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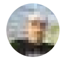

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Flowering, Seed setting and Self-incompatibility in *Poa labillardieri* (Poaceae)

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ABSTRACT

Flowering, seed setting and self-incompatibility in *Poa labillardieri* Steud., the common tussock grass of south-eastern Australia, were investigated by outdoor, greenhouse and laboratory studies. Flowering was found to progress from the terminal to the basal nodes of the panicle and within a branch from the distal to the proximal spikelets, but with significant irregularities and overlaps between the sequences. Progression within the spikelets was, however, without exception, from the basal to the terminal floret. The whole panicle flowered in two days, with about 30% of spikelets opening on the first day and the remainder on the second day. Anthesis was relatively slow under normal spring weather conditions, requiring on average 15 minutes for exsertion and 18 minutes for dehiscence. Under open pollination conditions mean seed setting percentage was about 25% in each of the four zones of the panicle (lower, middle and upper whorls, and terminal branches). A glasshouse experiment with eleven selected lines with open and self-pollination showed varying but generally high percentage seed setting with open pollination and zero or very low seed setting with selfing, indicating a high degree of self-sterility. Using the same eleven lines, a pollination experiment with excised pistils and observation of pollen tube growth by means of fluorescent microscopy showed that all cross-pollination combinations had normal pollen tube growth with penetration to the ovular region, while the self-pollinations showed complete gametophytic self-incompatibility. The implications of these findings for the biology of the species are discussed.

Keywords: *Poa labillardieri*, self-incompatibility, seed setting, panicle, flowering sequence

INTRODUCTION

The grass genus *Poa* with about 500 species (Clayton & Renvoize 1986) is one of worldwide distribution "occurring especially in temperate to cold climates irrespective of whether the latter results from latitude or altitude" (Vickery 1970). Taxonomically, the genus has long been regarded as difficult, seemingly consisting of a small number of well defined species and numerous groups or complexes, each composed of a wide array of intergrading forms. Bentham (1878), in developing a classification for the Australian *Poa* material, referred to the great variations encountered in some groups and the limitations imposed by having access only to herbarium specimens, many of them incomplete. Similarly, Bor (1952), working on *Poa* in India, found that in relation to the complexes of interconnected forms "one cannot rely on a single character to separate species in such groups, but combinations of more or less variable characters must be used" and went on to suggest that resolution of these difficulties would require an experimental approach. It is now generally accepted that many of the problems in the taxonomic treatment of *Poa* have their origin in the widespread occurrence of polyploidy, introgression and apomixis in the genus, as first pointed out by Stebbins (1950).

Large tussocks of *Poa* spp. are a conspicuous feature in the grassland and open forest formations of south-eastern Australia (including Tasmania) from the coast to an altitude of about 1,700m. In the classification of Bentham (1878) the omnibus name *Poa caespitosa* was applied to all of these tussocky forms, and passed into general use among botanists and agronomists (e.g. Maiden 1898, Breakwell 1923). By 1950, with progress in field ecology and pasture agronomy, the limitations of the omnibus name were becoming

apparent. Dr Joyce Vickery of the New South Wales National Herbarium took up the challenge and in 1970, after 16 years of research, published her now classic revision of the genus *Poa* in Australia. This study (Vickery 1970) was strongly field based and had the benefit of collaboration with Dr M.E. Phillips who for many years maintained an experimental garden of *Poa* tussocks at Cooma in the Southern Tablelands of New South Wales. The key result of the revision was that the omnibus species *Poa caespitosa* was shown to consist of more than twenty distinct species including the subject of this paper. The unfortunate result of this long hiatus in the recognition of the diversity of *Poa* species in south-eastern Australia is that in the absence of herbarium voucher specimens it is often difficult or impossible to relate many of the older ecological and pastoral studies of "*Poa caespitosa*" to current taxa.

Poa labillardieri Steud. is an endemic perennial tussock grass with a wide distribution in temperate south-eastern Australia from the coast to the adjoining tablelands and their inland slopes (Vickery 1970, Jacobs *et al.* 2008). It forms dense clumps about 0.7m high composed of harsh narrow (1-3.5mm) leaves and in the spring produces flowering culms reaching a height of 1.2m, the terminal part of each culm being a paniculate inflorescence typically 15-25cm long. Ecologically it shows a preference for flood plain grasslands and other periodically moist grasslands, but also occurs in open forest on sheltered slopes (Jacobs *et al.* 2008), particularly those facing south (Vickery 1970). Economically the grass is of little value as an animal fodder, so its tendency to spread in a weed-like manner under some grazing management systems in the frost-prone tablelands of New South Wales and Victoria is a cause for concern (Campbell *et al.* 1987). On the other hand, selected wild forms of the grass, particularly those with bluish-green (glaucous) leaves, are extensively used in low- maintenance landscape plantings in the lowland Sydney and Melbourne regions.

Under field conditions, natural populations of *Poa labillardieri* are characterized by high ploidy levels (tetraploid to dodecaploid), good pollen viability, ready hybridization between even numbered ploidy levels (May & Campbell 1991) and a high output of viable seed (Campbell *et al.* 1987). Despite the polyploid nature of the species, cytological studies by Ahmad (2005) and May & Campbell (1991) have not found any evidence of sexual apomixis. In contrast, vegetative apomixis in the form of occasional floret proliferation has been found on rare occasions in field material (Vickery 1970), although under controlled environment conditions it has been found possible to induce proliferation of all spikelets on an inflorescence (Ahmad *et al.* 2009).

Given the importance of *Poa labillardieri* in landscape gardening, there has been growing interest in the possibility of deliberate breeding of improved cultivars. Accordingly, since 2001 the Amenity Horticulture Research Unit of the University of Sydney's Plant Breeding Institute has undertaken a number of detailed studies on various aspects of the sexual and asexual reproduction in this species to serve as a basis for a rational programme of hybridisation and clonal propagation and to contribute to our knowledge of the reproductive biology of this widespread but poorly understood species. Based on a scanning electron microscope study, we have published a fully illustrated account of its floral morphogenesis (Ahmad *et al.* 2009), proceeding from initiation of the floral meristem through differentiation of panicle branch primordia and differentiation of the sexual organs to the flowering stage in the exserted panicle. This paper also included a study of spikelet proliferation, the different degrees of which were shown to depend on the timing of the deviation from the normal sexual pattern of development.

In the present paper we present observations on flowering behaviour, results of experiments on seed setting with both open-pollination and self-pollination, and the results of an exploration of self-incompatibility behaviour by means of observations on pollen tube growth on excised pistils.

