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Tamara N. Naumova · Michael D. Hayward
Marinus Wagenvoort

Apomixis and sexuality in diploid and tetraploid accessions of *Brachiaria decumbens*

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Abstract Meiotic and aposporous embryo sacs and the initial steps of parthenogenetic embryogenesis and endosperm formation were investigated in diploid and tetraploid accessions of *Brachiaria decumbens* in two environments, differing mainly in day length: early summer and late autumn. Both diploid and tetraploid accessions were facultative apomicts. Di(ha)ploids showed a much lower level of apomixis (10% to15%) than tetraploids (80% to 95%). No obligate sexual diploids were found; thus, their occurrence in natural populations is obscure. It is suggested that reproduction in *B. decumbens*, as in other agamic complexes of the Paniceae tribe, in general, approximates a diploid-tetraploid-(di)haploid reproductive cycle which does not involve triploids. The dihaploids were fertile and survived in nature. Development of the reproductive structures depended on the environment. In autumn, in contrast to early summer, many meiotic and aposporous embryo sacs degenerated during development, leading to a significant reduction in the proportion of parthenogenetic embryos. Whether this effect can be attributed to day length or simply to age remains to be investigated. The ratio of aposporous to sexual embryo sacs was relatively stable over the two seasons.

Key words Apomixis · Sexuality · Day length · Embryology · *Brachiaria* · Agamic complexes

T.N. Naumova · M. Wagenvoort (✉)
DLO – Centre for Plant Breeding and Reproduction Research (CPRO-DLO) P.O. Box 16, 6700 AA, Wageningen, The Netherlands
e-mail: M.Wagenvoort@CPRO.DLO.NL
Fax: +31–317–418094

T.N. Naumova
Komarov Botanical Institute, Prof. Popov Street 2, 197376 St. Petersburg, Russia

M.D. Hayward
Institute of Grassland and Environmental Research (IGER), Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

Introduction

The genus *Brachiaria* consists of about 70 species distributed across the tropics and subtropics of both hemispheres, but mostly in Africa and South Asia (Tsvelev 1984). *Brachiaria decumbens* Stapf. is a tropical, perennial savanna grass native to Africa. It spreads in nature by seed dispersal (Skerman and Riveros 1990). Natural tetraploid (2n=36) populations are widespread, whereas diploid populations are rare. Tetraploids are classified as obligate, aposporous apomicts which propagate clonally by seeds. Genetic variation is virtually absent in tetraploid populations (Pritchard 1967; Barnard 1969; do Valle 1987). *B. decumbens* is an important fodder crop, and is cultivated in the West Indies, Latin America, India and Australia. Knowledge of the mode of reproduction in natural populations and the reproductive biology of these plants is incomplete. In agriculture *B. decumbens* is propagated vegetatively by cuttings from stems or stolons. There are few cultivars. In the 1930s the cultivar Basilisk was introduced into Australia from Uganda (Skerman and Riveros 1990). In the 1960s two commercial varieties of *B. decumbens* were introduced in Brazil. They are now widely cultivated as monocultures, covering over 30 million hectares of Central Brazil. However, these cultivars suffer from harmful diseases and produce forage of low nutritive value (do Valle et al. 1989).

From earlier embryological studies it is known that apospory, followed by parthenogenesis, is the main type of gametophytic apomixis in *Brachiaria* and other Panicoid grasses (Warmke 1954; Brown and Emery 1958; Pritchard 1967; Rutishauser 1969). Aposporous embryo sacs originate from cells of the nucellus as a result of mitosis and are, therefore, diploid with the maternal genome. The Panicum-type embryo sacs start to develop when normal meiosis in ovules should occur. At the mature stage, these embryo sacs are monopolar, four-nucleate, with an egg apparatus containing three cells, namely the egg cell and two synergids, and one polar nucleus. Rare occurrences of an egg cell, one synergid and two polar nuclei have also been observed in *B. decumbens*

(Pritchard 1967; Lutts et al. 1994). The absence of antipodals is a characteristic morphological feature which differentiates the apomictic Panicum-type embryo sac from the meiotic Polygonum-type. It is used as the main criterion to screen for apomixis and sexuality in Panicoid grasses (Warmke 1954; Savidan 1982). In *B. decumbens*, haploid embryo sacs of the Polygonum-type can have a cluster of up to 20 antipodals originating from additional mitotic divisions (Lutts et al. 1994).

The *Brachiaria* germplasm collection gathered by the International Centre for Tropical Agriculture (CIAT) in Colombia and the International Livestock Centre in Africa (ILCA) in Kenya and Ethiopia under the auspices of the International Board for Plant Genetic Resources (IBPGR, now IPGRI in Italy) includes about 350 accessions of 10 different species. From these collections 28 accessions were grown at EMBRAPA, Campo Grande, Brazil, in 1987–1988 to study the mode of reproduction (do Valle and Savidan 1989; do Valle et al. 1989). The tetraploids of *B. decumbens* were found to be facultative apomicts with different levels of sexuality, and with irregular male meiosis as determined by cytogenetic and embryological examinations. Some diploid accessions of *B. decumbens* originating from Kenya and Rwanda were described as having obligate sexual reproduction with regular male meiosis (do Valle and Savidan 1989; do Valle et al. 1989; do Valle and Glienke 1991). However, in later cyto-embryological studies one of these diploid accessions of *B. decumbens* showed facultative sexual reproduction (Naumova et al. 1995).

