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Inflorescence Architecture in Grasses (Poaceae): Morphological Associations and
Pollination Consequences

by

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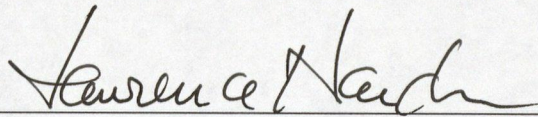
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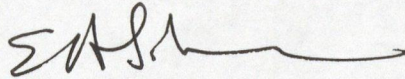
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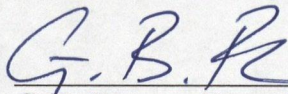
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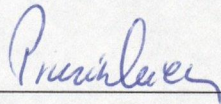
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ABSTRACT

Inflorescence architecture and floral morphology vary extensively within the Poaceae, but the biological and functional significance of this variation remains largely unknown. Given the potential for an intimate relation between form and function in wind-pollinated plants, I consider the association between inflorescence architecture and other aspects of reproductive morphology. A survey of grass species in Kananaskis, Alberta, found that species cluster into four distinct flower-size, inflorescence-architecture categories, and that strong correlations exist between various traits. In addition, manipulative studies that constrained the motion of inflorescences, revealed that compact and diffuse inflorescences employ different mechanisms to facilitate pollen removal and pollen receipt. Furthermore, aggregation of the inflorescence in two diffuse species reduced pollen removal, and had contrasting consequences on pollen capture between the two species. Overall, pollen-removal failure and pollen-transfer efficiency for six wind-pollinated species fell within the range observed for animal-pollinated plants.

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1 INTRODUCTION

1.1 Wind pollination

As a large quantity of pollen is wasted by anemophilous plants, it is surprising that so many vigorous species of this kind abounding with individuals should still exist in any part of the world; for if they had been rendered entomophilous, their pollen would have been transported by the aid of the senses and appetites of insects with incomparably greater safety than by the wind...It seems at first sight a still more surprising fact that plants, after having been once rendered entomophilous, should ever have again become anemophilous.

Darwin, 1876, p. 407

Evolutionary biologists have struggled with the evolution of wind pollination (anemophily) from insect-pollinated (entomophilous) ancestors. Wind pollination is typically viewed as a random and inefficient process, at the mercy of uncertain atmospheric conditions, and thus requiring large numbers of pollen grains to ensure reproductive success (Proctor et al. 1996). However, wind pollination is a derived condition in the angiosperms and has arisen independently in several families (Whitehead 1968). A recent survey estimated that 10% of angiosperm species rely on wind pollination, with 65 independent derivations from biotic to wind pollination (Linder 1998). The repeated evolution of wind pollination from animal pollination suggests that wind pollination is a more effective means of outcrossing in some situations.

Wind pollination is thought to evolve in response to shifts in the abiotic environment that render biotic pollination less advantageous (Faegri & van der Pijl 1979; Regal 1982; Cox 1991). Anemophily tends to be associated with particular ecological conditions. Regions of higher latitude, dry temperate environments, and island floras have the highest proportions of wind-pollinated plants. Wind pollination is more common in areas that are less diverse floristically, and in open vegetation. The physical requirements for successful wind pollination may explain these ecological and geographical correlates of anemophily (Whitehead 1968; Regal 1982). Pollen dispersal

will be most successful when intervening vegetation and raindrop capture do not deplete airborne pollen. Furthermore, pollen concentration in the air-stream attenuates rapidly with distance, thus small inter-plant distances favour successful pollen export (Bateman 1947; Faegri & van der Pijl 1979). However, high plant density may also reduce the distance of pollen dispersal by modifying the turbulence of the surrounding air (Okubo & Levin 1989; Dyer & Sork 2001).

