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Inflorescence architecture and wind pollination in six grass species

JANNICE FRIEDMAN†‡ and LAWRENCE D. HARDER
Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4

Summary

1. Inflorescence architecture and floral morphology vary extensively within the Poaceae, but the functional significance of this variation remains largely unknown. As grasses are wind-pollinated, their inflorescence diversity probably reflects alternate solutions to manipulating airstreams to enhance pollen export and import. We tested this hypothesis with two field experiments that contrasted pollen removal and receipt by compact and diffuse inflorescences.

2. In the 'aggregation' experiment, we tied together panicle branches of two species with diffuse inflorescences, creating more compact inflorescences. Aggregation reduced pollen removal from both species, probably by increasing boundary-layer thickness. The effects of inflorescence aggregation differed between the two species in a manner that is consistent with pollen-size differences, which could affect the ability of pollen grains to pass through the thickened boundary layer around stigmas.

3. The 'staking' experiment constrained inflorescence motion and revealed that culm characteristics contribute to the interaction between grass inflorescences and airstreams. In particular, inflorescence oscillation principally serves pollen removal for species with compact inflorescences, but is of primary importance in pollen receipt for species with diffuse architectures.

4. These results suggest that inflorescence architecture interacts with wind in a complex manner to facilitate pollination and supports the hypothesis that the extensive diversity of inflorescence architecture within the Poaceae represents evolutionary solutions to the physical constraints of wind pollination.

Key-words: Poaceae, pollen removal, pollen receipt

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Introduction

The grass family (Poaceae), which is one of the largest angiosperm families (10000 species: Watson 1990; Doust & Kellogg 2002), exhibits extensive, but perplexing, diversity in inflorescence architecture. Inflorescences function primarily in reproduction, so that if their architecture is adaptive it probably reflects selection for enhanced pollen export and receipt. Because chasmogamous grasses are almost exclusively wind-pollinated, their inflorescence diversity cannot reflect adaptation to contrasting pollen vectors. Instead, different architectures must be alternate means of manipulating airflow. For example, Niklas' (1987; 1988) studies demonstrated that at low airspeeds (10–50 cm s^{-1}) a compact panicle (*Setaria geniculata*) captured more pollen by sedimentation than by direct inertial collision, as pollen grains aggregated on the leeward side of the inflorescence, where they recirculated. In contrast, a diffuse panicle (*Agrostis hiemalis*) captured pollen only by direct inertial collision. Furthermore, the diffuse panicle swept a large volume of airspace with spikelets out of phase with each other, whereas the compact panicle swept a lesser volume and oscillated with a single motion. These results indicate that compact and diffuse grass panicles create different aerodynamic environments around their florets, and their contrasting harmonic motion determines the mode of pollen capture. Unfortunately, although the architecture of grass inflorescences has long been of interest to taxonomists, few experimental studies have investigated its functional role. The limited exceptions (reviewed by Niklas 1985) neither measured pollen removal and the proportion of successful pollen transfer, nor tested inflorescence function in natural conditions.

The effectiveness of wind pollination depends on interaction of fluid dynamics and biological structures (Ackerman 2000). Of particular importance is the boundary layer surrounding objects, within which airspeed

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†Author to whom correspondence should be addressed. E-mail: friedman@botany.utoronto.ca
‡Present address: Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

increases from 0 m s^{-1} at the object's surface to the ambient speed of unobstructed air at some distance from the object. The boundary layer complicates wind-pollination, because pollen grains in anthers must escape the boundary layer to disperse, and airborne pollen grains must pass through the boundary layer to reach stigmas (Niklas 1992). Many aspects of the design of grass flowers and inflorescences seem to be mechanisms for overcoming these physical problems (Whitehead 1983).

Pollen capture by wind-pollinated species requires that pollen grains entrained in the airstream lose enough momentum that they are not carried past a stigma, and so is determined by the airflow patterns around stigmas and the physical properties of pollen. The necessary airstream modification typically involves all structures that block airflow, which could include the flower plus subtending bracts in species with diffuse inflorescences, or the entire inflorescence in species with more compact inflorescences (Paw U & Hotton 1989).

In contrast to pollen receipt, pollen removal from anthers has not been studied mechanistically and the role of architecture in pollen removal remains to be explored empirically. However, fluid dynamic theory predicts that pollen removal is enhanced by traits that help move initially stationary pollen grains out of the boundary layer and into the airstream (Niklas 1992). Such traits include the long filaments of grass stamens, which project the anthers beyond the floret and elastic responses of inflorescences to wind displacement. Elasticity should be less important for pollen removal from diffuse inflorescences than from compact inflorescences, which have thicker boundary layers. In particular, elastic oscillation of compact inflorescences, should increase relative airspeeds near florets, narrowing the boundary layer and increasing the momentum of pollen in anthers.

In this study, we report the effects of two manipulations of inflorescence characteristics on pollen release and capture in otherwise natural conditions for six grass species with contrasting inflorescence architectures. In one manipulation we tied together branches of the diffuse panicles of two species, creating more compact inflorescences. This manipulation should alter the pattern of harmonic motion of inflorescence branches, decrease the volume of air through which the inflorescence sweeps, and change the aerodynamic environment around the florets. In particular, aggregated panicles should function more as bluff-bodied obstructions to airflow, creating more turbulence and capturing pollen by sedimentation, as well as by inertial collision (see Niklas 1987). Pollen removal should also be disrupted, because the increased density of florets may increase interference of floral parts with anthers, alter the boundary layer conditions around the florets, and reduce airflow around anthers. These effects may be more pronounced for florets in middle and lower positions, where the boundary layer conditions are disrupted more severely due to crowding from the gathered branches.

For the second manipulation, we tied the culms of three species with compact panicles and two species with diffuse panicles to stakes just below the inflorescence to limit their motion in the wind. Staking should particularly affect pollen capture by diffuse panicles, especially by upper florets, as it limits the air volume swept by the inflorescence and the associated opportunities for inertial collision with pollen. In contrast, staking should have little effect on the ability of compact panicles to create extensive downwind turbulence and capture pollen by sedimentation. We also expected that staking would hamper pollen removal less from diffuse inflorescences than from compact inflorescences, which may rely more on culm elasticity to project pollen into the airstream.