MATERIALS & METHODS

Plant material — Seeds of *P. labillardieri* were gathered from a field population of predominantly bluish-green (glaucous) leaved plants at Tuggeranong (latitude 35°27'S, longitude 149°07'E, altitude c.600 m), near Canberra in the Australian Capital Territory, in December 1999. The intact seed, consisting of the caryopsis (grain) enclosed in the persistent lemma and palea (Fig. 3. B & C), was found from a bulk sample to have an average weight of 0.26 mg per seed and, following after-ripening at room temperature, to have a germination percentage of about 90% at 20°C. Using this seed, a population of 66 plants was raised to maturity in 200 mm pots under outdoor conditions with sprinkler irrigation at the nursery of Leppington Speedy Seedlings and Supplies Pty. Ltd. at Leppington, New South Wales. On transfer to the Plant Breeding Institute in July 2001 (winter) the plants were accessioned as lines P1 to P66 for purposes of identification. They were then trimmed to 5 cm above soil level and the crowns cut into equal sized planting portions ranging from four in the smaller clumps to six or seven with the more vigorous plants. Each portion was reset in a 125 mm pot with a potting mix composed of nine parts of composted pine bark and one part of washed river sand (by volume). The reset plants were grown outside in a bird excluding cage at the Plant Breeding Institute, watered regularly and fertilized monthly. One year later, in mid-July 2002, four replicate pots of each of eleven lines, selected as

representative of the variations in vegetative morphology, were transferred to a greenhouse whose temperature was not allowed to fall below 14ºC at night. In response to the warmer conditions, these plants flowered in mid-August 2002 and were used at once for the polycross and self pollination experiments. The greenhouse population was maintained for a further year and used in September 2003 as the source of pistils and pollen for the *in vitro* self-incompatibility experiment. The plants not moved to the greenhouse flowered in the outdoor cage in mid-September 2002 and were used for the study of seed distribution within the panicle.

Following seed maturity in 2002, all 66 lines were confirmed by detailed morphological examination as falling within the circumscription of *P. labillardieri* adopted by Vickery (1970). However, in recognition of the past taxonomic difficulties with the tussock forming *Poa* grasses in south–eastern Australia and the likelihood of further revisions as understanding of the group increases, a representative voucher specimen (line P34) was deposited in the Australian National Herbarium (Herbarium Number: CANB 765300) to provide tangible evidence of the identity of the population studied.

Flowering behaviour & Flowering sequence — On each of ten plants of different lines growing in the outdoor cage enclosure described above, an inflorescence of average size approaching anthesis was tagged and observed several times per day over a period of seven days in the second week of September 2002. Outline diagrams of the inflorescences were prepared showing the individual branches and the sequence of flowering (exsertion of the anthers from at least one spikelet on the branch) was recorded by a numbering system. When the general pattern had been established, a detailed case study was carried out on one inflorescence of line P34 by preparing a diagram showing the exact location of every spikelet and recording their sequence of flowering (exsertion of the anthers from at least one floret in the spikelet) by numbers marked on the diagram.

Anthesis observations — For gaining some preliminary data on the process of anthesis under outdoor conditions, several inflorescence approaching anthesis were selected. As anthesis commenced, a number of spikelets with, between them, at least 20 opening florets at the same initial stage of opening were marked and observed frequently with a hand lens (x10). The time taken (in minutes) to reach various developmental stages was recorded. The stages adopted were: (i) commencement of anthesis - when the tip of an anther could be seen pushing between lemma and palea at the tip of a floret; (ii) end of filament elongation; (iii) beginning of anther dehiscence; (iv) completion of anther dehiscence. Immediately after the first set of observations was completed, another group of spikelets was selected and the procedure repeated. On the following day, some inflorescences of the same plants were detached just before anthesis was due to commence and the cut ends were at once plunged into water. These inflorescences were taken to the laboratory and when anthesis began in the panicle a similar set of observations was made by detaching spikelets at the appropriate stage, placing them on damp filter paper enclosed in a Petri dish and observing with the aid of a stereo-microscope (x10). As with the outdoor observations, the procedure was repeated with a second set of spikelets as soon as the first batch was finished. The temperature of the laboratory was maintained at 24 ºC.

SEED SETTING EXPERIMENTS

Distribution of seeds within the panicle — Open-pollinated plants growing in a block of more than 200 plants of *P. labillardieri* in the outdoor cage house were used for this experiment. In October 2002, fifteen lines were selected at random and from one replicate pot of each line a single panicle with mature seeds was removed. Each panicle was placed in a labelled paper bag and allowed to dry out at room temperature. To determine the distribution of spikelets, florets and seeds within a panicle, it was divided into four well defined morphological zones referred to as the lower whorl, middle whorl, upper whorl and terminal branches (see Figure 1). For each zone a count was made, with the assistance of a stereo-microscope at a magnification of 10x, of the number of spikelets, the number of florets and the number of seeds. The seeds (florets with caryopses enclosed) were distinguished from barren florets by their darker brown colour and by their firmness when touched with fine forceps.

Seed setting with polycross pollination and self – pollination — This experiment was conducted with the eleven selected lines moved to the warm greenhouse in mid-July 2002 as described under 'Source of Plant Material" above. The 44 pots (eleven lines each with four replicates) were placed randomly in a block on a bench in a large air-conditioned greenhouse containing no other plants of *P. Labillardieri* (Figure 3A). Open pollination took place naturally between the eleven lines in the polycross block, the air movement generated by the air-conditioning being sufficient to ensure a good distribution of the pollen. For self-pollination, after removal of the small flag leaf, individual inflorescences at the pre-anthesis stage were enclosed with glossy water-proof paper bags which were clipped tightly to a cotton wool band around the peduncle (see Figure 3A). Because of the large amount of pollen produced by each floret and the large number of florets open at the same moment in the bag, self-pollen was not considered as limiting, but as a precaution, for a week following bagging, each bag was shaken daily shortly after the noon anthesis to distribute the pollen. In addition, to check that the bags were not depressing seed set, a small number of plants were cross-