Specific morphological features characterize many wind-pollinated species. Some of these characters appear to be prerequisites for the evolution of wind pollination, whereas others evolve as a consequence of anemophily (Linder 1998). In general, wind-pollinated plants have small flowers, with reduced or absent perianth parts. Nectaries are usually absent or non-functional. The anthers are often exserted from the flower on long filaments, and they produce copious, dry pollen grains. The stigma is usually expanded and feathery, and the ovule typically produces few ovules, often only one. The inflorescence is often presented above or outside the vegetative structures of the plant, or flowering occurs before leaves develop. (Faegri & van der Pijl 1979). The presence of these morphological similarities in many unrelated wind-pollinated species may reflect the aerodynamic requirements for successful pollination (Whitehead 1968). I now review relevant aspects of the aerodynamics of wind-pollination during pollen liberation, transport and capture, and consider the importance of aerodynamics to the characteristics of wind-pollinated plants.

1.1.1 Pollen liberation

Pollen dispersal begins as pollen leaves the anther. The removal of pollen grains from the anther is complicated by the boundary layer of still air that forms as fluid flows over an object. The thickness of the boundary layer depends on the local Reynolds number. The Reynolds number is a dimensionless value that reflects the relationship

between the speed of a fluid (U), its density (ρ) and viscosity (μ), and the size of an intercepting object (l):

$$Re = \frac{\rho l U}{\mu}. \quad (1.1)$$

Local boundary layer thickness, δ_{bl} , depends on the local Reynolds number, and the characteristic length, or distance x from the leading edge.

$$\delta_{bl} \approx \frac{5x}{\sqrt{Re_x}} \quad (1.2)$$

(Vogel 1983; Denny 1993). At the interface between solid and fluid the speed of flow approaches zero, and asymptotically approaches the free-stream velocity with increasing distance from the solid. Thus, anthers experience higher wind speeds when they are suspended beyond the boundary layer of still air created by other floral organs. Not surprisingly, the anthers of wind-pollinated species are typically extended on long filaments enabling them to flutter in the slightest breeze.

For a spherical pollen grain to be liberated from a surface, the moment tending to hold the grain to the surface (M_1) must be less than the moment due to the force of airflow (M_2), or

$$M_1 = \frac{4}{3}\pi r^3 \rho g (\sin \theta) r < M_2 = 6\pi \mu r U (\cos \theta) r, \quad (1.3)$$

where $4/3\pi r^3$ is the volume of the pollen grain (cm^3), ρ is its density (g/cm^3), U is the wind velocity (cm/s), μ is the dynamic viscosity of air (0.00018 g/cm/s at air temperature 18°C), g is the gravitational acceleration constant (981 cm/s^2), and θ is the angle between the sphere's radius perpendicular to the surface and its point of contact with the surface (Gregory 1973; Niklas 1985). Therefore, the threshold wind velocity required to liberate a pollen grain is,

$$U_t = \frac{2r^2 \rho g \tan \theta}{9\mu}. \quad (1.4)$$

These equations reveal that four factors should influence pollen liberation: pollen size, pollen density, pollen shape (which influences θ) and airflow velocity.

Morphological features influence the likelihood of pollen entering airflow patterns. Pollen grains become liberated more easily occurs at high relative airspeed (eq. 1.3, large U), if the grains are small (small r), have low density (small ρ) and rest on a flat surface (small θ) (Whitehead 1983). Interestingly, the pollen of wind-pollinated species is not significantly smaller than that of animal-pollinated species, although there is less variance among species (Wodehouse 1935). The reason for this deviation from expectation will become apparent below.

The anthers of grasses (Poaceae) and sedges (Cyperaceae) produce pollen in an unusual manner, which may elevate the threshold airspeed for pollen liberation. At every stage during development, all prepollen cells and pollen grains remain in physical contact with the tapetum, so that a single layer of pollen is arranged in the anther locule. This pattern of production contrasts directly with the majority of angiosperms, in which developing pollen floats freely in the locular fluid and many interior grains never touch the tapetum. The pollen/tapetum arrangement of the Poaceae and the Cyperaceae has been studied from a systematic perspective; however its functional significance has never been tested (Kirpes et al 1996). This configuration necessarily restricts the number of pollen grains per locule. A monolayer of pollen may be advantageous for the release of pollen grains into the wind. Such a configuration may limit removal to wind speeds exceeding a threshold value, which favour longer dispersal distances (Harder 2000). Additionally, this arrangement may prevent grains from dehydrating before they leave the anther.