Methods

STUDY DESIGN AND SPECIES

We conducted our experiments near the Barrier Lake Field Station of the Kananaskis Field Stations, Alberta (51°02′ N, 115°03′ W), from June to August 2001 and 2002. The species involved are described in Table 1.

In total, seven trials were performed, as outlined below. For each trial, we selected 70 plants from populations of > 500 flowering plants before the onset of flowering (1–2 days prior to first floret opening), taking care to minimize disturbance of the surrounding vegetation.

Table 1. Mean (± SD) characteristics of species involved in the staking and inflorescence aggregation experiments. All species were studied in open meadows, except *Leymus innovatus* which lives in the understory of open forests

Species	Flowering time	Height (cm)	Inflorescence volume (cm^3)	Pollen diameter (μm)	Stigma surface area (mm^3)	Distance to neighbours (cm)	Windspeed during trials (m s^{-1})
Compact species							
Elymus repens	Morning	109 ± 6	4·2 ± 3·5	37·6 ± 1·4	4·2 ± 0·5	20·3 ± 7·7	1·10 ± 0·28
Leymus innovatus	Evening	92·2 ± 13·3	6·0 ± 2·9	34·4 ± 1·3	4·6 ± 0·8	58·1 ± 27·6	0·36 ± 0·11
Phleum pratense	Morning	63·5 ± 9·1	0·3 ± 0·2	22·2 ± 1·8	1·0 ± 0·3	21·5 ± 10·1	1·10 ± 0·45
Diffuse species							
Anthoxanthum nitens	Morning	25·1 ± 3·2	66·0 ± 14·6	22·6 ± 1·9	1·6 ± 0·5	16·2 ± 8·1	1·26 ± 0·14
Bromus inermis	Evening	78·8 ± 11·0	204 ± 61	33·4 ± 2·4	3·6 ± 0·8	25·3 ± 12·7	0·84 ± 0·19
Festuca campestris	Morning	51·7 ± 6·5	79·5 ± 31·3	28·5 ± 1·5	2·6 ± 0·6	35·5 ± 28·6	1·09 ± 0·42

The flowering culm of each plant was labelled with a jeweller's tag that hung at the base of the stem within the surrounding vegetation. The selected plants were assigned randomly to three treatments: 30 manipulated plants, 20 control plants, and 20 unaltered (natural) plants.

During any day of a trial, we measured pollen removal and receipt during only the first 5 h after anthesis, based on reports that anthesis occurs synchronously in grass populations and that anthers are depleted of pollen within a few hours (Gregor 1928; Beddows 1931; Dowding 1987; Huang *et al*. 2004). For all species that we studied, except *Festuca campestris* Rydb., flowering is tightly synchronized, and florets opened within 15–30 min of each other within the population. We measured pollen removal and pollen receipt from six florets on every plant, two each from the bottom, middle and top of the inflorescence. During each day of a trial, anthers and stigmas were collected from at least one floret from each plant with open florets. These organs were stored in separate microcentrifuge tubes containing 1·5 mL of 70% ethanol.

Altered inflorescence morphology

To create more compact inflorescences, we tied together branches of the open panicles of *Bromus inermis* Leyss. and *Anthoxanthum nitens* (Weber) Y. Schouten & Veldkamp. The branches of a single inflorescence on each manipulated plant were gathered to the main stem and tied in three places with 6-lb. test, nylon fishing line. The control plants had nylon line tied to the main stem of a single inflorescence in three places, but the branches of the inflorescence were not gathered up. Plants in the natural treatment were unaltered. To quantify the mass of nylon line added to inflorescences, we set up 30 additional plants (10 manipulated, 10 control and 10 natural) and then removed the inflorescences and weighed them. *Bromus inermis* inflorescences had a mean (± SD) mass of 1·00 ± 0·40 g. For control plants, the nylon line represented 0·94 ± 0·29% of the total mass, which did not differ significantly from the mass of line tied to manipulated plants (1·46 ± 0·99%: $F_{1,18} = 2{\cdot}37$, $P > 0{\cdot}1$). *Anthoxanthum nitens* inflorescences weighed 0·24 ± 0·06 g. Nylon line represented equivalent percentages of the inflorescence masses for control (1·80 ± 0·55%) and manipulated plants (2·32 ± 0·76%: $F_{1,18} = 3{\cdot}10$, $P = 0{\cdot}1$).

Restriction of inflorescence movement

To limit the movement of inflorescences in the wind, we tied the culms of three species with compact panicles (*Elymus repens* (L.) Gould., *Leymus innovatus* (Beal) Pilg. and *Phleum pratense* L.) and two with diffuse panicles (*Bromus inermis* and *Festuca campestris*) to two stakes on opposite sides of individual plants. For each manipulated and control plant, two wooden stakes (smooth doweling, 7 mm diameter) were pushed into the ground 5 cm on either side of the flowering stem. For manipulated plants, we also tied the middle of the inflorescence to the stakes with 6-lb test, nylon fishing line.

MEASUREMENTS

We quantified several environmental characteristics during our experiments. Wind velocity was measured with a TA3 hot-wire anemometer (Airflow Developments Ltd. Buckinghamshire, UK) when anther dehiscence started and once each hour during the remaining 5 h. All measurements were taken at inflorescence height, at a random location within the study site. Inflorescence height and spacing between plants strongly influence pollen capture (Bateman 1947; Levin & Kerster 1974; Griffiths 1950; Whitehead 1983). Therefore, for each plant, we recorded the heights of the top and bottom of the inflorescence, and the plant's distance to its five nearest conspecific neighbours.