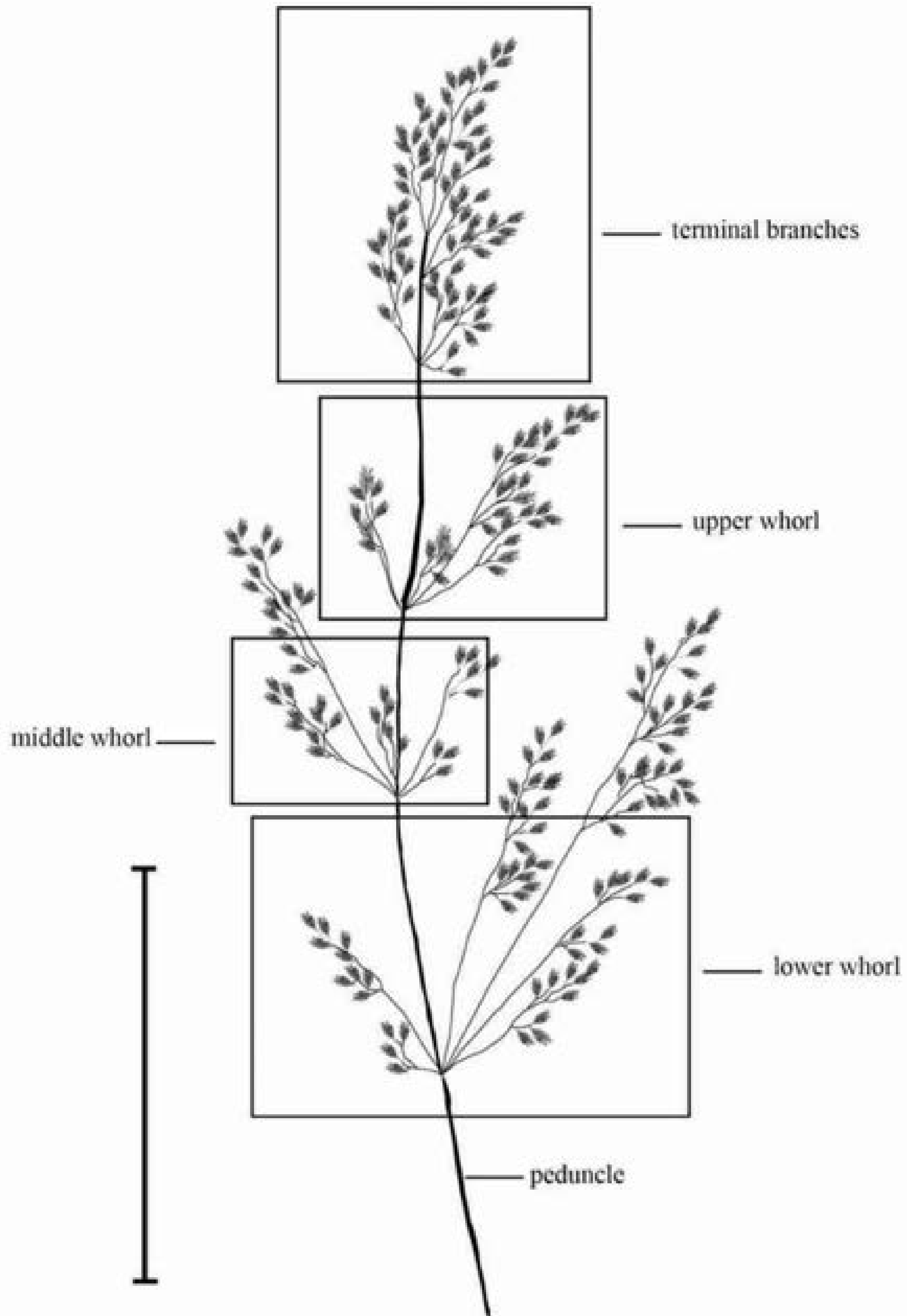

Figure 1 — Inflorescence of *Poa labillardieri* showing four zones. Scale bar = 100 mm

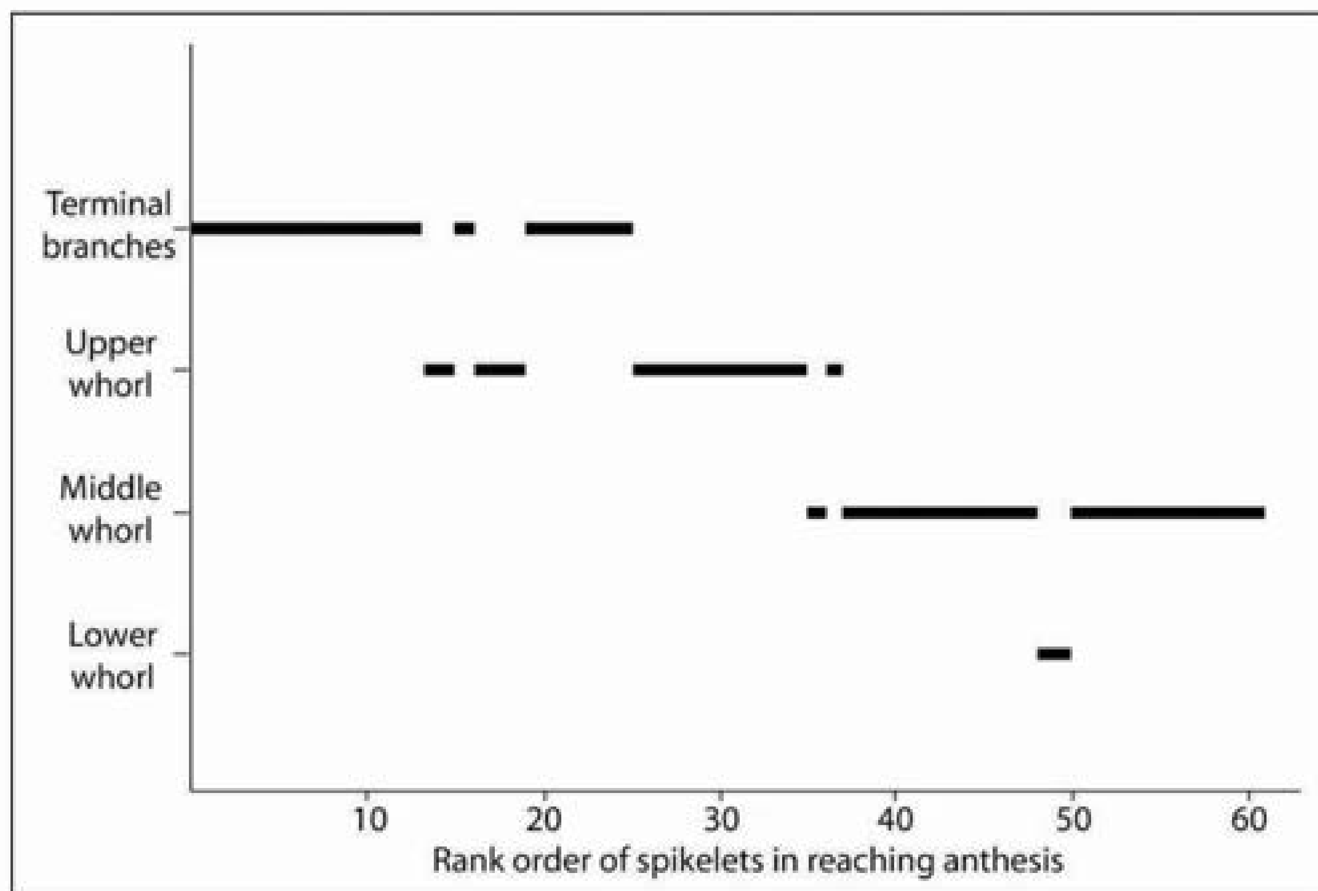

Figure 2 — Time and location sequence of the 61 spikelets reaching anthesis in a *Poa labillardieri* panicle on the first day of anthesis.

pollinated by bagging attached inflorescences at the correct stage from adjoining pots of different lines (Figure 3.A). All bags were removed after 4 weeks. Inflorescences were harvested about 6 weeks after anthesis when the seed was ripe but not yet at the shedding stage shown in Fig. 3.E. For yield analysis, from each pot the self-pollinated (bagged) inflorescence and an open pollinated inflorescence which had reached anthesis at about the same time were taken and scored for floret number and seed number, making a total of 88 inflorescences for analysis.