1.1.2 Pollen transport

Pollen dispersal distance depends largely on two interacting variables – the terminal velocity of the pollen grain and wind velocity (Niklas 1992). When a sphere falls through a column of fluid, it accelerates until the drag force acting on it precisely equals the object's net body force, then acceleration ceases and a constant terminal velocity (V_f) is achieved. This velocity is derived by balancing the drag (D) for the particle and the net body force of the particle. Drag is approximated by Stokes' Law for spheres at low Reynolds numbers,

$$D = 6\pi\mu rU,$$

and net body force equals the difference between the particles' weight and its buoyancy,

$$B = \frac{4}{3}\pi r^3 g(\rho - \sigma).$$

Therefore, the terminal velocity equals

$$V_f = \frac{2r^2 g(\rho - \sigma)}{9\mu}, \quad (1.5)$$

where, r is the radius of pollen, ρ is the density of pollen (1g/cm^3), σ is the density of air (0.00127g/cm^3), g is the gravitational acceleration constant (981cm/s^2), and μ is the dynamic viscosity of air (0.00018 g/cm/s at air temperature 18°C).

The terminal velocity of smooth spheres with diameters between 1 and $100\text{ }\mu\text{m}$ is satisfactorily predicted using Stokes' Law, and comparisons between observed and predicted terminal velocities are fairly reliable (Gregory 1973). Equation 1.3 reveals that larger spheres have a higher terminal velocity than small spheres of equivalent density. For any given mass, larger spheres have a lower terminal velocity. For wind-dispersed pollen a smaller V_f is beneficial, because grains take longer to settle out of the air column and thus will travel further horizontally. However, a tradeoff exists, because grains have

a preferred site of arrival (a stigma), and very small dispersules have so little inertia that they rarely collide with surfaces.

The simplest mechanistic model of transport distance (x) for a fluid-borne particle is the ballistic formulation:

$$x = \frac{HU}{V_f}, \quad (1.6)$$

which multiplies the mean horizontal wind velocity (U) by the time taken for a particle to fall with terminal velocity V_f from release height H (Greene & Johnson 1989; Okubo & Levin 1989). In this model, the concentration of particles emitted by a point source has a normal distribution in both the vertical and crosswind axes, and the variances of the vertical concentration increase with time. The long axis of the plume is tilted and has an angle of $\tan^{-1}(V_f/\mu)$ where μ is the mean horizontal wind speed averaged spatially and temporally (Pasquill & Smith 1983). Sutton's formula summarizes the relation between distance and dispersion, by defining the standard deviation of a particulate cloud. The formula predicts that the airborne pollen concentration will decrease at a rate inversely proportional to the square of the distance traveled (Sutton 1932). Okubo & Levin (1989) showed that the mode of the dispersion distribution for settling particles is inversely proportional to λ (a dimensionless quantity measuring the relative importance of turbulence and gravitation) and to the settling velocity of seeds, and directly proportional to the height of release and wind velocity. For light dispersules, $\lambda \gg 1$ and turbulent mixing becomes more important.

Several considerations are necessary if these models are to be applied to pollen transport in grasses. First, pollen can be released only when wind velocity exceeds a threshold value. Unfortunately, very little is known about the wind velocities necessary to dislodge pollen. The literature suggests that anther exertion is related to humidity, temperature and light conditions (Emecz 1962; Matsui et al. 1999; but see Liem 1980),

but the mechanisms involved in pollen removal from the anther (as opposed to anther exertion) have not been tested experimentally. Furthermore, for many grass species, female structures are located at similar heights to male structures. Therefore, the vertical dispersal distance is essentially zero, and the exact location of conspecific plants becomes important (Tufto et al. 1997). Finally, the pollen needs to be viable when it contacts the stigma. Grass pollen has a relatively short lifespan (less than one hour to one day: Dafni & Firmage 2000), and is the shortest lived of all Angiosperm pollen (Clayton & Renvoize 1986).