We measured pollen removal by comparing the pollen remaining in anthers of experimental plants after 5 h with the mean pollen production of freshly dehisced anthers from at least 20 plants not involved in the experiments. For the aggregated inflorescences, we collected anthers only from exposed, outer florets. In the laboratory, we sonicated microcentrifuge tubes with anthers in an ultrasonic bath to dislodge the pollen, and counted the pollen grains using an Elzone 5380 particle analyser (Micromeritics Inc. Atlanta, GA, USA), which counted particles only within the size range observed for each species. If too few pollen grains were present to use the particle counter (< 4000 grains), we hand-counted the pollen grains using a Wild Heerbrugg inverted microscope at 100× magnification.

To assess the effect of inflorescence manipulation on pollen capture, we counted pollen on stigmas that were collected 5 h after anther dehiscence. For the aggregated inflorescences, we collected stigmas only from exposed, outer florets. In the laboratory, we stained preserved stigmas with 1% basic fuschin, and counted the pollen grains that had been deposited under a compound microscope (20×). Our counts may include heterospecific grass pollen, as pollen from different grass species is difficult to differentiate (Driessen *et al*. 1989; Salih *et al*. 1997). In addition, all species may self-pollinate to some extent, although all species exhibited some protogyny. Stigmas always emerged first from florets, followed within < 30 min by the anthers. Thus, the stigma receptivity and anther dehiscence overlapped within florets and inflorescences for all species.

DATA ANALYSIS

We analysed pollen receipt and pollen removal with repeated-measures, general linear models (Neter *et al*. 1996: mixed procedure of SAS, release 8·2, SAS Institute Inc., 1999). Dependent variables were transformed as necessary to assure normally distributed residuals. Analyses of the inflorescence aggregation experiment considered species, treatment and floret position as categorical independent variables. The analyses for the staking experiment considered the effects of inflorescence architecture (compact or diffuse), species within architecture, treatment, and a floret's position within the inflorescence as categorical factors. All analyses also initially considered a variety of covariates, including plant height, mean distance to neighbours, mean daily wind speed, and any interactions with categorical factors. The analyses of pollen receipt also included the effect of the number of experimental plants in flower as a measure of pollen availability within the population.

All analyses initially assessed the effects of all factors and covariates and their interactions. Terms were excluded from the model by backward elimination ($\alpha = 0{\cdot}05$) if they did not explain a significant proportion of the variation in the dependent variable by themselves and they were not involved in a more complicated, significant interaction. However, the interactions between architecture and treatment, and between species within architectures and treatment, were always retained in the model to reflect the experimental design.

Because we measured six flowers per plant, we used restricted maximum likelihood (Jennrich & Schluchter 1986) to characterize the covariance between responses by individual plants. In all cases, a model of either compound symmetry or heterogeneous compound symmetry was more appropriate than one of independent responses ($P < 0{\cdot}05$). Denominator degrees of freedom for F-tests of the general linear models were calculated by Kenward & Roger's (1997) approximation, which can result in fractional degrees of freedom.

We analysed significant effects with contrasts (Kirk 1995). Analysis of the effect of treatment and its interaction with other terms considered two planned comparisons: one contrasting the natural and control treatments, and the other comparing manipulated plants with the mean of natural and control plants. These two a priori contrasts are orthogonal, and so were each tested with a Type I error rate of 0·05 (Kirk 1995). Because we are interested only in these specific treatment contrasts, we do not present the overall test of treatment effects, as it includes comparisons that are not of interest (e.g. control vs. manipulated plants). We interpret significant interactions with a posteriori Dunn–Šidák contrasts (Kirk 1995). All plotted values are ln-transformed and adjusted to account for the other components in the model.

Table 2. Summary of general linear models of the influences on pollen remaining in anthers and pollen receipt during the inflorescence aggregation experiment. Temperature, relative humidity, mean distance to neighbours, the number of plants in flower, and plant height were considered initially, but then excluded from the analyses because they did not affect the dependent variable significantly

Source of variation	Pollen remaining in anthers	Pollen receipt by stigmas
Species	$F_{1,204} = 63{\cdot}5$***	$F_{1,217} = 874{\cdot}8$***
Treatment		
Natural vs. Control	$F_{1,776} = 0{\cdot}32$	$F_{1,138} = 0{\cdot}26$
N + C vs. Manipulated	$F_{1,773} = 9{\cdot}3$**	$F_{1,67} = 8{\cdot}94$**
Treatment × Species	$F_{2,203} = 0{\cdot}4$	$F_{2,108} = 19{\cdot}8$***
Mean wind speed	$F_{1,693} = 10{\cdot}1$**	$F_{1,563} = 12{\cdot}2$***
Mean wind speed × Treatment	$F_{2,693} = 4{\cdot}0$*	
Flower type (Species)	$F_{1,721} = 32{\cdot}15$***	
Position		$F_{2,455} = 7{\cdot}0$***

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

Results

AGGREGATED INFLORESCENCES

Pollen production and removal

The two species involved in the inflorescence aggregation experiments produce different amounts of pollen. *Anthoxanthum nitens* produces both staminate flowers with three anthers and perfect flowers with two anthers. Staminate flowers produce significantly more pollen per anther than perfect flowers ($t_{25} = 3{\cdot}26$, $P < 0{\cdot}005$), so that staminate flowers produce about twice as much pollen per flower. *Bromus inermis* flowers produce significantly more pollen than both perfect and staminate flowers of *A. nitens* (*B. inermis* vs. *A. nitens* perfect: $t_{68} = 13{\cdot}26$, $P < 0{\cdot}001$; *B. inermis* vs. *A. nitens* staminate: $t_{68} = 10{\cdot}40$, $P < 0{\cdot}001$).

Different amounts of pollen remained in the anthers of unmanipulated *B. inermis* and *A. nitens* plants (Table 1). Despite greater pollen production by *B. inermis*, much less pollen remained in its anthers than in those of *A. nitens*. More pollen remained in the anthers of staminate flowers of *A. nitens* than in those of perfect flowers, but the proportions of pollen remaining did not differ between flower types.