Compatibility experiment with excised pistils — This experiment was conducted with pollen and excised pistils from the eleven lines composing the greenhouse population. Prior to fertilisation, the pistils of most grasses, including *P. labillardieri*, are readily removed by the slightest touch and are therefore suitable subjects for analysis of incompatibility by the convenient method of Lundqvist (1961). During September 2003 in inflorescences that were approaching anthesis in the greenhouse block, florets with fully developed anthers but un-elongated filaments were gently opened with very fine forceps and the entire flower lifted out. The anthers were then detached from the filaments and the emasculated pistils implanted base down in agar gel in 4.5 cm Petri dishes (Fig. 4.B). Lundqvist (1961) specified 2% agar, but using the product available to us (Agargel A-3301, Sigma-Aldrich, St. Louis, USA) this concentration produced a gel too stiff for effective implantation of the pistils. A 1% gel proved satisfactory. This was supplemented with sucrose and boric acid as per Lundqvist (1961) and adjusted to a pH of 5.8 using 1 M KOH or 1M HCl as necessary. For each self-fertilisation 75 pistils were used, 25 in each of three Petri dishes, while for each cross-pollination one Petri dish containing 25 implanted pistils was used. The experiment was designed as a complete 11x11 crossing matrix, giving 121 combinations composed of 11 self-pollinations, 55 forward crosses and 55 reciprocal crosses. In total, 3,575 excised pistils were used. Pollen was collected by excising mature anthers just before dehiscence and allowing them to dehisce and rehydrate on a microscope slide placed over moist filter paper in a Petri dish incubated at 22ºC for thirty minutes, after which anther debris was removed with forceps. Pollen was then dusted from the slide onto the pistils in the open dish with the aid of a small brush and the lid replaced to maintain humidity and exclude stray

pollen. The experiment was staged over eleven days: on any one day pollen from one line was applied to the requisite number of excised stigmas from all eleven lines.

After 24 hours of incubation at 18ºC, the growth of the pollen tubes was examined by a modification of the staining and fluorescence method of Martin (1959). The pistils were removed from a given Petri dish, placed in a small tube and stained with colourless 0.05% aniline blue in 0.1 M phosphate buffer (pH 7) for 20 minutes in a temperature controlled room at 24ºC. Five pistils were then removed at random from the 25 in the tube and viewed at magnifications of x100 and x200 with a Nikon Eclipse E800 compound microscope (Nikon Optical Co., Tokyo, Japan) using fluorescence optics with short-wavelength ultraviolet light and photographed with a Nikon Photo Head V-TP Sensicam camera (PCO Imaging, Kelheim, Germany) mounted on the same microscope. The callose lining of the pollen tubes and the callose plugs fluoresce brightly, providing an indication of the distance that the tubes had grown and the pathway taken (Martin, 1959).

Data Analysis — Standard error was calculated where appropriate using GenStat, 6th edition. For statistical tables reference was made to Fisher and Yates (1963) as necessary.

RESULTS & DISCUSSION

Flowering Behaviour

Panicle studies — During the period of these observations, the weather was mostly sunny with maximum temperatures in the vicinity of 22ºC. Typically, under these conditions, three days after emergence of the panicle from the sheath of the flag leaf, the peduncle and the nodes between the whorls reach their maximum extension (Fig. 4.A), the panicle branches spread widely, anthesis commences about noon and continues for some two hours. By early evening most of the florets which had opened are shut again and all are closed by the following morning. On the fourth day, commencing about noon, there is another burst of anthesis involving the spikelets whose florets had not opened the previous day. Very occasionally the opening of some spikelets in an inflorescence is deferred till the fifth day following panicle emergence.

Observations made on the flowering sequence in the ten different lines showed a consistent pattern with the first anther exsertions occurring in spikelets of the uppermost terminal branches of the panicle, followed by a progressive movement downward through the rest of the terminal branches, the upper whorl, the middle whorl and the lower whorl (see Fig. 1). The last spikelets to flower were always in the lower whorl. Within a given branch, the sequence was normally inwards from the distal spikelets to the proximal spikelets. Within a spikelet, regardless of position on the panicle, the direction of the opening sequence was invariably from the lower floret to the uppermost, this progression being very rapid and almost simultaneous in some instances. The overall pattern of anthesis could thus be summarised as basipetal with respect to the four zones of the panicle (as defined in Fig. 1), basipetal within a given branch and acropetal within a spikelet. This is similar to the pattern described by Jenkin (1959a) in panicles of *Festuca pratensis*, a grass belonging to a closely related genus.

It was evident, however, that with the exception of the sequence within a spikelet, this overall sequence does not provide an adequate description of the reality. As mentioned above, anthesis takes place over two days and occasionally three days. At the end of anthesis on the first day there were always numerous spikelets yet to flower in the upper three zones, whereas the simple overall model would suggest there should be none or very few in the terminal branchlets and upper node zones. Similarly, it was noticed frequently that on the long branches of the middle whorl at the end of the first day of anthesis the opened spikelets comprised the distal group and a few spikelets well down the branch, with numerous unopened spikelets in between, contrary to the concept of an orderly basipetal progression.

The detailed case study of the flowering behaviour of every spikelet on a single inflorescence of line P34 under outdoor conditions allows a much better analysis of the actual situation. In a well developed panicle with 212 spikelets, only 61 (28.8%) reached anthesis on the first day, all the rest opening on the second day. On both days anthesis

Table 1 — Distribution of flowering across the four zones of a *Poa labillardieri* panicle at the end of the first day of anthesis

Distribution	Number of spikelets	Number at anthesis	% at anthesis
Terminal branches	56	20	35.7
Upper whorl	42	16	38.0
Middle whorl	58	23	39.7
Lower whorl	56	2	3.6
Total	212	61	

commenced just before noon, with no further openings after 2pm. Table 1 provides an overview of the flowering behaviour at the conclusion of anthesis on the first day in terms of the four zones of the panicle. Rather than showing a steadily decreasing trend from top to bottom, the results show relatively low and almost equal percentages of spikelets at anthesis in the upper three zones (35.7%, 38% and 39.7% for the terminal branches, upper whorl and middle whorl respectively), together with a very low percentage (3.6%) for the lower whorl.

Since the exact sequence of opening of the spikelets had been recorded it is possible to gain a much clearer idea of what is going on by means of the graphical presentation in Figure 2. This shows a clear trend for a basipetal progression over the two hours but with a significant overlap between the top two zones and a departure from a strict basipetal sequence through the zones as shown by the gaps in the horizontal bars for the upper three zones. The two spikelets that opened in the lower whorl are also out of sequence and although both of them were in the distal regions of their respective branches, neither of them were terminal spikelets. Further consequence of the numbers recorded in Table 1 are that more than 70% of all spikelets flowered on the second day including more than 60% of those in the uppermost (terminal branches) zone.

Regarding sequences in the long branches of the lower, middle and upper whorls, in detail these often show large departures from the concept of a simple basipetal progression, the situation being further complicated by the presence of secondary branches and the sequences within these. Enough has been made of this analysis of the flowering sequence of a single panicle: it shows that the actual behaviour is vastly more complex than the overall trends revealed by cursory observation.