The objective of the present study was to further examine reproductive development in both diploid and tetraploid accessions of *B. decumbens* with special attention to the diploid accessions, and to consider the implications of the mode of reproduction at the population level. The influence of day length on the mode of reproduction is also examined.

Materials and methods

Plant material

Plants of *B. decumbens* Stapf. were grown from seeds obtained from the Brazilian Collection (kindly provided by Cacilda do Valle, EMBRAPA, Campo Grande, Brazil) under greenhouse conditions at CPRO-DLO, Wageningen, the Netherlands. A diploid accession originating from Kenya (D-4) and tetraploid accessions originating from Rwanda (D-62 and D-58) were the same as those examined by do Valle et al. (1989) and have the following SCPA and CIAT codes: D-4, BRA-004430 and 16493; D-62, BRA-001058 and 606, cultivar Basilisk (commercial); and D-58, BRA-000191 and 6012, cultivar Ipean (commercial) (do Valle et al. 1989). At the generative stage of the plants the glasshouse temperature was controlled (20–25°C), and the photoperiod was natural day length. Flowering was examined twice, in May-June when the average day length at Wageningen is 18 h, and in October-December when it is 11 h.

In May-June 1994, fresh ovaries were collected in bulk at anthesis, or 1–10 days later, from five plants of each of the three accessions. Embryo sac development was studied by the dissection technique of Naumova et al. (1993). The type of embryo sac (i.e. Panicum-type or Polygonum-type) was determined on fresh plant material. Whole embryo sacs were dissected from ovules and examined using Nomarsky optics. In *Brachiaria* it is common to find development of the embryo of an aposporous embryo sac before fertilization; embryo sacs which contained an embryo and no endosperm were therefore classified as Panicum-type. Older embryo sacs, in which the remnants of antipodal cells were still visible in the presence of an embryo and endosperm were classified as Polygonum-type.

Some plants of the 1994 sowing were maintained in the greenhouse until the end of 1996. In October-December of that year, spikes, which were fixed at anthesis or 2–3 days later, were examined with the clearing technique (see Clearing method), and fresh material was collected for flow cytometry (see Flow cytometry). These studies were carried out with plants 1, 5 and 7 of the diploid accession D-4, plants 2 and 9 of the tetraploid accession D-62, and the plant numbered 5 of accession D-58. Seed set under greenhouse conditions in both seasons was low. The occurrence of endosperm was used as a criterion for successful seed formation.

Clearing method

Our protocol for clearing female reproductive organs was based on the technique described by Young et al. (1979). Spikes at anthesis, or some days later, were fixed in FAA (formaldehyde 40%:ethanol 70%:acetic acid 98% in the ratio 3:7:1) and stored in 70% ethanol. Ovaries were removed from the flowers, and the ovules were dissected out. Individual ovules were dehydrated in an ethanol series with increasing concentrations (70%, 80%, 90%, 100%, 100%), 20 min per concentration, and cleared in mixtures of ethanol:methyl salycilate in the ratios 1:2, 1:1, 2:1 (1 h per mixture) and in pure methyl salycilate for 12 h. Cleared ovules were mounted on slides, covered with cover glasses and studied with a phase contrast microscope and Nomarsky optics. From 60 to 80 ovules were investigated per plant. The number of ovules, total number of embryo sacs, embryo sac type (Polygonum-type or Panicum-type), and whether each embryo sac type contained an embryo and endosperm were recorded. The percentage of apomixis was calculated as the ratio of the number of ovules with the Panicum-type embryo sac to the total number of ovules investigated. In the case of ovules with multiple embryo sacs, the extra sacs were not taken into account in the calculation of the percentage of apomixis. Also, ovules in which the embryo sacs had degenerated were not used for the calculations.

Flow cytometry

Flow cytometry was used to determine the ploidy level of the vegetative and generative structures (Naumova et al. 1993). DNA content was measured in fresh leaves, ovaries containing embryo sacs, and anthers. Samples consisted of 50–70 ovaries, 10–30 anthers, or segments of leaves. Nuclear DNA content was estimated by FCM according to the protocol of Arumuganathan and Earle (1991). Each type of plant material was carefully chopped with a razor blade in 750 µl nuclear extraction buffer, filtered through an 88-µm nylon gauze filter, and stained at least for 10 min in 20 µl propidium iodide (1.0 mg/ml water). A Coulter Epics XL-MCL flow cytometer was used for the measurements. Extraction of DNA-bound propidium iodide was performed by a 488-nm Argon Ion Laser, and the level of fluorescence was detected over a range of 605–635 nm. Cell cycle analysis software, MultiCycle for Windows, version 3.0 (Phoenix, Flow Systems, San Diego, Calif.) was used for fitting the curves of the fluorescence frequency distributions. The amount of DNA is proportional to the fluorescent signal and is expressed as arbitrary C values, in which 1 C comprises the DNA content of the unreplicated haploid chromosome complement.

