Filho and Ellis, (1991a,b). However, results presented by Ellis and Filho (1992) invalidated the hypothesis of Harrington (1972), because in wheat maximum potential seed longevity was attained many days after physiological maturity in all cultivars tested. They suggested ,therefore, that it would be preferable if the term mass maturity was used to describe the end of the seed-filling period instead of the term physiological maturity.

Hyde (1950) and Hyde *et al.*, (1959), working with perennial ryegrass and white clover considered three stages in the developing seed.

(1) The growth stage : This stage is characterised by a rapid increase in seed weight (both fresh and dry weight) with a high but constant seed moisture content of approximately 70% of fresh weight. Seed harvested during this stage is non-viable and is characterised by intensive cell division. This stage occupies a period of 10 days after pollination.

(2) The food reserve accumulation stage : Following the first stage, the rate of fresh weight increase begins to slow down. At the same time, dry weight increases linearly by about three times, reaching a maximum at the end of this stage. The amount of water in the seed also decreases slightly, falling from approximately 70% to 63%. This stage can be characterised by a uniform rate of growth and is

presumably determined by the rate of food reserves transferred from the parent plant to the seed. It occupies a period of 10-14 days after the growth stage. At the end of this stage, the seed is structurally complete and attains maximum viability.

(3) The ripening stage : During this period seed dry weight remains constant or changes little, but fresh weight and water content decrease rapidly as the seed tissues shrink in size. Finally, the seed moisture content reaches equilibrium with the relative humidity of the atmosphere decreasing from 63% to approximately 10%. This stage is completed over a period of 3-7 days after the second stage, but according to Delouche (1980) its duration can vary considerably due to weather conditions.

The maturity characteristics of the seed on the individual inflorescence have been investigated in many temperate pasture species by considering seed size, moisture content, endosperm consistency, viability, biochemical changes and abscission (Griffiths *et al.*, 1967). By comparison, relatively little similar work has been carried out on tropical species.

Although the fresh weight of individual seeds reach a maximum early in the maturation process, and decrease as the seed ripens further and loses water, optimum harvest time occurs subsequent to the attainment of maximum fresh weight. However, the uncertainties of weather from year to year make this a difficult index by which to judge correct harvesting time (Humphreys and Riveros, 1986).

Seed moisture content has been developed as a rapid method to assist in deciding optimum harvesting time. Hill (1973) for example recommended 43% seed moisture content as optimal for perennial ryegrass. According to Hill and Watkin (1975), seed moisture content and seed viability can be used as an indicator to predict the correct harvesting time for attaining maximum viable seed yield. However, the moisture content decrease does not always occur at a consistent rate : for example in *Paspalum dilatatum* harvested at 14, 21 and 28 days after peak flowering, percentage moisture contents was 35.5, 7.1 and 5.3 respectively (Bennett and Marchbanks, 1969). Also in tropical species, seed shedding can begin at very high seed moisture contents (>50%, Devahuti *et al.*, 1986). These factors can make seed moisture content an unreliable criterion for deciding correct or optimum time of harvest.

Endosperm consistency is also an indication of approaching maturity and is often helpful in judging the ripeness of grass and legume crops. The seed is sometimes described successively as "milky", "creamy", "cheesy", and "hard"; or at the "soft dough" or "hard dough" stages (Humphreys and Riveros, 1986). Brzostowski and Owen (1966) found that the "hard" stage was the best time to harvest *Cenchrus ciliaris*, since seed viability was 91%, compared with 31% at the milky stage and 42% at the cheesy stage.

There are three important aspects of seed quality which are affected by the stage of seed development - viability, vigour and storage life (Hyde, 1950). Humphreys and Riveros (1986), for example, emphasized that seed viability at the

time of harvest may provide a poor index of viability after storage. Maximum viability may be obtained relatively early in the maturation process, but at this stage, accumulation of food reserves in the seed has only just begun (Hyde, 1950).

Other seed character changes, such as colour, can also indicate seed maturity, particularly pigment changes in the seed coat or testa and its outer coverings. For example brown colour on the upper surface of the majority of pods in *Macroptilium atropurpureum* cv. Siratro is considered to be a good indicator of seed maturity (Humphreys and Riveros, 1986). Biochemical changes such as the level of free sugar content (Stoddart, 1964a) and the level of amino acids in the seed (Stoddart, 1964b) may also be used as an index of optimum harvest time in temperate grasses, although these is no knowledge of these relationships in tropical grasses.

Seed abscission occurs in most tropical pasture species after seed reaches maturity. This prevents the accumulation on the crop of successively ripened seeds (Humphreys and Riveros, 1986). For example, Javier (1970) reported that almost all spikelets of three varieties of *Panicum maximum* had shed their seed 20 days after panicle emergence, but in three other varieties 31 days were required before complete seed shedding, because of genetic diversity.

Seed development in *Brachiaria ruziziensis* on individual inflorescences has been studied by Devahuti *et al.*, (1986) who recorded % dry matter, % moisture content, % seed shedding and % viable seed. They reported that seed physiological maturity occurs approximately 3 weeks after first anthesis. About 5% of seed

started to shed by 10 days after anthesis and this increased to 60% by 21 days at physiological maturity.

4.3. MATERIALS AND METHODS

This experiment was conducted using Ruzi plants from the 14-h daylength treatment (CHAPTER ONE). A number of inflorescences were tagged when first anthesis was observed in the morning (9 am) and tagging was continued as necessary for two weeks to provide at least 30 inflorescences. Five inflorescences which had the same day of first anthesis were harvested at 10, 15, 20, 25, 30 or 40 days after first anthesis. At each harvest, all florets from each inflorescence were combined and divided into 3 portions by using the hand-quartering method (ISTA, 1993) and the following seed characteristics were measured : seed fresh and dry weight, seed moisture content (SMC), seed viability, and seed dormancy. Only florets containing a hard caryopsis (seed) determined by pressing the caryopsis between the thumb and index finger, were selected to determine fresh and dry weight, SMC and seed dormancy, but a random sample of all florets were used to determine seed viability.