Anthesis studies — Observations were made of the time taken for the process of anthesis in more than 80 florets on inflorescences of plants growing outdoors and on a similar number of florets in detached spikelets of the same plants under laboratory conditions. It was found that the complete process occupied an average total time of 33 minutes in the first case and 45 minutes in the second case. Variations between florets did not exceed one minute in either trial. From commencement of exsertion of the anther from the floret to the end of filament elongation took an average of 15 minutes under natural conditions and 25 minutes in detached spikelets. Elongation of the filaments was about 3 mm. Anther dehiscence and shedding of pollen started immediately after the completion of filament elongation in the florets of both attached and detached spikelets. Completion of anther dehiscence (x shape of anthers) took, on average, 18 minutes outdoors and 20 minutes in the laboratory. It will be noted that although time for completion of anther dehiscence was similar in both cases, time for elongation of filaments was nearly doubled in the laboratory. Temperature conditions were similar in both locations and although light levels were much lower in the laboratory, it is probable that disruption of water supply as a consequence of detachment of the spikelets was the principal factor in the increased elongation time.

Seed Setting:

Distribution of seed set within the panicle (outdoor experiment) — The panicle is generally pyramidal, 10-25

Table 2 — Seed set percentage and mean number of spikelets, florets and seeds, by panicle zones, for open pollinated inflorescences of *Poa labillardieri*.

Position from base of panicle upwards	Mean spikelet number*	Mean floret number*	Mean number of florets per spikelet	Mean seed number*	Average% seed setting
Lower whorl	36.7±3.2	107.0±11.1	2.92	26.2±4.8	24.5
Middle whorl	37.7±3.5	117.3±11.5	3.11	31.7±5.8	27.0
Upper whorl	30.0±3.6	97.0±12.6	3.23	24.5±3.4	25.3
Terminal branches	55.8±5.7	188.5±17.5	3.38	47.2±8.4	25.0
Whole inflorescence	160.2±14.8	509.7±47.3	3.18	129.6±19.7	25.4

*Values are means ± SE of inflorescences taken randomly one per plant from 15 randomly selected lines.

Table 3 — Observed and expected mean numbers of seeds in the four panicle zones of 15 *Poa labillardieri* inflorescences.

	Lower Whorl	Middle Whorl	Upper Whorl	Terminal branches	
Observed values	26.2	31.7	24.5	47.2	χ^2=0.5498
Expected values	29.7	30.5	24.3	45.2	

cm long, with three loosely spreading whorls of long branches in the lower three quarters and a cluster of short branches in the upper quarter. For purposes of analysis, it is divided into four zones reflecting its structure viz. the lower whorl, the middle whorl, the upper whorl and the terminal branches (see Figure 1). Developmentally, we have previously shown (Ahmad *et al.* 2009) that although the lower branches of the panicle are the first to form, the terminal branches are the first to develop spikelets, so there is interest not only in defining the reproductive performance of the panicle as a whole, but also in ascertaining whether the four zones differ in their behaviour.

An overview of the reproductive performance of 15 open pollinated *Poa labillardieri* accessions grown in an outdoor bird-proof enclosure is shown in Table 2. Mean spikelet number per inflorescence was roughly equal for the three whorls which between them contributed about two thirds of the total spikelets, the remaining third coming from the terminal branches zone. Mean floret numbers were distributed in approximately the same proportions, the terminal branches again contributing a little more than one third of the total. Mean floret numbers per spikelet were relatively constant ranging from 2.92 in the lower whorl to 3.38 in the terminal branches. Looking at the data for the individual panicles, the majority of spikelets had either two or three florets, but some had as few as one and others as many as five. In general floret per spikelet numbers were consistent across the panicle for individual plants, panicles with high numbers in the lower whorl having high numbers in each portion of the panicle and the converse.

Mean seed numbers were broadly similar in the three whorls and somewhat greater in the terminal branches zone, but when the mean seed set per zone was calculated the percentage of florets yielding seed was seen to be very similar across all four zones at about 25% (Table 2). To test the null hypothesis that seed set was uniform across the four zones, expected mean seed numbers were calculated by multiplying the overall mean seed set of 25.4% by the mean floret number per zone, and comparing these with the actual numbers by means of a Chi-square analysis (Table 3). The value for Chi-square was far below the level required for a statistically significant departure from a uniform distribution of seed set across the panicle. The earlier development of spikelets in the terminal branches during floral morphogenesis (Ahmad *et al.* 2009) may have some connection with the primacy of this zone on the first day of anthesis (Figure 2), but there is no evidence of any continuing advantage for this zone in terms of seed set percentage.

Given the lack of any obvious gradient in seed set from the lower to the upper part of the panicle, it is clear that for experimental purposes spikelets in any zone of the panicle could be used as a representative sample for controlled pollination work. However, for genetic studies where emasculation and hand pollination of specific florets is required, spikelets on the lower whorl would be favoured because their wider spacing reduces the risk of contamination while the long branches devoid of spikelets in the basal third (see Figure 1) facilitate isolation by small bags.

Seed setting with self- and open-pollination (greenhouse experiment) — In this experiment total floret numbers and total seed numbers were obtained for each inflorescence and percentage seed set calculated from these values. As shown in Table 4, self-pollination resulted in no seed set (lines P43 and P14) or very low seed set, the highest yielding line (P25) having a mean value of only 1.6%. Within a line, the individual replicates (plants) showed maximum variation in seed set percentage of less than double the small mean value. These results are in contrast to those obtained by Jenkin (1959b) who found that within lines of *Lolium perenne* of comparable mean self-fertility to those in our study, the values for individual plants ranged up to nearly nine times the small mean value. It is concluded that self-incompatability is strongly expressed in all eleven lines selected for assessment from this population of *Poa labillardieri* plants.

With open-pollination in the poly-cross block, all lines had a higher seed set than under self–pollination, but there was great variation between the accessions, ranging from 4.49% and 4.83% for lines P6 and P30 respectively to more than 35% for lines P25 and P43. There was no relationship between seed setting under self- and cross pollinated conditions: lines with complete or almost complete self sterility being amongst both the least and the most prolific lines under open-pollination. The reason for the poor performance of some lines with open-pollination in the polycross block has not been investigated, but may well be associated with differences in the compatability of the high polyploids involved. Ahmad (2005) has provided counts for six of the eleven lines showing them to be septaploids ($2n=7x=49$) and octoploids ($2n=8x=56$) but unfortunately counts are not available for the low seed set lines P6, P14 and P30.