1.1.3 Pollen capture

The effectiveness of particle capture is described in terms of collection efficiency, or the ratio of the number of pollen grains captured to the number of grains that would pass through the airspace if the filtering structure (e.g., stigma) were not present (Vogel 1983a),

$$E = 2A_f \left(\frac{r_p}{r_e} \right)^2, \quad (1.7)$$

where A_f describes streamlines (directly proportional to wind velocity), r_p is the pollen grain radius, and r_e is the radius of the filtering element. The feathery stigmas of many anemophilous species have higher collection efficiencies than would solid stigmas, because they have smaller radii, and because they produce proportionally thinner boundary layers (Niklas 1985).

Equation 1.5 is based on the assumption that all particles are neutrally buoyant and follow streamlines. However, pollen grains have inertial momentum and will diverge from streamlines diverted around an object. Whether this inertial effect is sufficient to result in a collision will depend on the density of the grain, the thickness of the boundary

layer around the object and the flow velocity. Increased wind speeds produce both smaller boundary layers and greater momentum, and thus increase collection efficiency.

For anemophilous plants, the size of an obstruction to airflow and the settling velocity of pollen can be expected to vary in a manner that optimizes pollen capture. Collection efficiency is accepted to be a non-linear function of settling velocity (Gregory 1973; Paw U 1984), and the relationship between E and V_f is usually expressed as a function of the Stokes number,

$$S = \frac{V_{set} U}{Dg} \quad (1.8)$$

where D is the diameter of the receptor and U is mean wind speed (Paw U & Hotton 1989). The relationships between S and E are derived from experiments (Gregory 1973) or numerical integration (Paw U 1984). Paw U & Hotton (1989) considered a log-linear relationship, a logistic form, and a polynomial form, and through partial differentiation of the various relationships between Stokes number and collection efficiency, they determined that the lower and upper bounds for optimal pollen deposition resulted when Stokes number equaled 1 and $e^1=2.718$, respectively. Using Paw U and Hotton's (1989) model, the optimal interactor size for maximum deposition is

$$D = \frac{2(\rho - \sigma)r^2 U}{9S_o \mu}, \quad (1.9)$$

where $S_o = 1$ and 2.718 .

The reproductive morphology of wind-pollinated species seems to create a unique aerodynamic environment in the immediate vicinity of reproductive organs. Using wind-tunnel experiments, Niklas (1985) demonstrated that the morphology of ovulate organs influences the airflow disturbance through which pollen travels. Because the geometry of female organs is species-specific, airflow disturbance may be species-specific. Thus, pollen morphology and ovulate organs may act synergistically to generate aerodynamic

environments that increase a species' efficiency of pollen capture (Niklas & Buchmann 1987). Indeed, Linder & Midgley (1996) found considerable pollen discrimination by anemophilous species in their field study of four sympatric species.

The geometry of the inflorescence and its capability and range of harmonic motion also affect pollen capture (Niklas 1987). The volume of airspace through which an inflorescence moves positively affects its probability of intercepting airborne pollen grains. The oscillatory motion of inflorescences will be affected by the drag created by the inflorescence. The drag force per unit length can be described by:

$$D = \frac{1}{2} C_D \rho S_x U^2, \quad (1.10)$$

where C_D is the dimensionless drag coefficient (a function of the Reynolds number and accounts for the peculiarities in the behaviour of drag), ρ is the air density, S_x is the size of the plant intercepting the airflow at position x , and U is the mean wind velocity (Vogel 1983a; Finnigan & Mulhearn 1978). Thus, higher wind speeds, and wider obstructions, create more drag. However, the form of a flexible object, such as a diffuse grass inflorescence, becomes a function of wind speed, and the reconfiguration of the inflorescence shape can substantially reduce drag (Vogel 1983a). Experimental studies of flow-structure interactions that have a mechanical and theoretical interpretation are in their infancy, and were explored only recently for fibre-bending in a two-dimensional field (Alben et al. 2002; Steinberg 2002).