Seeds (caryopsis within the glumes) were weighed to determine fresh weight. Dry weight was then determined using the air-oven method, with samples being dried at 103^0C for 17 hours (ISTA, 1993). Percentage SMC was calculated using the weight lost after drying, on a fresh weight basis. Approximately 10 seeds were used per replicate (two replicates per treatment).

The Topographical Tetrazolium (TZ) testing (ISTA, 1993) was used to determine seed viability. All florets (approximately 150) were cut longitudinally through the mid-section of the embryo and through part of the endosperm, leaving the two halves attached by the uncut section of endosperm. They were then placed in 1% 2,3,5-triphenyl tetrazolium chloride solution and held in the dark for 24 hours. Viable seeds were evaluated by examining the embryo staining pattern (ISTA, 1993), as described for monocotyledons.

For dormancy measurement, germination tests (ISTA, 1993) were conducted by dividing samples into 3 seed lots (the number of seeds used to conduct this experiment was not consistent due to differences in % seed set at each time of harvest). From 5-10 seeds per replicate (two replicates per treatment) were usually used and tested on germination paper (TP method) by 3 different treatments i.e. a sheet of germination paper with water, a sheet of germination paper soaked in 0.2% KNO_3, and seeds following treatment with concentrated sulphuric acid (Barnard, 1969) for 15 minutes and washing in water for 2 minutes placed on a sheet of germination paper with 0.2% KNO_3. Blotters were placed in a germination cabinet at $30^0C/25^0C$ day and night temperatures respectively, 70% RH and 12 hour light/day. Germination was evaluated at 7 days for the first count and 21 days for the final count. Tests were examined and the following categories recorded; germinated seeds (normal and abnormal seedlings), and ungerminated seeds (fresh ungerminated seeds and dead seeds).

4.4. RESULTS

4.4.1. Seed fresh weight, Dry weight and Moisture content

From 10 days after first anthesis (DAFA), seed fresh weight slowly increased and reached a peak at 30 DAFA (Table 4.1 and Figure 4.1). Dry weight per seed fluctuated as seed developed (Table 4.1 and Figure 4.1), but maximum dry weight per seed also occurred at 30 DAFA (Table 4.1 and Figure 4.1).

Percentage seed moisture content decreased by just over 2% per day between 10 and 20 DAFA (Table 4.1), but then declined more slowly, reaching 25% at 40 DAFA.

4.4.2. Viability

Seed viability increased from 15% at 10 DAFA to 36% at 20 DAFA, with little change thereafter (Table 4.1).

4.4.3. Germination

There was no seed germination in the water only and KNO_3 treatments because of post-harvest dormancy. However, seeds were successfully germinated when pre treated with concentrated sulphuric acid. The numbers of normal seedlings increased markedly to a maximum of 35% at 30 DAFA (Table 4.1).

TABLE 4.1. Fresh and dry seed weights, seed moisture content, seed viability and seed germination in Ruzi grass, 10, 15, 20, 25, 30 and 40 days after first anthesis

DAFA[1]	Fresh weight per seed (g)	Dry weight per seed (g)	% SMC	Viability (%)	Germination (%)
10	0.0080 (±0.0004)	0.0050 (±0.0003)	56.2 (±2.4)	15 (±4.2)	18.8 (±8.8)
15	0.0066 (±0.0008)	0.0053 (±0.0001)	45.6 (±1.4)	22 (±5.7)	-
20	0.0073 (±0.0005)	0.0048 (±0.0003)	34.3 (±2.2)	36 (±19.8)	30.0 (±14.1)
25	0.0082 (±0.0003)	0.0055 (±0.0002)	32.9 (±2.0)	35 (±7.1)	-
30	0.0090 (±0.0005)	0.0066 (±0.0003)	26.7 (±1.8)	34 (±12.7)	35.8 (±10.1)
40	0.0085 (±0.0004)	0.0064 (±0.0002)	24.7 (±2.0)	38 (±11.3)	33.4 (±9.4)

DAFA[1] = Days after first anthesis

\- = no results due to contamination with mould

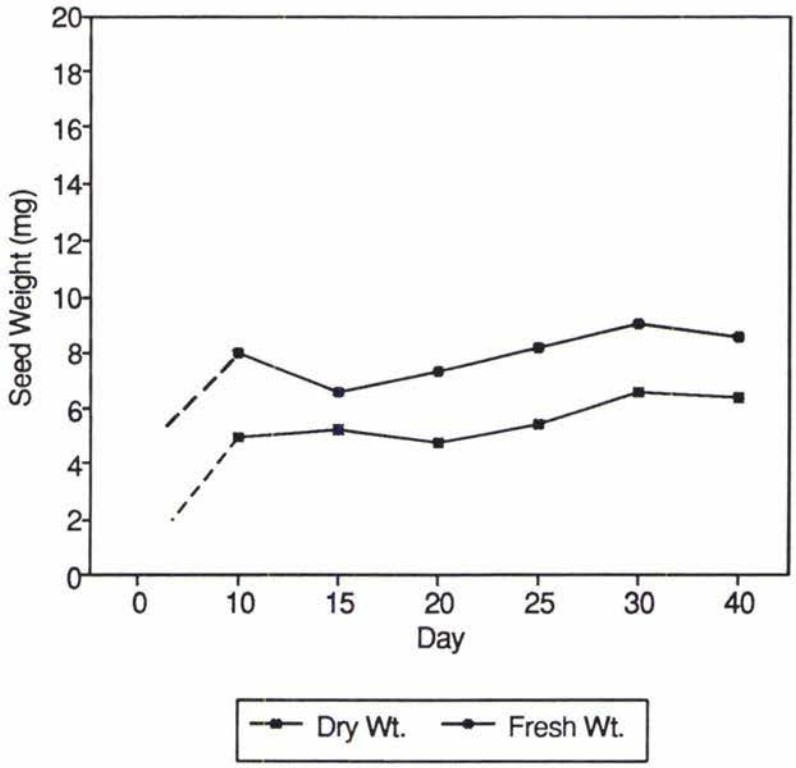


Figure 4.1. Fresh weight and dry weight of Ruzi grass seed development from 10-40 days after first anthesis (---estimated seed weight changes before data recorded).