Estimated total seed output per plant has been calculated for each line as the product of the mean seed number per inflorescence and the mean number of inflorescences per plant. Inflorescence number per plant is an important variable for breeding purposes, with some genotypes producing inflorescences on almost every tiller while others have very few fertile tillers (Figure 3.D). As shown in Table 5, the mean number of inflorescences of the eleven selected lines ranged from 4 to more than 20 per plant, while the calculated seed output per plant showed an almost nineteen–fold difference between the highest (P25) and lowest (P38). Somewhat surprisingly, there was no

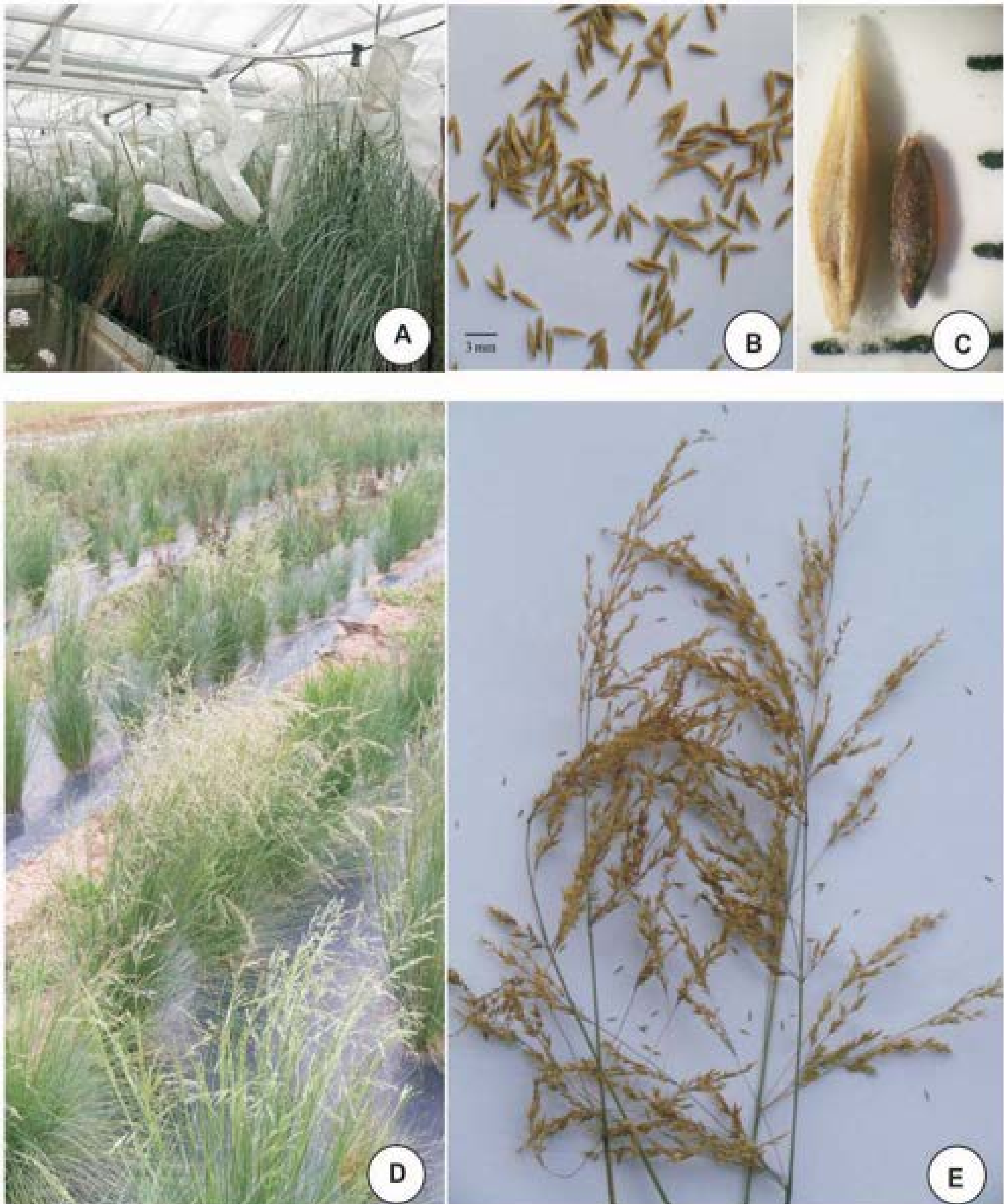

Figure 3. A-D. — A. Inflorescences bagged for self and cross compatibility studies, B. Seeds of *Poa labillardieri : scale bar 3 mm*, C. Intact seed and caryopsis of *Poa labillardieri* : scale to right of image with 1 mm divisions, D. *Poa labillardieri* plants at anthesis stage growing in the field at the Plant Breeding Institute's Lansdowne Farm, E. Inflorescences at the seed shedding stage.

Table 4 — Seed setting of selected *Poa labillardieri* lines.

Line No.	Selfed		Open-pollinated**	
	Mean No. of seeds per inflores.*	% Seed setting	Mean No. of seeds per inflorescence*	% Seed setting
P6	1.00 ± 0.4	0.20	46.00 ± 4.4	4.49
P14	0.0	0.0	66.50 ± 4.2	10.43
P24	2.25 ± 0.5	0.33	137.25 ± 8.1	24.55
P25	7.00 ± 1.1	1.60	190.25 ± 5.3	37.18
P30	11.25 ± 1.1	1.51	35.50 ± 3.4	4.83
P34	3.25 ± 0.7	0.46	90.00 ± 4.8	18.60
P38	7.00 ± 1.1	0.74	176.25 ± 4.8	34.71
P42	8.00 ± 0.7	1.34	116.25 ± 2.6	19.46
P43	0.0	0.0	232.00 ± 4.5	36.77
P44	4.25 ± 1.0	0.78	201.50 ± 3.7	26.00
P53	7.50 ± 1.3	0.98	145.25 ± 3.8	28.88

* Values are means ±SE for four inflorescences, one from each of four replicate pots.

**Open pollination among the 11 lines in an isolation (polycross) block

Table 5 — Estimated total seed output per plant of selected *Poa labillardieri* lines.

LineNo.	Mean No. of seeds per inflorescence*	Mean No. of inflorescences per plant*	Estimated total No. of seeds per plant
P6	46.00 ± 4.4	14 ± 1.3	644
P14	66.50 ± 4.2	17.0 ± 6.2	1131
P24	137.25 ± 8.1	4.0 ± 0.7	549
P25	190.25 ± 5.3	21.8 ± 2.9	4138
P30	35.50 ± 3.4	13.5 ± 2.5	479
P34	90.00 ± 4.8	26.8 ± 7.3	2408
P38	176.25 ± 4.8	1.3 ± 0.3	220
P42	116.25 ± 2.6	11.5 ± 3.9	1337
P43	232.00 ± 4.5	5.3 ± 1.2	1218
P44	201.50 ± 3.7	4.8 ± 1.6	957
P53	145.25 ± 3.8	20.3 ± 6.8	2941

*Values are means ±SE for four inflorescences, one from each of four replicate pots.

correlation between inflorescence number per plant and seed number per panicle. Thus, line P25 had a mean seed number of 190 per panicle and almost 22 inflorescences per plant in contrast to line P38 with176 seeds per panicle but only 1.3 inflorescences per plant (Table 5).