Table 1 Mode of reproduction in sexual (D-4) and apomitic (D-62, D-58) accessions of *B. decumbens* (early summer 1994), determined by dissection

[a] In *parentheses*, the number of embryo sacs with endosperm

Code	Number of ovules	Total number of embryo sacs	Polygonum-type[a]	Panicum-type[a]	Apomixis percentage
D-4	26	30	22 (3)	8, 4 ovules (0)	15.4
D-62	38	46	4 (2)	42, 34 ovules (2)	89.5
D-58	41	68	2 (1)	66, 39 ovules (2)	95.1

Table 2 Mode of reproduction of *B. decumbens* accession D-4 (late autumn 1996) determined by clearing

[a] In *parentheses*, the number of embryo sacs with endosperm

Code	Number of ovules	Total number of embryo sacs	Polygonum-type[a]	Panicum-type[a]	Apomixis percentage
D-4 plant 1	65	65	57 (0)	8 (0)	12.3
D-4 plant 5	68	68	59 (0)	9 (0)	13.2
D-4 plant 7	61	61	57 (2)	4 (0)	6.6
D-4 total	194	194	173 (2)	21 (0)	10.8

Results

Determination of embryo sac type by the dissection technique

In plants of accession D-4, 22 ovules had meiotic embryo sacs of the Polygonum type, and each of four ovules had two aposporous embryo sacs of the Panicum type (Table 1). This corresponds to 15.4% apomixis in the diploid accession D-4. Panicum-type embryo sacs showed no endosperm development, and parthenogenetic embryos (Fig. 1E) of different developmental stages (pro-embryo, multicellular and globular) were found in two embryo sacs. No seed formation can be expected from ovules with Panicum-type embryo sacs, because the parthenogenetic embryos will degenerate at the late globular stage without nutrition. In some Polygonum-type embryo sacs of accession D-4, both embryo and endosperm development was observed. In such ovules, seed formation can be expected.

In plants of accession D-62, 34 out of 38 ovules had a total of 42 Panicum-type embryo sacs. Multiple Panicum-type embryo sacs developing in one ovule are common in *Brachiaria* (Lutts et al.1994; Naumova et al. 1995). Aposporous parthenogenetic embryos developed in three (7%) of these embryo sacs. Endosperm development occurred in two older Panicum-type embryo sacs due to fertilization, and this may allow the formation of seeds. Four (10.5%) of the 38 ovules had a Polygonum-type embryo sac. Endosperm development was observed in two of these embryo sacs and, therefore, seed formation in this accession can also be expected from Polygonum-type embryo sacs (Table 1).

Plants of accession D-58 also contained two types of embryo sacs. The Panicum-type was observed in 39 (66 embryo sacs) of the 41 ovules examined. Only two (5%) Polygonum-type embryo sacs were detected. Aposporous parthenogenetic embryos developed in 58% of the Panicum-type embryo sacs. Endosperm development took place in both embryo sac types (Table 1) and in these cases seed formation can be expected.

Determination of embryo sac type by the clearing technique

The clearing technique has advantages for embryo sac analysis over the dissection method. Firstly, it is quicker and a more routine method and, secondly, it can be used on fixed material. The use of ovules instead of ovaries strongly enhances the clearing process, allowing a more reliable analysis of embryo sac type. The results of this analysis for three different plants of the diploid accession D-4 are presented in Table 2. Ovules containing both types of embryo sacs (i.e. Polygonum- and Panicum-type) or multiple embryo sacs were not found. Polygonum-type embryo sacs (Fig. 1A) occurred most frequently. Endosperm development was rarely observed, but the embryo was always present. Panicum-type embryo sacs (Fig. 1B–D) were found with a frequency of 12–13% in two plants, whereas in plant 7 a lower frequency (6.6%) was observed. The difference in the frequency of the Panicum-type embryo sacs could be due to the developmental stages of the plants studied. Plant 7 was at the end of the generative period, and only a few spikes were available for analysis. No endosperm formation was found in the 21 Panicum-type embryo sacs, and a two-celled parthenogenetic embryo was observed in one of them (Fig. 1C). Very often, one or more large nucellar cells were observed close to the chalazal part of the Panicum-type embryo sacs (Fig. 1B,D,F). These cells were different in their morphology than the antipodals of the Polygonum-type embryo sac (Fig. 1A), but the presence of such cells should be taken into account during embryo sac type estimation to avoid incorrect analyses.

In accession D-62, a total of 149 ovules from two plants were studied, and 60 ovules were studied from one plant of D-58 (Table 3). The Panicum-type embryo sac (Fig. 2A) was most frequently observed. Multiple embryo sacs of the Panicum-type (Fig. 2C) were detected in several ovules, but the occurrence of both Polygonum and Panicum types in the same ovule was not observed. Certain morphological differences in the structure of the egg apparatus were seen: an egg cell, together with one synergid and two polar nuclei were found in 15 out of 149 and