4.5. DISCUSSION

Florets containing a caryopsis (seed) which had developed successfully to the "hard" endosperm stage were different in colour (light brown) from other florets within the raceme as shown in Plate 4.1. Most of these seeds could be easily removed or readily shed from the raceme by 10 DAFA, which is similar to the 7 days reported by Devahuti *et al.*, (1986). It was therefore difficult to retain seeds on the raceme through to the required later harvesting times, particularly in inflorescences bearing many racemes which lodged readily. In addition it was also hard to collect at least 5 inflorescences which had the same day of first anthesis, because of limited plant numbers. Accordingly, only 5-15 seeds were used in each replicate (two replicates per treatment).

Some seeds were viable by 10 days after first anthesis in the present study. However, Devahuti *et al.*, (1986) reported viable seeds at 3 DAFA. Generally, freshly harvested seed of tropical grasses show post-harvest dormancy which may prevent germination, because of an impermeable seed coat (Davidson, 1966; McLean and Grof, 1968). The period of seed dormancy breakdown is normally 4 to 6 months, but can extend to 18 months if the seed is stored at low temperature (Devahuti and Sirisomparn, 1985). Barnard (1969) recommended treating the seed with concentrated sulphuric acid for 15 minutes to break dormancy. Although this treatment of fresh seeds at 10 days after anthesis did enhance germination, the seedlings produced were thinner compared to seedlings from more developed seeds. It is likely they needed more developmental time to improve food reserve

Plate 4.1. Florets containing a caryopsis (seed) (light brown); florets without caryopsis (green).

accumulation to reach maximum seed weight. It is likely that seeds at this early age would have low levels of food reserves as suggested by Hyde *et al.*, (1959) in perennial ryegrass and white clover.

It appears from the data that % seed germination was sometimes higher than % seed viability. However, these two tests used different "seeds" (see Materials and Methods). Only florets containing a hard caryopsis (seed) was able to tolerate concentrated sulphuric acid. Therefore, % seed germination was based on the number of seeds (florets containing caryopses), but % seed viability was based on the total floret numbers (with or without caryopses). The very small number of seeds tested also made both sets of data highly variable.

Hyde (1950) and Hyde *et al.*, (1959) noted that stage 1 (growth stage) contained no viable seeds and occupied a period of 10 days after pollination. Although, the present seed development study did not begin until 10 DAFA nearly 50% of the viability potential and germination capacity of the seed was detected at this time. Seed dry weight reached a maximum 30 DAFA, which could be considered to be the end of stage 2 (the food reserve accumulation stage), and the starting point of stage 3 (the ripening stage). This achievement of physiological maturity is about 9 days longer than that reported by Devahuti *et al.*, (1986). However the small number of seeds available in the present study and the presumably cooler environment compared with that used by Devahuti *et al.*, (1986) may have been responsible for these differences. This suggests the results should be treated with caution.

It has already been noted that the field study on Ruzi grass in Thailand by Devahuti *et al.*, (1986) produced different time frame results from those in the present study. For example, they found that maximum seed dry weight occurred 21 days after anthesis, at 20% seed moisture content, and the onset of viable seed was detected only three days after the onset of anthesis. Such differences between this and the present study may have been due to different environmental conditions, in terms of temperature, light, relative humidity and wind, affecting the rate of seed development.

Seed shedding is an extremely important factor affecting seed production in Ruzi grass, as in many other tropical species (Boonman, 1973; Hare and Waranyuwat, 1980; Devahuti *et al.*, 1986; Oliveira and Humphreys, 1986). Unfortunately it was not possible to examine this aspect accurately in the present glasshouse study, due to its protected environment. However, about 8% seed shedding was recorded by 30 days after first anthesis (CHAPTER THREE). From the data obtained, it would appear that the best time to harvest Ruzi grass seed is approximately 30 days after first anthesis, when seed has reached its maximum dry weight, its maximum viability and germination percentage. However, at this time seed moisture content is still approximately 27%, which means that seed would need to be carefully dried to a safe storage moisture content to ensure the retention of seed viability in storage.

CHAPTER FIVE

GENERAL DISCUSSION, LIMITATIONS, AND CONCLUSIONS

5.1. GENERAL DISCUSSION

The reproductive behaviour of Ruzi grass *(Brachiaria ruziziensis)* in Thailand suggests it is a qualitative short-day plant with a critical daylength of approximately 12½-h. However, the present study has shown that there is no "trigger" daylength requirement for reproductive initiation, as Ruzi grass produced flowers in all daylength treatments (14, 13, 12 and 11-h). Furthermore, the ability to flower occurred irrespective of whether daylength was decreasing as in the present study, or increasing, as noted in an earlier observation trial. However, the data did show that the shorter the daylength the greater the seed yield (i.e. potential seed yield was greater at 11-h), which confirms the conclusion of Dirven *et al.*, (1979), that Ruzi grass is a quantitative short-day plant.

The question therefore arises - why does Ruzi grass in Thailand appear to behave as a qualitative short-day plant ? The answer probably lies in the fact that plants fail to flower under the longer daylength (13-h) in June because they are still in a juvenile vegetative stage following sowing, in April/May, at the beginning of the "wet" season. It is likely these young plants (4-6 weeks old) can not respond to the photoperiod stimulus that would otherwise be conducive to flowering. Such a period of "juvenility" varies considerably between tropical grasses and is probably based on their source of origin. Humphreys and Riveros (1986) emphasised that the

duration of the juvenile phase is longer and more important in plants with a determinate habit such as the grasses. For example, in *Paspalum plicatulum* flowering, in response to 10-h days, will not occur until plants are at least 60 days old (Chadhokar and Humphreys, 1974). Similarly, Tompsett (1976) found that *Andropogon gayanus* would not flower in 28 cycles of 8-h days if plants were less than six weeks old at the start of the treatment. From the present study, it appears that Ruzi plants need about 87-128 days from sowing to the onset of floral initiation - suggesting a "juvenility" requirement intermediate between *Andropogon gayanus* and *Paspalum plicatulum.* These data therefore support the suggestion of a non reproductive response in 4-6 week old Ruzi grass plants in Thailand under the 13-h daylength.