Inflorescence aggregation affected pollen removal consistently for both species (Table 2). Control and natural plants had equivalent numbers of pollen grains remaining in anthers, indicating that the added mass of thread used to tie inflorescences did not affect pollen removal. In contrast, inflorescences that had been aggregated had significantly more pollen remaining in anthers (mean ± SE = 446 ± 42 grains) than control and natural plants (362 ± 41 grains).

Fewer pollen grains remained in anthers of both species as mean wind speed increased (Table 2). This effect involved primarily plants with aggregated inflorescences ($b \pm$ SE: $-1{\cdot}13 \pm 0{\cdot}27$, $t_{704} = 4{\cdot}23$; $P < 0{\cdot}001$), as pollen removal did not vary significantly with mean wind speed for unmanipulated plants (control plants: $-0{\cdot}33 \pm 0{\cdot}28$, $t_{677} = 1{\cdot}16$, $P > 0{\cdot}5$; natural plants: $-0{\cdot}08 \pm 0{\cdot}28$, $t_{699} = 0{\cdot}28$, $P > 0{\cdot}5$). Thus low wind speeds seem least favorable for pollen removal in manipulated plants.

Pollen receipt

Pollen receipt by *B. inermis* and *A. nitens* responded differently to inflorescence aggregation (Table 2, Fig. 1a). Stigmas on plants in control and natural treatments captured equivalent amounts of pollen for both species ($P > 0{\cdot}5$ in both cases). For *B. inermis*, stigmas on aggregated plants captured significantly more pollen than stigmas on unmanipulated plants ($t_{124} = 6{\cdot}93$, $P < 0{\cdot}001$; Fig. 1a). In contrast, stigmas on aggregated plants of *A. nitens* captured significantly less pollen than those on unmanipulated plants ($t_{28{\cdot}4} = 2{\cdot}28$, $P < 0{\cdot}05$; Fig. 1a).

Pollen capture varied significantly with both a floret's position within the inflorescence (Table 2, Fig. 1b) and mean wind speed (Table 2). Lower and middle florets received significantly less pollen than upper florets (middle vs. lower: $t_{377} = 1{\cdot}07$, $P > 0{\cdot}5$; upper vs. middle: $t_{660} = 2{\cdot}45$, $P < 0{\cdot}05$; upper vs. lower: $t_{421} = 3{\cdot}70$, $P < 0{\cdot}001$). For both species, pollen capture varied positively with mean daily wind speed.

STAKED INFLORESCENCES

Pollen production and removal

Overall, the three species with compact panicles produced significantly less pollen per flower than the two species with diffuse panicles ($F_{1,107} = 14{\cdot}70$, $P < 0{\cdot}001$; Table 1). This difference resulted primarily from the low pollen production by *Phleum pratense*. All five species have three anthers per flower.

Five hours after flowers opened, less than 10% of pollen remained in anthers. The amount of pollen remaining varied significantly among inflorescence architectures and among species within architectures (Table 3). Overall, more pollen remained in anthers of diffuse panicles than of compact panicles, even after accounting for differences in production. Essentially all pollen had been removed from the two species with compact inflorescences from open habitats (*Elymus repens* and *P. pratense*), whereas between 1% and 10% of pollen remained in the compact species from a wooded site (*Leymus innovatus*) and the two diffuse species (both from open habitats). Species within each inflorescence type differed significantly in the amount of pollen remaining, except *E. repens* and *P. pratense*.

Inflorescence staking had mixed effects on pollen removal (Table 3, Fig. 2a). The quantity of pollen remaining in anthers did not differ significantly between control and natural plants (Table 3), indicating that the presence of the stakes did not affect pollen removal. Overall, equivalent amounts of pollen were removed from staked plants and unmanipulated plants (treatment contrast, Table 3). However, this result obscures differential responses by species with compact vs. diffuse inflorescences (treatment × architecture contrast, Table 3), as staking significantly reduced pollen removal compared to unmanipulated plants for compact species, but not for diffuse species (Fig. 2a). These differences were consistent features of compact and diffuse inflorescences, as equivalent amounts of pollen remained in anthers of different species with the same architecture (Table 3).

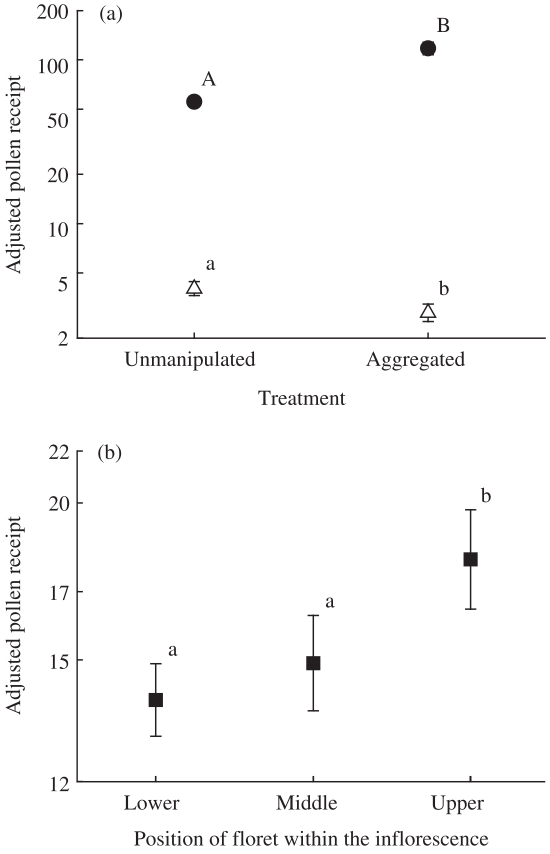


Fig. 1. Influences of (a) treatment and (b) floret position on the mean (± SE) number of pollen grains received by stigmas for *B. inermis* (●) and *A. nitens* (△) during the inflorescence aggregation experiment. Letters indicate the outcomes of Dunn–Šidák multiple comparisons. For panel (a) capital letters depict *B. inermis,* whereas lower-case letters represent *A. nitens*. For panel (b) the two species are combined because the effects of species and position did not interact significantly. See Table 2 for statistical details.