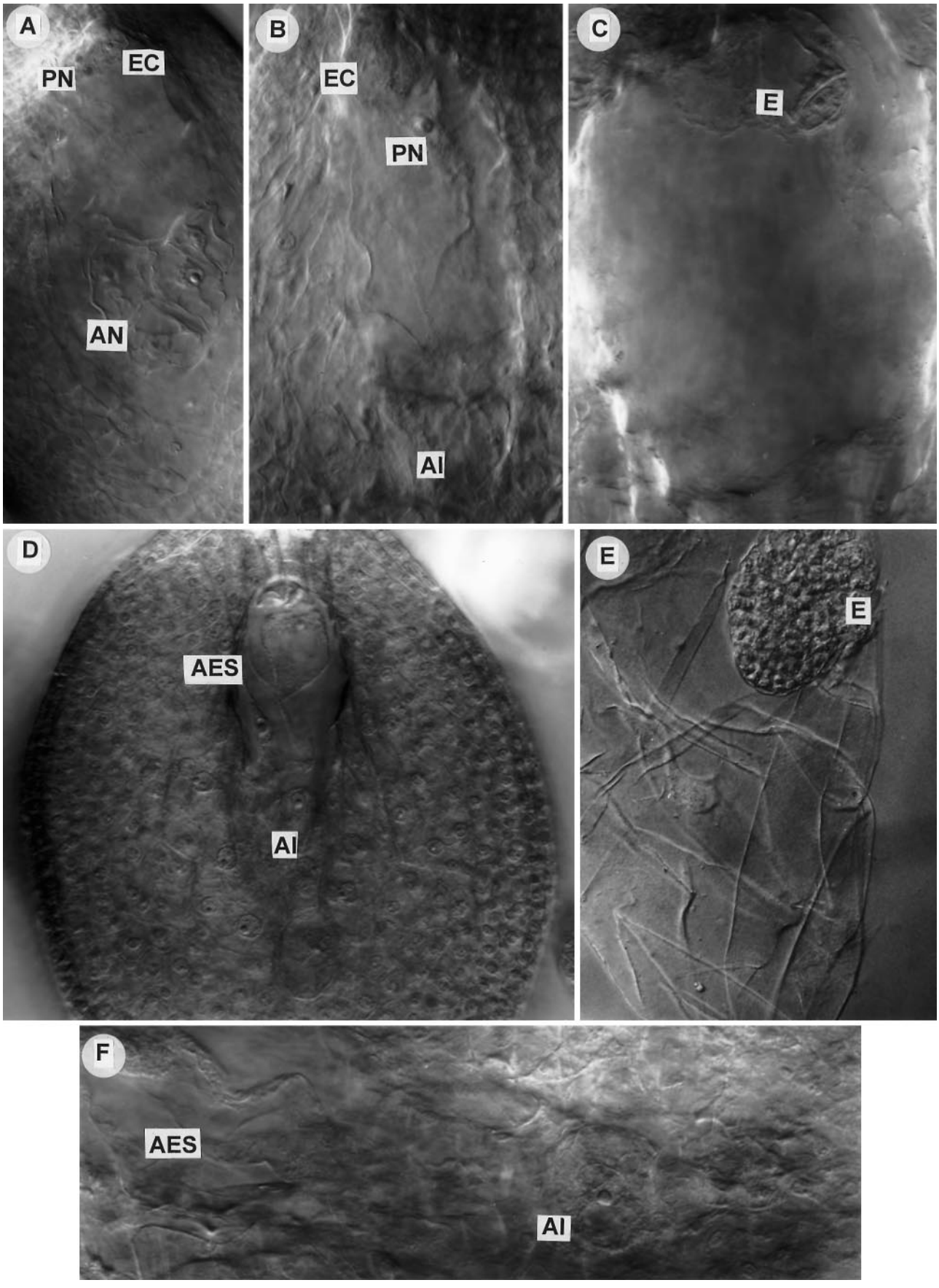
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Table 3 Mode of reproduction of *B. decumbens* D-62 and D-58 accessions (late autumn 1996) determined by clearing

[a] In *parentheses*, the number of embryo sacs with endosperm

Code	Number of ovules	Total number of embryo sacs	Polygonum-type[a]	Panicum-type[a]	Apomixis percentage
D-62 plant 2	87	127	12 (0)	115 (75 ovules) (3)	86.2
D-62 plant 9	62	84	17 (0)	67 (45 ovules) (6)	72.6
Total:	149	211	29 (0)	182 (120 ovules) (9)	80.6
D-58 plant 5	60	116	9 (0)	107 (51 ovules) (11)	85.0

Table 4 Mode of reproduction of *B. decumbens* during early summer (1994) and late autumn (1996)

Code	Numberof ovules		Percentage of ovules multiple embryo sacs		Percentage of Panicum-type embryo sacs with parthenogenetic embryos	
	1994	1996	1994	1996	1994	1996
D-4	26	194	15.4	0.0	25.0	4.7
D-62	38	149	34.0	32.0	7.0	0.0
D-58	41	60	44.0	48.0	58.0	1.7

in 7 out of 60 embryo sacs in accessions D-62 and D-58, respectively. The cells of a three-celled egg apparatus had characteristics that are common in grasses. The polar nucleus was close to the egg apparatus (Fig. 2B). One two-celled embryo was found in the 60 ovules of accession D-58 examined. A larger egg cell with optically dense cytoplasm and a large nucleus with a well-shaped nucleolus were often observed in Panicum-type embryo sacs (Fig. 2A,D). Initial stages of endosperm development were found in some cases. The endosperm nuclei were large, of irregular shape and contained several nucleoli (Fig. 2E,F). At this developmental stage these endosperm nuclei did not resemble the nuclei of normal grass endosperm post-fertilization. Polygonum-type embryo sacs (Fig. 2G) were found in both asexual accessions. The percentage of apomixis varied from 72.6% to 86.2% among the two plants of accession D-62. In accession D-58 apomixis frequency was 85%. In none of the 38 Polygonum-type embryo sacs of accessions D-62 and D-58 was an embryo or endosperm observed.