Under the shortest Thailand daylength in December (11½-h), plants also do not flower, presumably due to moisture stress conditions brought about by the onset of the dry season in October. Although the results of this study showed that Ruzi grass can produce seed in daylengths ranging from 11-14 h, higher potential seed yield did occur in the shorter daylengths. In view of these constraints (juvenility and moisture stress), it would appear that the present management of "closing" a field crop of Ruzi for seed in August/September and harvesting at the end of the rainy season in November is most appropriate. However, the feasible explanation presented above needs to be confirmed, particularly in terms of the possible effects of plant juvenility, since the data recorded in this study were obtained under glasshouse conditions in New Zealand which differed significantly from the field situation in the Thailand environment. Also plants were not cut during this

glasshouse experiment, unlike the field situation where Ruzi grass is grown to produce significant forage for animals prior to being allowed to produce seed. Confirmation therefore necessitates experiments being carried out in Thailand under natural daylength conditions with irrigation to overcome moisture stress effects during the dry season. In addition, the cutting management and closing time relationship should be also considered, to more closely relate to management practices in tropical pasture seed production in Thailand.

Until recently, most of the Ruzi grass seed production in Thailand has come from smallfarmers who produce approximately 60% of the total production with 40% coming from government stations and other sources (Phaikaew *et al.*, 1993). However, it must be remembered that grass seed production is only a minor activity in the farmers' programme. The average farm size is only 4 ha,with the main production effort being concentrated on rice and cassava production plus 4 or 5 cattle for working or beef production. Under the present government scheme only 0.32 ha of Ruzi grass can be grown for seed under contract with a guaranteed price. Therefore, the paddock of Ruzi grass will normally be grazed by animals until the end of the first peak of rainfall in June (Appendix 1.2), then the pasture will be closed and topdressed in preparation for the second peak of growth in September (Appendix 1.2) for seed production and subsequent, harvesting early in the dry season in November. Although it is possible to produce seed earlier, the difficulties of harvesting and drying seed during the rainy season make this impractical and hence farmers prefer to delay seed harvesting until the dry season and use the earlier forage for feeding animals. However, as shown in this study, it would certainly be

possible to grow and harvest Ruzi seed during the so-called dry season if water for irrigation was available. With an increasing number of farmers installing small dams on their farms this is a real possibility, particularly in view of the relatively small seed area involved. However such a potential requires careful consideration in terms of the capital costs involved in irrigation and also possible alternative uses for water.

A high number of tillers producing inflorescences is considered to be a basic requirement in grass seed crops. Unfortunately, only a small proportion of the tillers formed in many tropical grasses seem capable of producing an emerged head at harvest time (Boonman, 1971b). Studies on the management of tropical grass seed crops to maximize the number of tillers producing inflorescences and to reduce the spread of seedhead maturity between individual tillers are required (Loch, 1986). The results of the present tiller development study showed that total tiller numbers were low, even though they continued to show a steady increase through to harvest, by which time approximately 30% were reproductive and 70% vegetative. This is in contrast to the general pattern of tillering in grasses which rises to a maximum roughly coinciding with the period of initial head emergence (IHE), followed by a decline as reported by Langer *et al.*, (1964) and Boonman (1971). Whether this suppression of tillering during flowering is due to apical dominance or to insufficient light (Dorrington, 1970) is not clear. However, the importance of the early-formed tillers to total seed production has been demonstrated by many workers (Haggar, 1966; Bahnisch and Humphreys, 1977). Humphreys (1979) explained this as a competitive advantage of early heads in terms of size and preferential position in the

crop for light and nutrients. In addition, Loch (1983) indicated that early-formed tillers are important simply because there is considerable delay before significant tillering activity is renewed. In a field situation, an increased population of tillers of similar age could be achieved by burning, mowing, application of nitrogen or irrigation, and this could also favour synchrony of flowering (Humphreys and Riveros, 1986). Despite this Dirven *et al.*, (1979) did not agree that tiller-density could be improved in Ruzi grass. In their opinion, higher seed yields could only be obtained if more seeds were formed per inflorescence. They suggested that the most likely way to improve seed yields of tropical grasses, was to grow photoperiod-sensitive cultivars in areas with pronounced differences in daylength during the growing season.

This negative view of Dirven *et at.*, (1979) on the inability to improve tiller density of Ruzi grass, must surely be questioned, particularly in light of the marked variation in fertile tiller numbers per unit area recorded by Brunse and Sukpituksakul (1992) in Thailand. Their results suggest that there is considerable scope to increase fertile tiller density, and hence potential seed production, by adjusting closing date, nitrogen levels and by irrigation.

The results of this experiment indicate that daylength had no effect on the number of vegetative and reproductive basal tillers, but did strongly affect the number of inflorescences and inflorescence components (i.e. raceme numbers/inflorescence, floret numbers/raceme and thus floret numbers/inflorescence). Many aerial tillers were formed in this study as has been

reported by other workers (Dirven *et al.*, 1979; Boonman, 1971b). Dirven *et al.*, (1979) considered that in the absence of cutting, aerial tillers are probably caused by high relative humidity and an optimal supply of nutrients. These conditions would seldom occur in practice, as farmers would normally defoliate Ruzi stands several times during the vegetative stage for animal feeding prior to closing for seed production. Hence the opportunity for the development of aerial tillers would be significantly reduced, and they would probably make little or only minor contribution to seed yield compared with seed production from basal tillers. However, the question arises - would seed production be increased if the grass crop was not used or used to only a limited extent for forage production for livestock and allowed to develop aerial tillers ? From the results obtained it would appear that in the absence of defoliation, a large number of aerial tillers can be produced which can make a major contribution to seed production, particularly in the shorter daylength. However in the field situation it is probable that "lodging" of these extended aerial tillers would present a major problem at harvest and lead to significant seed loss, thereby negating any potential increase in seed yield. However, this aspect is still interesting and warrants further investigation to determine whether there is a place for the development and exploitation of aerial tillers in seed production.