Table 3. Summary of general linear models of the influences on pollen remaining in anthers and pollen receipt during the staking experiment. Temperature, relative humidity, mean distance to neighbours, and plant height were considered initially, but then excluded from the analyses because they did not affect the dependent variable significantly

Source of variation	Pollen remaining in anthers	Pollen receipt by stigmas
Architecture	$F_{1,826} = 351{\cdot}9$***	$F_{1,1310} = 8{\cdot}7$**
Species (Architecture)	$F_{3,608} = 47{\cdot}8$***	$F_{3,528} = 50{\cdot}1$***
Treatment		
Natural vs. Control	$F_{1,313} = 0{\cdot}22$	$F_{1,311} = 0{\cdot}04$
N + C vs. Manipulated	$F_{1,305} = 3{\cdot}73$	$F_{1,306} = 20{\cdot}98$***
Treatment × Architecture		
N + C vs. M Compact	$F_{1,294} = 3{\cdot}88$*	$F_{1,292} = 2{\cdot}99$
N + C vs. M Diffuse	$F_{1,315} = 0{\cdot}64$	$F_{1,313} = 21{\cdot}43$***
Treatment × Species (Architecture)	$F_{6,306} = 0{\cdot}9$	$F_{6,291} = 0{\cdot}5$
Mean wind speed	$F_{1,\,1787} = 14{\cdot}7$***	$F_{1,1544} = 0{\cdot}01$
Mean wind speed × Architecture	$F_{1,\,1791} = 15{\cdot}3$***	
Mean wind speed × Species (Architecture)	$F_{3,\,1766} = 19{\cdot}7$***	$F_{4,1676} = 4{\cdot}6$**
Position	$F_{2,1683} = 0{\cdot}7$	$F_{2,1664} = 0{\cdot}8$
Position × Architecture	$F_{2,1683} = 4{\cdot}8$**	$F_{2,1664} = 5{\cdot}0$**
Count		$F_{1,1674} = 8{\cdot}3$**
Count × Architecture		$F_{1,1318} = 16{\cdot}26$***
Count^2		$F_{1,1692} = 6{\cdot}8$**

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

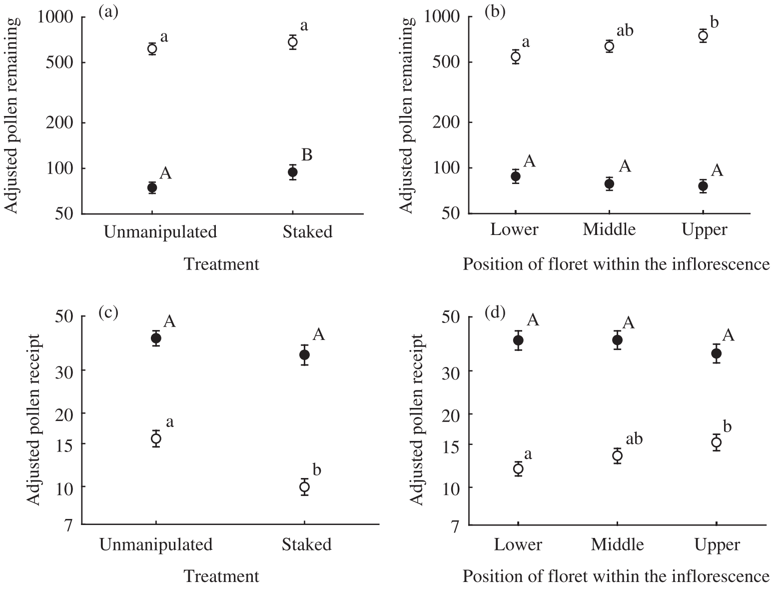


Fig. 2. Influences of treatment (a and c) and floret position (b and d) during the staking experiment on mean (± SE) pollen remaining in anthers (a and b) and mean (± SE) pollen receipt by stigmas (c and d) on compact panicles (●) and diffuse panicles (○). Letters indicate the outcomes of Dunn–Šidák multiple comparisons for each architecture, with capital letters for compact panicles and lower-case letters for diffuse panicles. See Table 3 for statistical details.

A floret's position within the inflorescence affected pollen removal for diffuse inflorescences, but not for compact inflorescences (position × architecture, Table 3; Fig. 2b). Within diffuse panicles, significantly more pollen remained in anthers of upper florets than in lower florets (upper vs. lower: $t_{1727} = 2{\cdot}74$, $P < 0{\cdot}05$), but middle florets did not differ significantly from these extremes ($P > 0{\cdot}5$ in both cases).

Daily mean wind speed affected the amount of pollen remaining in anthers differently for the two architectures and for species within each architecture (Table 3). The pollen remaining in anthers varied significantly with mean wind speed for only two species; decreasing for *L. innovatus* (partial regression coefficient, $b \pm \mathrm{SE} = -0{\cdot}082 \pm 0{\cdot}222$, $t_{1843} = 4{\cdot}87$, $P < 0{\cdot}001$), and increasing for *B. inermis* ($b \pm \mathrm{SE} = 2{\cdot}928 \pm 0{\cdot}559$, $t_{1792} = 5{\cdot}27$, $P < 0{\cdot}001$). The absence of an effect of mean wind speed for other species should be interpreted cautiously. In particular, removal of essentially all pollen from the anthers of *E. repens* and *P. pratense* during the experiment limited our ability to detect the effect of wind speed for these species.

Pollen receipt

Pollen capture by stigmas varied significantly among inflorescence architectures and among species within architectures (Table 3). In general, stigmas on compact panicles captured more pollen than stigmas on diffuse panicles (Fig. 2c). Within each inflorescence type, all species differed significantly in the amount of pollen captured, except *E. repens* and *L. innovatus*.