Influence of environment on the mode of reproduction

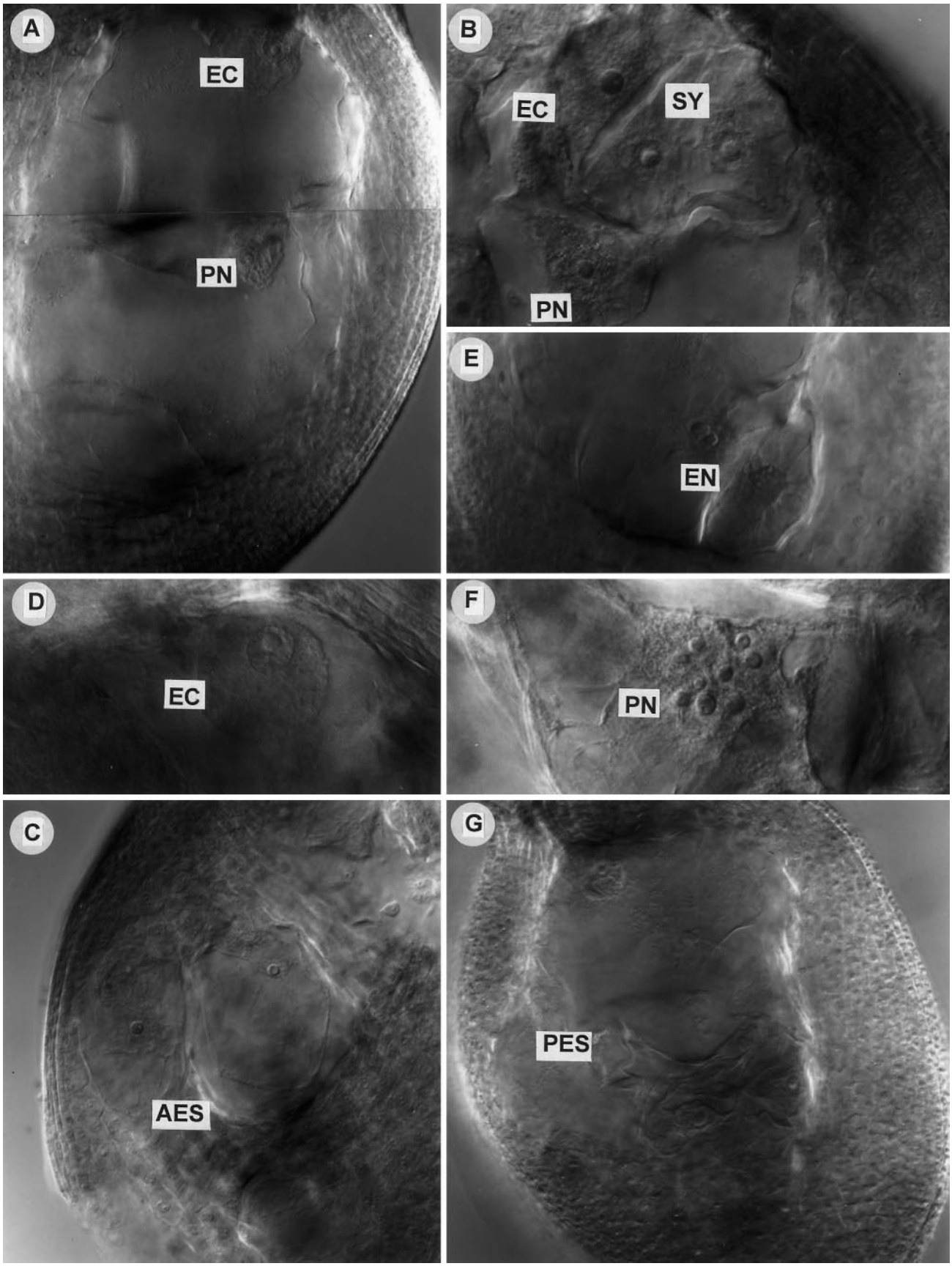
Differences in reproductive behaviour of accessions of *B. decumbens* were determined by comparing plants grown in the two environments, which differed principally with respect to day length, (18 h in early summer and 11 h in late autumn). The number of aborted embryo sacs of the Polygonum- and Panicum-types was much larger in late autumn than in early summer. Many of them degenerated over a range of developmental stages. The frequency of aborted embryo sacs was estimated to be between 70% and 80%. Consequently, seed set was expected to be very low in late autumn. In diploid accession D-4, Panicum-type embryo sac formation decreased from 15.4% (Table 1) in early summer to 10.8% (Table 2) in late autumn. In the tetraploid accessions D-62 and D-58, Polygonum-type embryo sac formation increased from an average of 7.6% (Table 1) to 18.2% (Table 3) during this period. However, these differences in the frequencies of meiotic and aposporous embryo sacs were not statistically significant for the three accessions examined. In the diploid accession there were fewer ovules with multiple embryo sacs, but in the tetraploids the number was relatively stable (Table 4). Formation of both meiotic and aposporous embryo sacs in autumn 1996 was strongly inhibited.

In autumn, initiation of parthenogenetic embryo formation was much lower than in spring (Table 4). Both tetraploid accessions, D-62 and D-58, showed a statistically significant decrease in parthenogenetic embryo formation from early summer to late autumn (respectively, χ^2=13.17 and 71.26, P<0.001). In the diploid accession D-4, the frequency of Panicum-type embryo sacs with parthenogenetic embryos decreased from 25% in early summer to 4.7% in late autumn. However, this difference was not statistically significant (χ^2=2.56, P>0.05), possibly because of the small number of embryo sacs investigated.