Although a prolonged period of anthesis and stigma exsertion within single heads is one of the problems in tropical grasses which can cause both low seed yield and quality (Boonman, 1971a), the present study suggests that this characteristic does not really appear as important in Ruzi grass, where all florets/raceme (1-5

racemes/inflorescence) exserted anthers within 1-2 weeks and approximately 80% of florets within an inflorescence completed anthesis in approximately week. This is unlike *Setaria sphacelata* where the duration of flowering a single head has been found to continue for up to 7 weeks (Boonman, 1971a). However when one considers the total population of heads per tiller or per plant there is a very prolonged period of time involved as head emergence in Ruzi grass continued to occur over several months. This protracted period of inflorescence emergence agrees with the findings of Boonman (1971a) who recorded head emergence in *Panicum maximum* cv. "Solai"; cv. "Makueni" and *Setaria sphacelata* cv. "Nandi 1" over a period of up to 3 months or longer. It is likely that this was caused, in the present study, by increased head number arising from aerial and basal tillers produced in response to continuous supplies of water and nutrients.

Seed set is one of the most important factors affecting components of seed yield. Generally, seed set in tropical grasses is very low (Burton, 1943; Boonman, 1971a; Langer, 1972; Humphreys and Riveros, 1986). In this study, daylength did not significantly affect percentage seed set, which was consistently around 20%. Humphreys and Riveros (1986) indicated that the degree of seed setting is very susceptible to weather conditions and also dependent upon internal plant controls. In addition, percentage seed set is also genetically controlled. It is possible that low seed set in this study may have been due to some degree of selfing because the few plants available produced pollen at different times, suggesting less efficiency for cross-pollination to occur. As stated in the earlier, *B. ruziziensis* produces 30% of spikelets with a caryopsis when cross-pollinated but only 0.5% when selfed (CIAT,

1972). Although it was not possible to determine the main factors affecting seed set in this study, it was observed that different plants showed very different levels of seed setting ability. Obviously this characteristic warrants further attention in a plant breeding programme.

5.2. LIMITATIONS OF THIS STUDY

5.2.1. Plant numbers

Plant numbers were low in this study, particularly for the seed production measurements, due to limited space in the glasshouse. Although, twenty plants per treatment were grown to determine this aspect, only 40-50% of total plants produced inflorescences and were recorded at harvest. The remainder did not flower. This large variation between plants was evident in many of the characters measured and probably explains why some workers have used clonal material, with a narrow genetic base, rather than contend with the significant variations arising from plants grown from commercial seed, as in the study. However, it must be recognised that clonal material does not represent the normal population that occurs in practice and can lead to biased conclusions. It is suggested, therefore, that these problems of plant variability would be better resolved by increasing plant numbers and providing greater space.

As more than one experiment was conducted within the same plant treatments (e.g. daylength effect and seed development in the 14-h daylength), harvesting time

was, of necessity, delayed until the finish of the seed development study 60 days after anthesis by which time all seed was shed. Hence only potential seed yield was recorded. Again, higher plant numbers would have allowed these two studies to be separated successfully. A further limitation in the present study, brought about by limited plant numbers, was the relatively few seeds available for viability, germination tests and seed development. Thus the data may be unreliable. For example ISTA (1993) requires 4 x 100 seeds for a germination test. In this work germination was determined using less than 50 seeds. Therefore, greater plant numbers would overcome this deficiency.

5.2.2. Environment in the glasshouse

Although every endeavour was made to maintain a constant day and night temperature of 25^{0}C in the glasshouse, it was possible that changes in the outside seasonal temperatures had an effect on glasshouse temperature to a small extent. It is therefore possible that daylength was not the only factor affecting the plant responses recorded but that temperature and/or possibly light intensity variations were also involved. Such errors can only be effectively removed by the use of sophisticated climate controlled facilities.

5.3. CONCLUSIONS

Based on the results in the present study and the subsequent discussion, the following conclusions can be drawn:

(1) The results of this experiment tend to agree with Dirven *et al.*,(1979) that Ruzi grass (*Brachiaria ruziziensis*) is a quantitative short-day plant which produces higher seed yield in shorter daylengths (11-h) than in longer daylengths (14-h). However the results also show that Ruzi grass does not need a critical daylength to trigger the reproductive process, as flowering occurs freely in daylengths from 11-14 hours, irrespective of whether daylength is increasing or decreasing.

(2) There was compensation between raceme numbers per inflorescence and the proportion of basal and aerial tillers produced. As daylength declined raceme numbers per inflorescence arising from basal tillers tended to decrease, with a corresponding increase in the number of racemes in inflorescence on aerial tillers.

(3) Floret numbers per raceme was a more important factor influencing seed yield than raceme numbers per inflorescence.

(4) Ruzi grass produced fewer inflorescences from basal tillers than from

aerial tillers. The greater aerial tiller numbers produced resulted in their greater contribution to total seed yield. However this was a somewhat artificial situation because the plants were not defoliated.

(5) The results of this experiment, in relation to the pattern of seed production in Thailand, seem to suggest why Ruzi grass appears to require a critical daylength to trigger reproductive development. The extremes of availability of water encountered in the Thailand climate and a juvenile vegetative requirement appear to be involved in controlling plant response to daylength.

(6) Basal reproductive tiller numbers and vegetative tiller numbers were not significantly affected by daylength and basal tillers continued to show a steady increase through to harvest. However, aerial reproductive tiller numbers tended to increase as daylength declined.