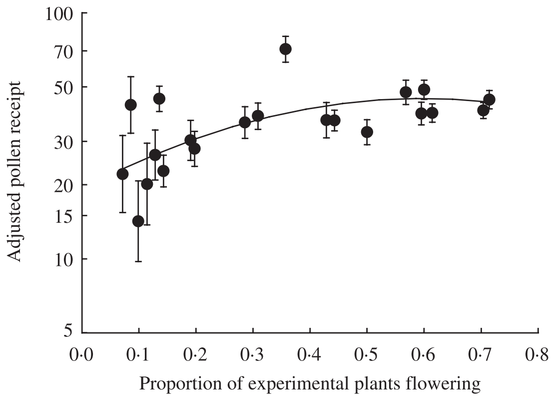
Staking strongly affected pollen receipt, as stigmas on manipulated plants captured significantly less pollen than control or manipulated plants, which did not differ from each other (Table 3, Fig. 2c). This effect was strongest for the two species with diffuse panicles, for which staking significantly reduced pollen receipt, whereas staking did not significantly affect pollen receipt for compact species (treatment × architecture interaction, Table 3; Fig. 2c). These differences were consistent features of compact and diffuse inflorescences, as pollen capture did not differ significantly between species within architectures (Table 3).

Pollen capture varied with floret position within the inflorescence for species with diffuse panicles, but not for those with compact inflorescences (position × architecture, Table 3; Fig. 2d). Stigmas at upper positions captured significantly more pollen than stigmas at lower positions ($t_{1785} = 2{\cdot}78$, $P < 0{\cdot}05$), but middle florets did not differ from upper or lower florets ($P > 0{\cdot}5$ in both cases). To understand this interaction, we investigated the treatment × position × architecture interaction, because this effect was almost statistically significant ($F_{4,531} = 2{\cdot}29$, $P = 0{\cdot}06$). Pollen capture did not differ significantly for any combinations of treatment and position for species with compact architectures. In addition, control and natural plants with diffuse inflorescences had equivalent pollen capture at all positions. However, manipulated plants with diffuse inflorescences differed significantly from control and natural plants at upper and mid positions, but not at lower positions (C + N vs. M, diffuse, upper: $t_{928} = -3{\cdot}63$, $P < 0{\cdot}005$; C + N vs. M, diffuse, mid: $t_{810} = -3{\cdot}44$, $P < 0{\cdot}01$; C + N vs. M, diffuse, lower: $t_{144} = -2{\cdot}31$, $P = 0{\cdot}25$; Fig. 2d).

Daily mean wind speed affected pollen capture differently for the species within each architecture (Table 3). Although none of the partial regression coefficients for the effect of wind speed differed significantly from zero, they differed significantly between species pairs within each architecture. Of the diffuse species, pollen capture varied negatively with mean wind speed for *B. inermis*, but positively for *F. campestris*, generating a significant difference between the partial regression coefficients ($t_{1355} = 2{\cdot}91$, $P < 0{\cdot}05$; partial regression coefficient for *B. inermis*, $b \pm \mathrm{SE} = -0{\cdot}932 \pm 0{\cdot}377$, $t_{1368} = -2{\cdot}47$, $P > 0{\cdot}05$; partial regression coefficient for *F. campestris*, $b \pm \mathrm{SE} = -0{\cdot}029 \pm 0{\cdot}235$, $t_{1590} = -0{\cdot}13$, $P > 0{\cdot}9$). For the compact species, the interaction resulted from a negative association for *L. innovatus* and a positive association for *E. repens* ($t_{1749} = 2{\cdot}89$, $P < 0{\cdot}05$; partial regression coefficient for *L. innovatus*, $b \pm \mathrm{SE} = -0{\cdot}810 \pm 0{\cdot}341$, $t_{1290} = -2{\cdot}38$, $P > 0{\cdot}05$; partial regression coefficient for *E. repens*, $b \pm \mathrm{SE} = 0{\cdot}402 \pm 0{\cdot}301$, $t_{1676} = 1{\cdot}34$, $P > 0{\cdot}5$).

Pollen capture generally varied positively and nonlinearly with one measure of pollen availability, the proportion of experimental plants in flower (Count effects, Table 3; Fig. 3), but it was not affected by a plant's mean distance to its five nearest neighbours. Pollen capture increased more strongly as the proportion of

(a) compact panicles



(b) diffuse panicles

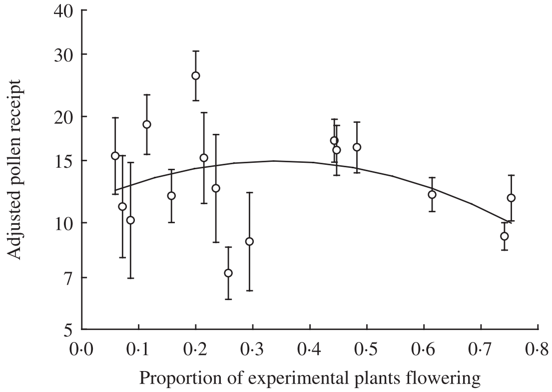


Fig. 3. The relation of the number of pollen grains captured by stigmas (C) of grass species with (a) compact and (b) diffuse panicles to the proportion of conspecific plants in flower (F) during the staking experiment. The fitted lines depict quadratic regressions based on a repeated-measures analysis (compact, $\hat{C} = 2{\cdot}95 + 2{\cdot}85F - 2{\cdot}39F^2$; diffuse, $\hat{C} = 2{\cdot}43 + 1{\cdot}63F - 2{\cdot}39F^2$). See Table 3 for details of overall analysis.

plants in flower increased from 0·05 to 0·3 for species with compact inflorescences than for those with diffuse inflorescences (count × architecture interaction: partial regression coefficients; compact species 2·85 ± 0·77, diffuse species 1·63 ± 0·81). However, the common significant quadratic effect (count^2) indicates that the increase in pollen capture slowed with further increases in the proportion of plants flowering for both architecture types. Pollen receipt varied more among sampling dates when few experimental plants flowered than when many flowered (Fig. 3).