◀ **Fig. 1A–F** Ovules and embryo sacs of the diploid accession D-4 of *B. decumbens*. (**A–D**, **F** obtained with the clearing technique). **A** Ovule with Polygonum-type embryo sac, antipodals and egg apparatus are well presented ×1400. **B**, **C** Two ovules with Panicum-type embryo sacs; antipodals are absent, egg cell and polar nuclei (**B**) ×1400 and 2-celled embryo (**C**) are present ×800. **D**, **F** Ovule with Panicum-type embryo sac (**D**) ×700: the large and spherical cells, initials of additional aposporous embryo sacs, are present in the chalazal part of the nucellus (**D**, **F**) ×700, ×1600. **E** Panicum-type embryo sac with a multicellular parthenogenetic embryo; endosperm is absent (obtained with embryo sac dissection technique) ×800. *AI* Aposporous initial cell, *AN* antipodals, *AES* aposporous embryo sac, *E* embryo, *EC* egg cell, *EN* endosperm nuclei, *PES* embryo sac of the Polygonum-type, *PN* polar nucleus, *SY* synergid

Flow cytometry determination of DNA amount and ploidy levels of leaves, ovaries and anthers

To determine the ploidy of vegetative and generative cells in individual plants of each accession, flow cytome-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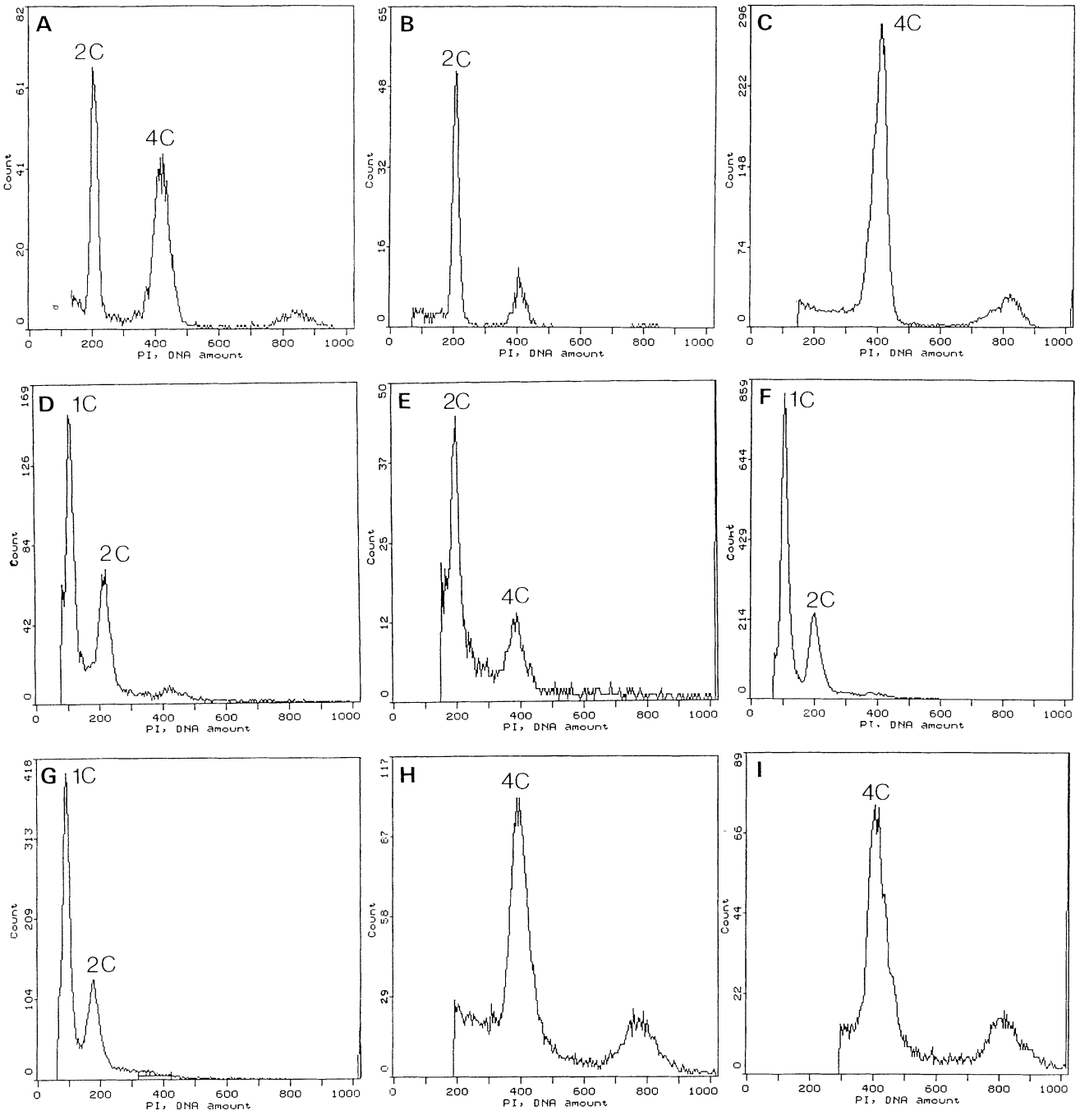

◀ **Fig. 2A–G** Ovules, embryo sacs and initiation of endosperm development in the tetraploid accessions D-62 and D-58 of *B. decumbens* obtained with the clearing technique. **A** Ovule with a mature embryo sac of the Panicum-type, ×800. **B** Egg cell and two synergids of a mature embryo sac of the Panicum-type; the polar nucleus is in close contact with the egg apparatus, ×1600. **C** Ovule with four aposporous embryo sacs, ×700. **D** Egg cell of increased size in an embryo sac of the Panicum-type, ×1600. **E**, **F** Nuclei of irregular shape and with numerous nucleoli during initiation of endosperm development in embryo sacs of the Panicum-type, ×1 600. **G** Ovule with embryo sac of the Polygonum-type, ×800. *Abbreviations*: see **Fig. 1**