These findings have considerable horticultural importance. Superior lines of *P. labillardieri* can be clonally propagated, but the extremely tough and almost woody crowns of the plant cannot be sub-divided readily and usually require the use of a saw to achieve this goal. This is a time consuming process and yields propagules consisting of clumps of tillers, thereby giving a low multiplication ratio. This approach is not appropriate for economical mass scale production. An alternative approach, which has attracted industry interest, is to develop seed propagated synthetic strains with a defined (limited) range of variation and desirable characteristics for landscape horticulture. Such an approach is extensively used in the breeding of improved strains of *Lolium* spp. and other important pasture grasses for temperate and cool-temperate climate areas. As summarised by Simmonds (1979) efficient production of a synthetic strain (SYN) requires the identification of a small number (typically five to nine) of highly compatible lines having high self sterility, a limited range of variation for specified

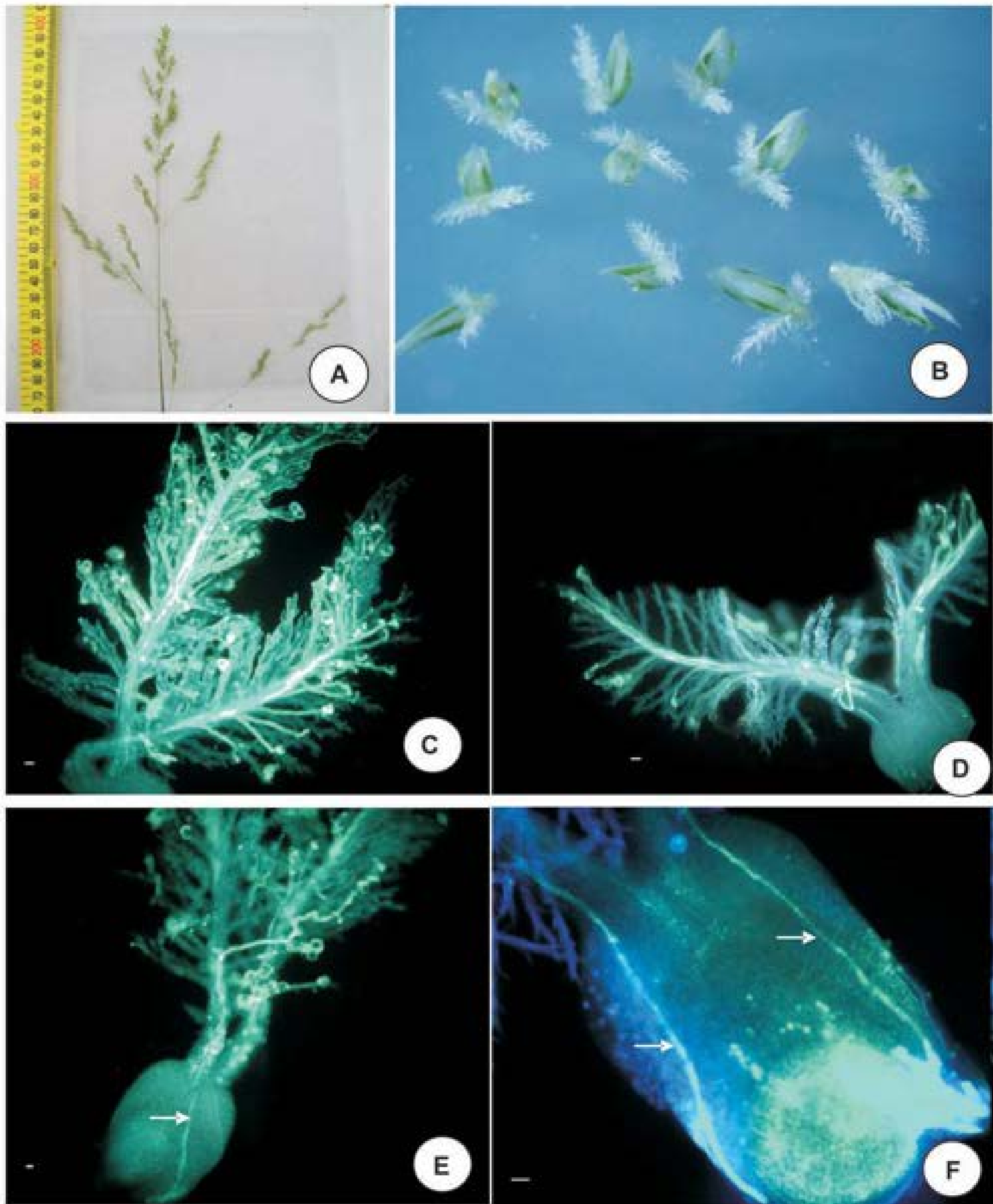

Figure 4. A-F. — A. Inflorescence of *Poa labillardieri* at pre-anthesis time. B. Emasculated excised pistils in Petri dish. C-F. Pollen tubes of *P. labillardieri* stained in decolourised aniline blue and observed with UV light 24 hours after pollination, cross pollinations. Penetration of pollen tubes to the base of the ovary indicated by arrows in E and F. Lines used for crosses: C P24xP42, D P34xP42, E P24xP42, F P53xP42. Scale bar in Figures 4C-4F= 20μ.