(7) The morphological changes occurring from vegetative to reproductive development were divided into five stages. It took approximately 22 days from the appearance of early raceme initiation in the "double ridge" stage to the point of inflorescence exsertion.

(8) Anthesis began in the middle region of the uppermost raceme and expanded afterwards to the upper and lowermost raceme(s). All anthers exserted within 1-2 weeks, but approximately 80% of florets

within an inflorescence completed anthesis in a week, suggesting that Ruzi grass does not have a characteristically prolonged anthesis within the individual inflorescence. However, it does have a prolonged head emergence period, although this can be highly variable within individual plants.

(9) Seed development studies suggested that although some variation due to seed shedding may occur, harvesting should not be carried out before 20-25 days after anthesis (maximum viability), and should not be left any longer than 30 days (maximum dry weight).

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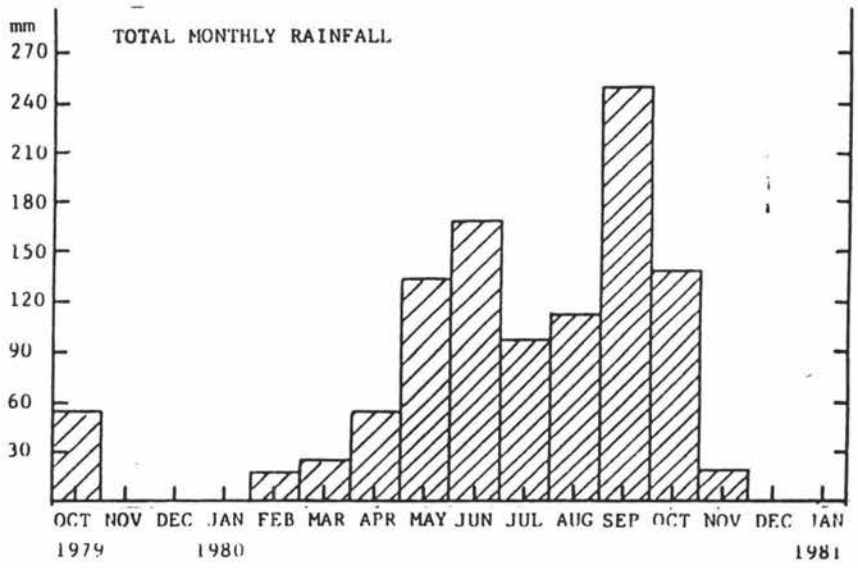
E05365 **MASSEY UNIVERSITY** GRID REFS NZMS 1, 1:63360 N149103311 NZMS 260, 1:50000 T24316874 LAT. 40 23S LONG. 175 36E HT. 75 M.

	PERIOD	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	YEAR
TEMPERATURE OF THE AIR. DEGREES CELSIUS														
AVERAGE DAILY MAXIMUM	1971-1980	22.0	22.8	21.1	18.3	14.9	12.6	12.2	13.0	14.5	16.3	18.3	20.5	17.2
MEAN	1971-1980	17.5	18.1	16.7	14.1	11.1	8.9	8.5	9.3	11.0	12.5	14.3	16.0	13.2
AVERAGE DAILY RANGE	1971-1980	9.0	9.6	8.9	8.1	7.6	7.5	7.3	7.4	7.1	7.7	8.2	8.9	8.1
AVERAGE DAILY MINIMUM	1971-1980	13.0	13.2	12.2	10.2	7.3	5.1	4.9	5.6	7.4	8.6	10.1	11.6	9.1
AVERAGE MONTHLY/ANNUAL MINIMUM	1971-1980	6.2	6.8	4.9	3.5	0.4	-0.7	-1.4	-0.6	1.1	2.0	3.4	5.3	-2.3
LOWEST RECORDED	1971-1980	3.5	2.5	1.9	1.4	-1.5	-3.3	-3.2	-2.2	-1.3	0.9	0.5	2.6	-3.3

E05231 **OHAKEA** GRID REFS. NZMS 1, 1:63360 N143882542 NZMS 260, 1:50000 S23120091 LAT. 40 12S LONG. 175 23E HT. 48 M.

	PERIOD	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	YEAR
SOLAR RADIATION. MEGAJOULES/SQUARE METRE MEAN GLOBAL/DAY	1954-1980	24.6	21.5	16.0	11.4	7.5	5.9	6.5	9.3	13.6	18.2	22.7	24.6	15.2

Appendix 1.1. Mean temperature at Massey University (1971-1980) and solar radiation at Ohakea (1954-1980). (data obtained from CRI, Palmerston North)



Appendix 1.2. Total monthly rainfall in Thailand (1979-1980) (after Juntakool, 1983).

Appendix 3.1. The number of florets with anthers exerted per day for inflorescences with 2 racemes (In. = Inflorescence, Ra. = Raceme)

In. no.	Ra. no.	Days															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1	3	14	4	5	2	6	0	2	2	1	1					
	2			1	20	5	2	3	7	0	3	1					
2	1	3	5	4	3	6	1	1									
	2				3	8	1	2	2	1	1	1					
3*	1	5	8	4	2	4	3	0	1	1							
	2	2	6	5	3	5	2	2	2	2							
4	1	7	4	5	2	3	2										
	2		2	6	3	5	1	0	3								
5	1	2	3	5	6	5	1	0	0	0	0	1					
	2					9	3	3	1	1							
6	1	2	8	6	5	2	4	5	0	1	0	1	0	0	1		
	2							4	7	4	6	0	1	0	0	2	
7	1	12	8	5	6	2											
	2			13	6	4	5	2	2	0	1						
8	1	4	5	8	4	6	5	4	5	0	1						
	2				1	4	4	0	4	5	0	0	1	1	1		
9	1	5	2	3	6	2	0	2	2								
	2						10	3	6	3	0	1					
10	1	3	6	4	2	2	0	3	1	0	1	1					
	2					3	9	2	1	2							
11	1	9	2	5	3	4	3	2	0	1	1	0	0	2			
	2							7	8	3	3	2	3	1			
12	1	6	4	6	3	1	3	1									
	2				1	8	4	4	3	3	2	2	1				
13	1	1	2	12	5	2	0	1	1	0	2	0	0	2	0	1	1
	2							14	1	3	0	3	1	2	2	2	
14	1	1	7	4	4	1	2	4	3	0	0	0	2				
	2								8	5	6	2	2	2			
15	1	10	5	4	6	1											
	2		1	6	5	6	4	1	1	1	0	0	0	1			
16	1	5	8	2	6	1	3	0	3								
	2					6	9	5	3	3	0	1					
17	1	2	10	2	8	3	2	1	0	0	1	1					
	2			3	7	9	3	2	4	3	1						
18	1	1	13	9	4	1	3	5	4	0	0	0	1				
	2				7	8	4	7	5	2	2	1	1	2			