Discussion

CONTRASTING PERFORMANCE OF DIFFUSE AND COMPACT INFLORESCENCES

Based on his observations of pollen receipt by two grass species in a wind-tunnel, Niklas (1987, 1988) concluded that compact and diffuse panicles create contrasting aerodynamic environments, which allow different mechanisms of pollen capture. These aerodynamic effects probably also govern pollen removal from anthers, although this has not been examined previously. Our experiments considered features of this hypothesis under natural conditions. We contrasted compact and diffuse architectures explicitly in our aggregation experiment, which converted the diffuse inflorescences of two species into more compact architectures. Our staking experiment further examined whether reduced culm mobility affects the pollination of species with compact and diffuse inflorescences differently, as is expected if culm characteristics contribute to the interaction between grass inflorescences and airstreams. The effects of both manipulations are largely consistent with Niklas' hypothesis, but they also reveal some new perspectives on the role of inflorescence architecture in wind pollination.

By bringing originally horizontal inflorescence branches into more vertical positions and reducing the spacing between flowers, inflorescence aggregation probably affected the interaction of anthers and stigmas with the boundary layer in at least two ways. Most obviously, aggregation would create a single, large boundary layer around the entire inflorescence, in contrast to the thinner boundary layer that typically surrounds the isolated individual spikelets of a diffuse inflorescence. In addition, aggregation reoriented flowers, altering the projection of anthers and stigmas into the boundary layer. In particular, the anthers of species with diffuse inflorescences generally hang freely below the isolated florets on lax filaments, so that they project well into the boundary layer. In aggregated inflorescences, the lax filaments resulted in anthers hanging alongside the repositioned florets. This orientation contrasts sharply with that of the stamens of species with compact inflorescences, such as *Phleum pratense*, which have more rigid filaments that hold the anthers horizontally, projecting them as far as possible into the boundary layer of the vertical inflorescence (pers. obs.).

Such influences on boundary-layer thickness would affect both pollen removal from anthers and pollen receipt by stigmas. Aggregation probably diminished pollen removal because the thicker boundary layer around aggregated inflorescences reduced the frequency of airflow velocities around anthers that exceeded the stationary inertia of pollen grains. This conclusion is consistent with the interacting effect of mean wind speed on the treatment groups (Table 2), whereby low wind speeds were least favorable for pollen removal in manipulated plants. Increased boundary-layer thickness caused by inflorescence aggregation should also reduce the opportunity for pollen grains carried in the airstream to be deposited on stigmas by direct inertial collision. However, this effect on pollen capture should be less severe for species with large pollen, as large grains travelling in an airstream have greater momentum and are therefore less likely to remain entrained in laminar airflows around aggregated inflorescences. This influence of pollen size may explain why inflorescence aggregation reduced pollen receipt by *Anthoxanthum nitens*, but not by *Bromus inermis* (Fig. 1a), as *B. inermis* pollen is 48% larger in diameter and 118% larger in volume (and presumably mass) than that of *A. nitens* (Table 1). Together, these results imply that boundary-layer effects may significantly influence the evolution of inflorescence architecture.

The greater proximity of spikelets caused by inflorescence aggregation could have also increased interference between pollen removal and receipt within and between flowers, perhaps increasing self-pollination. With six florets per spikelet, *Bromus inermis* may be more susceptible to such effects than *Anthoxanthum nitens*, which has three small florets per spikelet, of which some are perfect and others are staminate. A higher incidence of self-pollination by aggregated inflorescences of *B. inermis* may explain why inflorescence aggregation increased pollen capture by this species (Fig. 1a).

Unlike the aggregation experiment, the staking experiment reduced the harmonic motion of inflorescences, rather than affecting the ability of the inflorescence to deflect streamlines. The results of the staking experiment suggest that diffuse and compact inflorescences employ oscillatory motion in contrasting ways under natural conditions, primarily serving male success of compact inflorescences vs. female success of diffuse inflorescences (Table 3 and Fig. 2). Staking reduced pollen removal in compact species, which may rely on harmonic motion to reduce the large boundary layer created by their dense inflorescences. In contrast, staking did not affect pollen removal from diffuse species, probably because only the central rhachis of the inflorescence was tied to the stakes, so that the elongate inflorescence branches of diffuse inflorescences could still move in the wind. Such secondary mobility may allow adequate wind velocity for pollen grains to be removed from the anthers of diffuse species.

Staking reduced pollen receipt by species with diffuse architectures, but not by compact species. Flowers in diffuse inflorescences capture pollen primarily by inertial collision, and pollen capture is enhanced by the inflorescence sweeping through and sampling much of the surrounding airstream (Niklas 1987). Staking eliminated inflorescence oscillation, so that staked plants with diffuse panicles could sample much less air, reducing the opportunities for inertial collision with pollen. In contrast, staking probably did not affect pollen capture by compact species because the panicles of all three compact species are large enough (Table 1) to create downstream vortices at the wind speeds that they experienced (Vogel 1983). Given such leeward turbulence, staking would not alter the opportunity for pollen grains to recirculate within this region, and settle onto stigmas.

In addition to the specific responses to inflorescence aggregation and staking, our experiments revealed that pollination varied consistently among floret positions within diffuse inflorescences, but not within compact inflorescences. During both experiments, upper florets of diffuse inflorescences captured more pollen than lower florets (see Figs 1b and 2d), whereas during the staking experiment less pollen was removed from upper florets than from lower florets, with no significant difference between treatments (Fig. 2b). In general, pollen removal and receipt require different aerodynamic conditions, with high relative airspeeds promoting pollen removal, but reducing pollen receipt (Niklas 1985). That pollen removal and receipt vary inversely with each other as a function of floret position, implies that the aerodynamic environment experienced by florets varies significantly within diffuse inflorescences. In diffuse species, upper florets may capture more pollen because they sweep through a larger volume of air during oscillation. This explanation is supported by the significant interaction between unmanipulated and staked diffuse plants at upper and mid positions, but not at lower positions. This result exposes the need for more detailed analysis of the effects of inflorescence architecture on aerodynamic conditions within inflorescences.