Fig. 3A–I DNA histograms obtained from nuclei of leaves, ovaries and anthers of diploid (D-4) and tetraploid (D-62, D-58) accessions of *B. decumbens*. **A–C** DNA histograms from leaves. **A** Mixture of leaves of D-4 and D-62. **B** Leaves of D-4 **C** Leaves of D-62. **D–E** DNA histograms of anthers with pollen grains. **D** Anthers of D-4 **E** Anthers of D-62. **F–I** DNA histograms of ovaries with embryo sacs. **F** Ovaries of D-4. **G** Ovaries of D-4 from flowers without anthers. **H** Ovaries of D-62. **I** Ovaries of D-58

try was used. The accuracy of DNA measurements was checked with a mixture of leaves from plants of the putative diploid and tetraploid accessions D-4 and D-62, respectively (Fig. 3A). The two high peaks, at values 200 and 400, represent the 2C (diploid) and 4C (tetraploid)

levels, respectively. Separate measurements from leaves of each accession prove that accession D-4 is diploid (Fig. 3B) and D-62 is tetraploid (Fig. 3C). D-58 also turned out to be tetraploid (data not shown). In all types of tissue investigated, the second and third peaks represent cells in the G-2 stage. The frequency of these cells was very low (Figs. 3A–I).

The histogram of anther DNA content showed two peaks in accession D-4: the 1C peak for nuclei from haploid pollen grains and the 2C peak for nuclei from the somatic cells of the pollen sac wall either for G-2 nuclei from pollen grains or microspores (Fig. 3D). In accession D-62 the 2C peak is of nuclei from the pollen grains and the 4C peak is either nuclei from the somatic tissue or G-2 nuclei of pollen grains (Fig. 3E). The histograms are based on 32 000 and 34 000 nuclei for accessions D-4 and D-62, respectively. The results show that pollen grains in diploid accession D-4 generally contain haploid nuclei and in tetraploid accession D-62 they contain dihaploid nuclei. Therefore, in diploid and tetraploid accessions of *B. decumbens* pollen grains originate as a result of meiosis.

DNA measurements in ovaries were performed to verify the occurrence of meiotic Polygonum-type embryo sacs among the ovary tissues (nucellus and integuments) (Fig. 3F–I). These measurements could not be used for identification of aposporous Panicum-type embryo sacs, which are expected to show the same patterns as leaves and maternal ovary cells. In each sample 50000–100000 nuclei were measured. The histogram for D-4 (Fig. 3F) showed two peaks: a 1C peak which indicated the presence of a number of haploid nuclei in ovaries belonging to the meiotic embryo sacs, and a 2C peak which represents the DNA content of somatic tissue nuclei and Panicum-type embryo sacs. Histograms for the ovaries of accessions D-62 and D-58 (Fig. 3H,I) had a sharp 4-C peak and a flat peak of the double DNA amount, possibly G-2 cells, indicating a cycling population of tetraploid cells. This shows that all ovary and embryo sac cells had the same DNA content. Based on the embryo sac analyses, a 2-C peak was also expected representing the haploid nuclei of the Polygonum-type embryo sacs.

According to previous reports, the upper flowers of the panicle in the genus *Brachiaria* are bisexual and fertile, and the lower flowers are staminate and sterile (Tsvelev 1984). However, no data on anthecology of *B. decumbens* have been reported. We observed many variations in flower morphology of both diploid and tetraploid accessions. One of the abnormalities was the absence of anthers in flowers of a plant of accession D-4. The ovaries of these flowers were examined by flow cytometry (Fig. 3G). The histogram showed the same ploidy levels as ovaries from normal flowers of accession D-4 (Fig. 3F). Therefore, in spite of the absence of anthers, formation of the ovary and meiotic or unreduced embryo sacs was normal.

Discussion

It is only recently that quantitative cyto-embryological estimations of the mode of reproduction on a large amount of plant material from grasses have become available (Naumova et al. 1993). The results for *B. decumbens* described in this study, obtained using an improved clearing technique, and the use of a large number of ovules instead of ovaries for the investigations, have given reliable data. The results show that dihaploids, which were earlier described as obligate sexual diploids (do Valle and Savidan 1989; do Valle et al. 1989; do Valle and Glienke 1991), are in fact facultatively sexual, because of the occurrence of aposporous embryo sacs. This phenomenon was observed during two different seasons, in spring (15.4% apospory) and in autumn (6.6–13.2% apospory).

The observation that the diploid accession of *B. decumbens* does not have an obligate, but instead a facultative sexual reproductive system, justifies further discussion. The genera *Brachiaria*, *Panicum* and *Paspalum* all belong to the Paniceae tribe of the subfamily Panicoideae, which exhibits a high degree of anatomical and taxonomical diversity. Plants of these genera exist in nature in the form of agamic complexes and show many similarities in reproductive biology (Stebbins 1950; Tsvelev 1984; Zuloaga 1986). All the genera constitute very important forage crops, which need to be genetically improved. But in this respect, the occurrence of apomixis is a major problem.