Appendix 3.2. The number of florets with anthers exserted per day for inflorescences with 3 racemes

In. no.	Rac. no.	Days												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	1	10	2	4	1	3	6	2						
	2		2	3	4	3	6	3	0	1	2			
	3			2	6	5	5	4	1	1	1			
2	1	3	6	2	4	2	2	2						
	2				2	4	6	3	1	1				
	3					3	7	1	3	3	1			
3	1	4	7	1	0	3	1	0	1	1				
	2				2	6	2	2	1	3				
	3								4	9	1	2	5	
4	1	4	5	7	0	0	5	2	3					
	2			5	2	3	3	7						
	3						8	9	2	1				
5	1	2	12	2	5	2	1	2	2	1	1			
	2			11	4	6	5	2	1	7	1			
	3					6	5	7	2	6	2	0	2	1
6	1	6	3	2	3	4	3	4	1	1				
	2			8	4	2	2	2	4	3	1			
	3					10	4	6	2	4	1			
7	1	13	5	7	2	2	2	0	1	0	1			
	2				1	14	6	1	2	3	0	3		
	3					10	9	7	0	2	4	2	2	1
8	1	9	8	3	0	1	1							
	2		7	7	1	8	1	1	1					
	3			2	15	4	2	2	2	0	2			
9	1	1	4	11	5	6	0	1						
	2			2	5	7	5	3	2	2	1			
	3					11	3	4	7	1	1			
10*	1	9	13	11	4	3	4	2						
	2		5	10	11	7	8	2	1					
	3		6	15	13	0	9	0	1					
11	1	2	22	5	9	3	2							
	2			1	29	9	5	2	1	2	1			
	3				9	7	5	9	14	3	2			

Appendix 3.3. The number of florets with anthers exserted per day for inflorescences with 4 racemes

In. no.	Ra. no.	Days																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1*	1	6	4	0	4	0	2	1	0	1	1	0	0	1	0	1		
	2		5	5	3	3	0	1	0	0	0	0	1	3				
	3		3	6	3	2	3	3	2	0	1	0	0	1	1	0	0	2
	4				7	2	6	5	0	0	2	3	0	2	2	1		
2*	1	1	5	0	9	2	8	3	2	1	0	1						
	2				8	3	9	1	3	2	1	1						
	3					1	9	5	4	4	2	3						
	4					1	11	8	3	2	2	0	1					
3*	1	1	5	8	2	2	1	2	2	2								
	2			1	7	3	2	4	3	1	0	1						
	3			1	6	7	4	1	4	2	2							
	4						9	7	4	1	2	0	0	2	1			
4*	1	6	9	6	4	2	2											
	2	6	13	12	4	5	3	2										
	3		2	16	11	2	6	1										
	4				19	2	17	2	0	2								
5*	1	3	13	8	10	2	3	3										
	2		11	10	7	9	10	4										
	3			2	10	9	11	3	0	2								
	4			6	5	11	7	6	0	2								

Appendix 3.4. The number of florets with anthers exserted per day for inflorescences with 5 racemes

In. no.	Ra. no.	Days												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1*	1	1	8	1	8	3	7	2	0	1				
	2			3	7	3	9	1	1	2	0	2		
	3			1	8	2	9	0	3	4	1			
	4					7	12	0	0	4	0	4		
	5							12	7	6	1	1	0	1
2*	1	3	5	1	5	3	4	0	3	2	0	1		
	2	3	7	2	6	1	3	1	1	1	1			
	3				3	4	6	2	4	2				
	4		4	5	5	2	3	4	0	3	1			
	5				1	7	5	5	2	4	2			
3*	1	1	13	2	8	7	0	0	4					
	2		9	9	2	8	0	3	4	1				
	3		2	3	5	5	20	0	4	4	0	1		
	4			9	11	12	2	6	2	1	3			
	5					1	6	19	8	4	5	1		
4*	1	6	5	5	14	8	3							
	2			6	18	7	4	4						
	3			1	17	10	0	8						
	4			5	19	8	9							
	5				21	4	15	2						
5*	1	3	5	10	11	2	1							
	2		3	16	7	3	2							
	3		2	6	10	7	4							
	4				12	10	5	4	0	1				
	5				9	9	8	7	1	0	0	1		

Appendix 3.5. Floret numbers, percentage fertile florets, percentage sterile florets and percentage florets shed at harvest 30 days after first anthesis in inflorescences bearing 1 raceme.