The inverse relation of pollen removal and receipt to floret position suggests heterogeneity in the sex roles of florets within diffuse inflorescences. Although upper florets captured more pollen than lower florets, their female success probably would not differ, because all florets received at least 13 pollen grains, on average. As each floret contains only one ovule, this intensity of pollen receipt is probably sufficient to assure fertilization. In contrast, the reduced pollen receipt by upper flowers may indicate poorer male function than that realized by lower florets. Such variation could select for contrasting patterns of allocation to female vs. male function within grass inflorescences.

The results of both our aggregation and staking experiments confirm Niklas's (1987, 1988) hypothesis that diffuse and compact grass inflorescences rely on contrasting aerodynamic mechanisms to effect pollination. This conclusion is further supported by comparison of the proportion of pollen removed from anthers that reaches stigmas (pollen-transfer efficiency). Based on plants in our natural treatments, the three compact species realized higher pollen-transfer efficiency than the three diffuse species (Fig. 4). This result may illustrate that a compact inflorescence allows for more efficient cross-pollination. Alternatively, compact species may capture more self-pollen than diffuse species, due to the inflorescence being thrust into the region of leeward turbulence where pollen grains recirculate (Niklas 1987). Unfortunately, we could not distinguish self-pollen from outcross-pollen, so the implications of inflorescence architecture for mating patterns remains unclear. This finding appeals for studies investigating the mating systems of grasses with different architectures.

EFFECT OF POLLINATION ENVIRONMENT

In addition to the intrinsic effects of inflorescence architecture, wind pollination depends on prevailing environmental conditions. During our experiments,

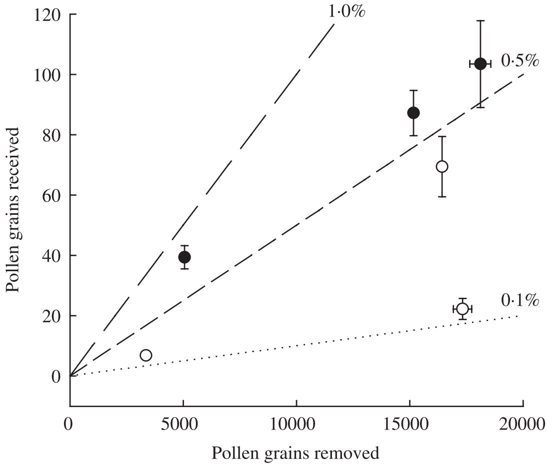


Fig. 4. Relation of the mean (± 95% CI) number of pollen grains captured by stigmas to mean (± 95% CI) pollen removal from anthers for unmanipulated plants of three grass species with compact panicles (●) and three species with diffuse panicles (○). Lines indicate specified percentages of removed pollen that reached stigmas. See Table 1 for species identifications.

pollination varied with the daily number of experimental plants in flower and daily mean wind speed (Tables 2 and 3), but not with a plant's mean distance to its five nearest conspecific plants. Thus, reliance on wind as a pollen vector exposes grasses to significant environmental variation in reproductive success.

The influence of the proportion of plants flowering on pollen receipt during the staking experiment (Fig. 3) implies that pollen import varies with pollen density in airstreams. When few plants flowered, pollen receipt varied extensively, both among plants and between days. This variation and the lack of influence of the proximity of a plant's five nearest neighbours suggest that pollen receipt depends on wind directionality when a floret was in anthesis and the specific location of neighbouring flowering plants when airstreams carry relatively little pollen. Plants downwind of flowering individuals probably experience relatively high pollen capture compared to plants with no upwind neighbours in flower. Pollen capture increases overall and becomes less variable as the proportion of plants releasing pollen in the population increases. The proportional benefit of increased flowering density was greater for compact species than for diffuse species, perhaps because diffuse species sweep a greater air volume (Niklas 1987). The quadratic relations of pollen receipt to flowering density (Table 3, Fig. 3) indicate that the benefit of greater pollen availability saturates for unknown reasons, particularly for species with compact architectures. Consequently, plants that flower during peak flowering in a population may realize relatively lower male success than those that flower when a smaller proportion of plants contribute to the pollen pool in airstreams.

Daily mean wind speed had complex effects on pollen removal and receipt, especially during the staking experiment, when the effect of wind speed differed among species within inflorescence types. Pollen removal and receipt probably respond differently to daily differences in wind speed. Pollen removal should increase asymptotically with increased wind speed, because of an elevated probability that pollen grains will experience airflows sufficient to overcome their inertia (Gregory 1973; Niklas 1992). The results of both experiments are consistent with this expectation, except for *Bromus inermis* during the staking experiment, for which pollen removal decreased with increasing wind speed. This result seems anomalous, as the same species did not exhibit this unexpected pattern during the aggregation experiment. In contrast to pollen removal, pollen receipt is probably maximized at some intermediate wind speed at which the benefit of increased velocity for enhancing the number of pollen grains moving past stigmas is counteracted by a reduced chance of a pollen grain leaving the airstream and impacting a stigma (Gregory 1973). The specific velocity that optimizes pollen receipt probably differs among species, depending on their floret and inflorescence characteristics and the density of airborne pollen. Consequently, the diverse relations of pollen removal to daily mean wind speed that we observed may have resulted because the range of wind speeds fell largely below the optimum for some species and above the optimum for other species.

Our results support the hypothesis that the extensive diversity of inflorescence architecture within the Poaceae represents alternate evolutionary solutions to the advantages and physical constraints of wind pollination. However, the simple dichotomy between compact and diffuse inflorescences, which was the focus of our study, represents only a fraction of the inflorescence diversity seen within the grass family. Clearly, many other aspects of this diversity await functional explanation.

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