Two kinds of model systems have been proposed for the reproductive cycle of Panicoid grasses: first, the diploid-tetraploid- (di)haploid cycle and, second, the diploid-tetraploid-diploid cycle. The first system was described in the *Bothriochloa-Dichanthium* agamic complex (de Wet 1968; de Wet and Harlan 1970), and in *Panicum* (Savidan and Pernès 1982). It has been assumed that this model system consists of basic sexual diploids in addition to a complex superstructure of largely apomictic polyploids. Apomictic tetraploids tend to be slightly sexual and only apomicts are known to occur at the higher ploidy levels. Diploids are extremely rare, (about 2% of the total population of a *Bothriochloa-Dichanthium* complex), and are found to be less competitive than the polyploid, apomictic genotypes. Cytologically, it was shown that there are two types of diploids: natural, sexual, fertile diploids and di(ha)ploids which originate from tetraploids. The latter could have arisen from allotetraploids or from autotetraploids which were completely sterile or slightly fertile, respectively. The fertile diploids were cytologically similar to natural diploids (Harlan and de Wet 1963; de Wet and Harlan 1970). No aposporous dihaploids were found in nature in the *Bothriochloa-Dichanthium* complex or in agamic complexes of *Panicum*. In *Panicum* several dihaploids arose from artificial interspecific crosses. A few of these dihaploids formed aposporous embryo sacs but none of them produced seeds after self- or cross-fertilization (Savidan and Pernès 1982). The dihaploids in *Panicum*

and in the *Bothriochloa-Dichanthium* complex were poorly adapted for survival in nature.

The second model system proposed for the reproductive cycle of Panicoid grasses, the diploid-tetraploid-diploid cycle, was described in *Paspalum* (Hanna and Burton 1986; Norrmann et al. 1989; Quarin 1992). The functional components of this model system are diploids, facultatively apomictic tetraploids, triploids and aneuploids. The diploids, probably dihaploids, are not obligate sexuals and are able to develop both meiotic and aposporous embryo sacs. The occurrence of partly fertile triploids in agamic complexes of species belonging to this model system differentiates it from the first system discussed. Quarin (1992) suggested that autotetraploids could arise by a two step process. In a diploid plant, an occasional unreduced egg cell is fertilized by a reduced sperm nucleus to form a triploid (2x+x=3x). The triploid, in its turn, could give rise to the formation of an autotetraploid (3x+x=4x). This model of reproduction is close to the model system described for *Ranunculus auricomus* (Nogler 1982, 1984).

The existence of obligate sexual diploid founder populations is the most important functional element of both model systems, but data about their actual existence are limited and unclear. Hanna et al. (1973) and Nogler (1984) reported that it is surprising that such diploids are often disregarded in embryological investigations, and that no one has demonstrated their use to produce controlled hybrids. Cyto-embryological and quantitative descriptions of obligate, sexual, natural diploids of the Panicoid grasses are lacking and reports of diploids originating from tetraploids are limited and controversial. Therefore, both model systems of reproduction of agamic complexes of the Panicoid grasses include natural obligate sexual diploids, but their existence does not exclude the occurrence of facultative, sexual dihaploids.

The aposporous embryo sacs found in a diploid accession of *B. decumbens* examined in this study point to the occurrence of dihaploids in natural populations of this species. These dihaploids were fertile and are not rare in *B. decumbens* since they survive in nature and probably arose from apomictic tetraploids. Our finding of facultative sexual dihaploids in accession D-4 disagrees with the results of do Valle et al. (1989), who discovered only obligate sexual plants in the same accession. The use of a refined clearing method for the embryo sac analyses enabled us to detect aposporous dihaploids in this natural population of *B. decumbens*. More accessions, however, must be analysed before general conclusions about the reproductive cycle in natural populations of *B. decumbens* may be drawn. As triploids have not yet been found in natural populations of *B. decumbens* (C.B. do Valle, personal communication), the reproductive system of this species resembles the first model system presented for the *Bothriochloa-Dichanthium* complex. It has a diploid-tetraploid-di(ha)ploid cycle, where obligate sexual diploids are not observed and dihaploids, originating from tetraploids, are facultatively sexual. The tetraploids are facultatively apomictic and must have originated from obligate sexual diploids. The degree of apomixis in general is higher in tetraploids than in diploids. But the sexual process, as a mechanism for recombining genetic variation, can occur at the tetraploid level as well. In the agamic complex of *Panicum maximum* two pools were described, each characterized by its own breeding system, sexual or through facultative apomixis (Noirot 1993). The level of isozyme polymorphism in the two pools was similar, and was explained by reciprocal gene flow between them (Assienan and Noirot 1995).

The present results demonstrate fluctuations in the frequency of aposporous embryo sacs in both diploid and tetraploid accessions. These changes, however, were not significantly different between the two seasons. The percentage of ovules with multiple embryo sacs was highly variable in the diploid accession but relatively stable for the tetraploid over the two day length regimes. Our results agree with those of Hussey et al. (1991) who found no influence of photoperiod on the frequency of sexual embryo sacs in facultative apomictic buffelgrass.

In this study, environment had a strong influence on the development of parthenogenetic embryos in Panicum-type embryo sacs. Development of aposporous embryo sacs was strongly inhibited during autumn, while during early summer parthenogenetic embryos were frequently produced. Whether these effects can be attributed to day length effects or simply to age differences requires further investigation with plants of similar age under controlled environmental conditions. However, a similar tendency was observed in other grasses, where a positive correlation was established between length of photoperiod and the formation of sexual embryos (Knox and Heslop-Harrison 1963; Knox 1967; Evans and Knox 1969). The data obtained here suggest that similar events control embryo formation from both zygotes and parthenogenetic egg cells.

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