Infl.[1] no.	Total florets	No.of fertile florets	% fertile florets	No. of sterile florets	% sterile florets	No. of florets shed	% florets shed
1	36	17	47.2	19	52.8	0	0
2	42	14	33.3	28	66.7	0	0
3	33	12	36.4	18	54.6	3	9.1
4	45	1	2.2	28	62.2	16	35.6
5	22	3	13.6	19	86.4	0	0
6	26	11	42.3	12	46.2	3	11.5
7	30	14	46.7	16	53.3	0	0
8	37	11	29.7	21	56.8	5	13.5
9	30	0	0	12	40.0	18	60.0
10	26	4	15.4	21	80.8	1	3.8
11	22	2	9.1	20	90.9	0	0
12	23	1	4.4	16	69.6	6	26.1
13	23	4	17.4	19	82.6	0	0
14	25	0	0	19	76.0	6	24.0
15	25	0	0	24	96.0	1	4.0
16	47	5	10.6	42	89.4	0	0
17	36	2	5.6	34	94.4	0	0
18	35	2	5.7	31	88.6	2	5.7
19	30	8	26.7	22	73.3	0	0
20	45	11	24.4	34	75.6	0	0
21	42	8	19.1	26	61.9	8	19.0
Av.[2]	32.38 ±8.28	6.19 ±5.44	18.6 ±15.8	22.90 ±7.66	71.3 ±16.8	3.29 ±5.19	10.1 ±15.5

[1] = Inflorescence numbers

[2] = Average

Appendix 3.6. Floret numbers, percentage fertile florets, percentage sterile florets and percentage florets shed at harvest 30 days after first anthesis in inflorescences bearing 2 racemes.

Infl. no.	Total florets	No.of fertile florets	% fertile florets	No. of sterile florets	% sterile florets	No. of florets shed	% florets shed
1	107	43	40.2	64	59.8	0	0
2	58	12	20.7	38	65.5	8	13.8
3	55	48	87.3	7	12.7	0	0
4	131	2	1.5	129	98.5	0	0
5	82	3	3.7	79	96.3	0	0
6	42	4	9.5	38	90.5	0	0
7	42	9	21.4	33	78.6	0	0
8	43	8	18.6	35	81.4	0	0
9	57	10	17.5	47	82.5	0	0
10	66	1	1.5	58	87.9	7	10.6
11	52	5	9.6	47	90.4	0	0
12	50	8	16.0	32	64.0	10	20.0
13	52	13	25.0	38	73.1	1	1.9
14	80	2	2.5	68	85.0	10	12.5
15	62	13	21.0	49	79.0	0	0
16	59	1	1.7	58	98.3	0	0
17	46	7	15.2	28	60.9	11	23.9
18	63	12	19.1	45	71.4	6	9.5
19	54	21	38.9	33	61.1	0	0
20	27	0	0	18	66.7	9	33.3
21	40	0	0	15	37.5	25	62.5
22	50	1	2.0	16	32.0	33	66.0
23	46	5	10.9	20	43.5	21	45.7
Av.	59.30 ±22.75	9.91 ±12.44	16.7 ±19.2	43.26 ±26.05	70.3 ±22.3	6.13 ±9.15	13.0 ±20.3

Appendix 3.7. Floret numbers, percentage fertile florets, percentage sterile florets and percentage florets shed at harvest 30 days after first anthesis in inflorescences bearing 3 racemes.

Infl. no.	Total florets	No.of florets	% fertile florets	No.of sterile florets	% sterile florets	No. of florets shed	% florets shed
1	64	2	3.1	57	89.1	5	7.8
2	110	16	14.6	79	71.8	15	13.6
3	90	10	11.1	80	88.9	0	0
4	92	5	5.4	84	91.3	3	3.3
5	95	3	3.1	90	94.7	2	2.1
6	93	5	5.4	88	94.6	0	0
7	126	92	73.0	34	27.0	0	0
8	123	100	81.3	23	18.7	0	0
9	97	40	41.2	57	58.8	0	0
10	156	5	3.2	151	96.8	0	0
11	67	1	1.5	66	98.5	0	0
12	56	3	5.4	53	94.6	0	0
13	80	16	20.0	64	80.0	0	0
14	77	2	2.6	75	97.4	0	0
15	99	22	22.2	40	40.4	37	37.4
16	139	37	26.6	98	70.5	4	2.9
17	93	27	29.0	66	71.0		0
18	62	0	0	58	93.6	4	6.5
19	53	12	22.6	24	45.3	17	32.1
20	99	5	5.1	84	84.9	10	10.1
Av.	93.55 ±27.46	20.15 ±28.48	18.8 ±22.9	68.55 ±29.01	75.4 ±24.8	4.85 ±9.13	5.8 ±10.7

Appendix 3.8. Floret numbers, percentage fertile florets, percentage sterile florets and percentage florets shed at harvest 30 days after first anthesis in inflorescences bearing 4 racemes.

Infl. no.	Total florets	No.of fertile florets	% fertile florets	No. of sterile florets	% sterile florets	No. of florets shed	% florets shed
1	118	7	5.9	111	94.1	0	0
2	140	5	3.6	131	93.6	4	2.9
3	103	9	8.7	79	76.7	15	14.6
4	155	108	69.7	47	30.3	0	0
5	163	116	71.2	37	22.7	10	6.1
6	116	77	66.4	38	32.8	1	0.9
7	210	3	1.4	207	98.6	0	0
8	200	2	1.0	198	99.0	0	0
9	116	4	3.5	112	96.6	0	0
Av.	146.78 ±38.48	36.78 ±48.81	25.7 ±32.6	106.67 ±64.17	71.6 ±33.0	3.33 ±5.50	2.7 ±4.9

Appendix 3.9. Floret numbers, percentage fertile florets, percentage sterile florets and percentage florets shed at harvest 30 days after first anthesis in inflorescences bearing 5 racemes.

Infl. no.	Total florets	No.of fertile florets	% fertile florets	No. of sterile florets	% sterile florets	No. of florets shed	% florets shed
1	206	54	26.2	149	72.3	3	1.5
2	208	143	68.8	65	31.3	0	0
3	160	83	51.9	45	28.1	32	20.0
4	165	76	46.1	84	50.9	5	3.0
5	281	3	1.1	278	98.9	0	0
6	153	25	16.3	122	79.7	6	3.9
7	144	1	0.7	143	99.3	0	0
8	200	0	0	104	52.0	96	48.0
9	77	2	2.6	74	96.1	1	1.3
10	212	5	2.4	207	97.6	0	0
Av.	180.60 ±54.10	39.20 ±48.68	21.6 ±25.5	127.10 ±71.28	70.6 ±28.3	14.30 ±30.31	7.8 ±15.4