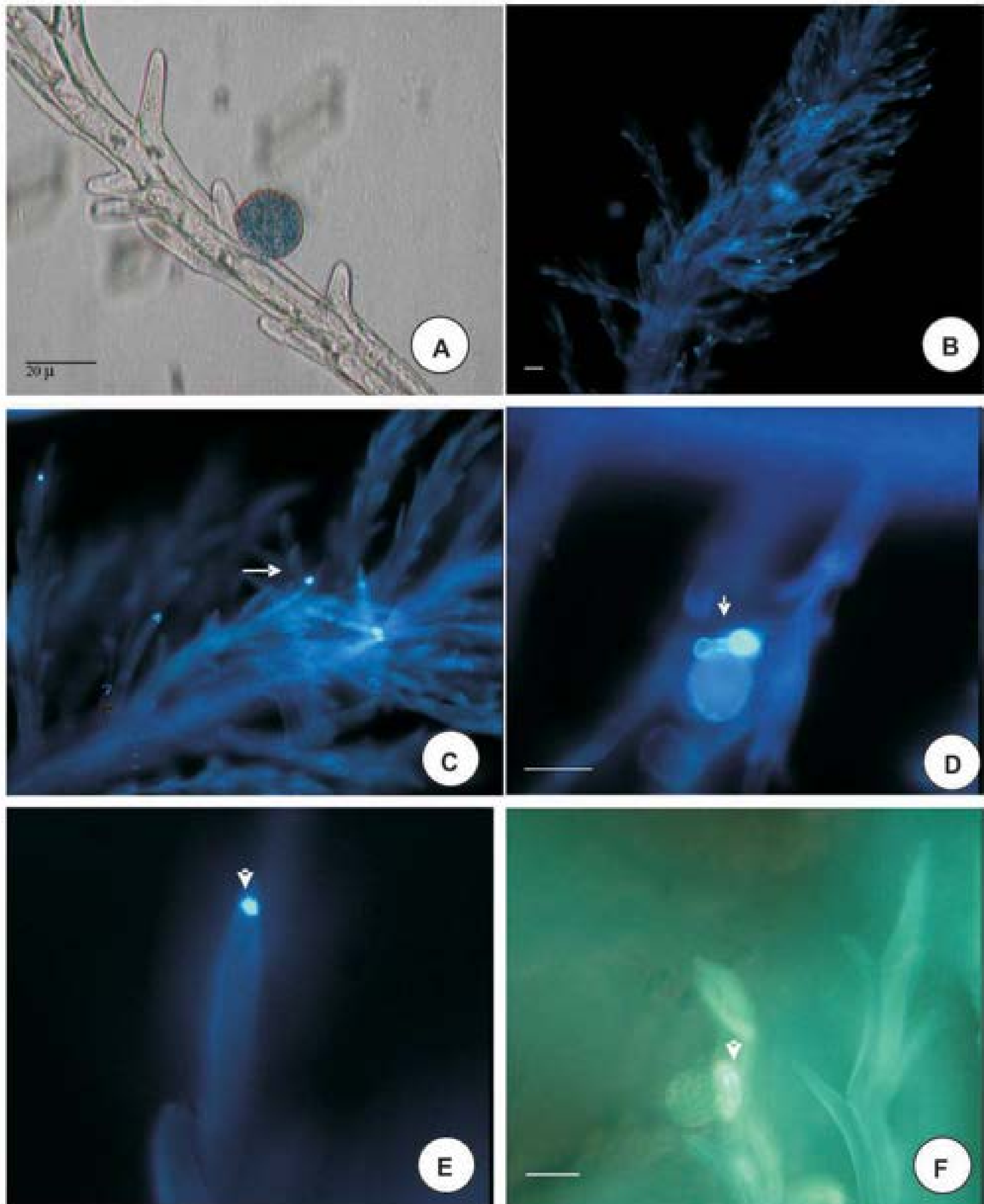

Figure 5. A-E. — A. Pollen grain in contact with a stigmatic papilla. B to F. Pollen tube inhibition in self-pollinated pistils. 5. D, E and F. More highly magnified views showing swelling and rupture of tube tip with formation of callose plug before pollen tube entry (arrowheads). B and F line P43, C,D and E line P14. Scale bar A - F = 20μ.

morphological characters, approximately equal seed production per plant and a limited and defined range of variation in the polycross progeny. On the basis of the data presented in Tables 4 and 5 covering eleven selected lines, it is evident that with the evaluation of a larger number of forms it should be possible to bring together small groups of lines meeting all of the necessary criteria to produce a number of distinct synthetic strains to be propagated by seed for horticultural purposes.

Compatability experiment with excised pistils — To further explore the findings set out in Table 4, a pollination study using excised pistils was carried out a year later at the next flowering time. As explained in the Methods and Materials section, this was a large experiment involving all 121 crosses between the eleven lines listed in Table 4, using the pistil culture technique of Lundqvist (1961) and the fluorescent staining and observation method of Martin (1959). The tri-nucleate pollen grains are about 20 µm in diameter and are relatively large in relation to the stigmatic papillae (Fig. 5.A).

Cross-pollinated pistils showed brilliantly fluorescing pollen tubes throughout the whole pistil (Fig. 4. C-F). In these figures, the pollen used in each case was from line P42, but there were three different seed parents (lines P24, P 34 and P 53). Penetration of pollen tubes to the ovular area of the ovary is clearly shown in Figures 4. E & F. All 110 out-crosses (55 forward crosses and 55 reciprocal crosses) showed a similar pattern of tube growth with penetration of multiple tubes to the ovular area of the ovary. Nothing unusual was noticed about tube growth on stigmas of lines P6 and P30, the lines which had shown low seed set in the previous experiment, suggesting that events at or after fertilisation are responsible for the poor seed output. Across the whole of the crosses, no obvious differences were noted in the manner of pollen tube growth.

Self- pollinated pistils appeared dark under fluorescence microscopy at x100 except for small bright spots caused by the fluorescence of callose plugs at the top of some papillae (Fig. 5.B and 5.C). Under higher magnification it was seen that with self-pollination the pollen tube typically grew tangentially to the grain for about 10 µm before forming a swollen end which then ruptured to produce a strongly fluorescing callose deposit where it terminated on a stigmatic papilla without penetration (Fig. 5.D & E). A very small proportion of the selfed pollen tubes succeeded in penetrating the stigmatic papillae but none reached beyond the fine branches of the stigma before swelling and terminating in a callose plug. This behaviour on self-pollination is a clear indication of the special type of gametophytic self-incompatibility found in the grass family (Briggs & Walters 1997, Connor 1979, Heslop-Harrison 1979). According to Heslop-Harrison (1979) the production of the callose plug on the surface of the papilla follows the arrest of tube growth within a few minutes of its commencement.

The demonstration of gametophytic self-incompatibility by the convenient excised pistil method confirms the results obtained in the previous experiment with the self-pollinated inflorescences. Given the total self-incompatability shown in this experiment as against the low level of apparent self-fertility found with self-pollination in the greenhouse experiment (Table 4) there is need for further comment. The most probable explanation is that the pollen exclusion system was not perfect and that a small quantity of cross pollen gained access to the stigmas. Another possibility is that there was some lessening of the integrity of the gametophytic incompatibility system as the stigmas aged: this would not have been a factor in the excised pistils experiment because all pistils were collected just before anthesis and the study was completed 24 hours later. A final possibility is that given the frequent occurrence of various degrees of apomixis (agamospermy) in the genus *Poa* (Akerberg & Nygren 1959), a low level of facultative apomixis is present in *P. labillardieri*. These are issues for resolution by further experiment, but the simplest explanation, less than perfect exclusion of cross pollen, needs to be ruled out before time is spent on more elaborate studies.

GENERAL DISCUSSION

This experimental investigation of some aspects of the reproductive biology of the widely distributed but poorly understood tussock grass of south-eastern Australia, *Poa labillardieri*, shows it to be an out-breeding, open-pollinated species of high ploidy level maintaining a high degree of heterozygosity by means of a strong gametophytic self-incompatability system. This stands in contrast to the frequently reported pseudogamous apomixis found in many *Poa* species such as *P.pratensis* (Akerberg & Nygren 1959, Connor 1979).

Flowering behaviour in broad terms followed the usual pattern reported for paniculate inflorescences in the Festucoid grasses, but when examined in detail it was found that apart from the acropetal sequence within spikelets the behaviour in both the panicle as a whole and in the individual branches departs widely from an orderly basipetal pattern. Heslop-Harrison (1979) emphasises the generally speedy and precise nature of the sequence of events in the pollination of grasses, but the flowering behaviour of the *P. Labillardieri* inflorescence, particularly the sequence in the branches, seems to be far from precise. This raises interesting questions about the signalling processes involved in panicle co-ordination for further exploration by morphological, histological and molecular approaches.

The findings reported in this paper relate to plants derived from a wild population at one locality. Further work is required with material derived from other populations representative of the extensive geographical range of the species to establish the validity or otherwise of these findings for the species as a whole.

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