Niklas (1987) showed that a diffuse grass panicle sweeps through a large volume of airspace and creates little turbulence, favouring the capture of pollen by upstream inertial collision. By contrast, a compact panicle oscillates with a higher frequency and less displacement, but creates extensive leeward turbulence favouring capture by downstream sedimentation. Different inflorescence architectures thus affect the

harmonic motion, which contributes to the manner in which airborne pollen is likely to be captured.

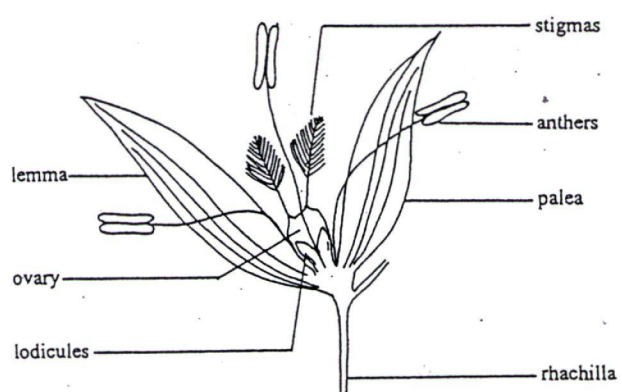
1.2 Inflorescence architecture in the grass family (Poaceae)

The grass family (Poaceae), which is almost entirely wind pollinated, is one of the largest families of the angiosperms with respect to numbers of genera (600 - 700) and species (10000: Watson 1990; Doust & Kellogg 2002). Given this species richness, corresponding diversity in the morphology of inflorescences and spikelets is not surprising (Clayton 1990). However, single genera (e.g., *Muhlenbergia*, *Eragrostis*) also exhibit extensive variation in reproductive morphology.

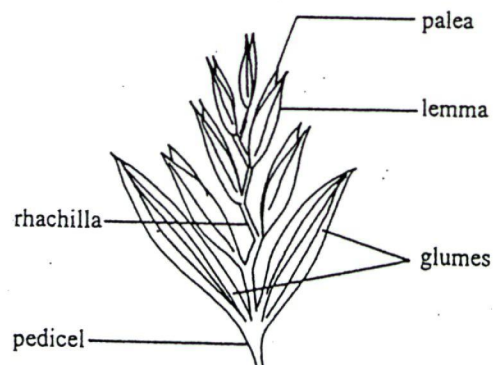
The grass flower, or floret, typically comprises three stamens, a uniovulate pistil bearing two feathery stigmas, and two tiny lodicules enclosed in two bracts – the lemma on the outer side and the palea on the inner side (Fig.1a). During flowering the lodicules swell, spreading the lemma away from the rest of the floret and exposing the sexual organs (Heslop-Harrison & Heslop-Harrison 1996). Florets are borne alternately along a rachilla, at the base of which are two empty bracts called glumes. Together the floret, rachilla and glumes form the spikelet (Fig.1b). Spikelets are borne in an inflorescence whose branches lack subtending leaves or bracts (Connor 1979). An enormous wealth of variation exists in inflorescence types, ranging from a many-branched, diffuse panicle to a dense spike (Fig. 1c, 1d: Clifford 1986). These differences can be characteristic of specific tribes, but analysis of the phylogenetic distribution of inflorescence architecture suggests that different designs have arisen repeatedly and independently (Stebbins 1982; Kellogg 2000b). Indeed, early descriptions of the grass family relied on inflorescence typology as diagnostic characters, and typically inferred relationships quite different from current conceptions of Poaceae phylogeny (Pohl 1986). Such convergent evolution of architectures implies adaptation to a similar environment, and suggests that functional

Figure 1.1. Morphology of a) a typical grass floret and b) a typical grass spikelet, and examples of the range of inflorescence architecture, as illustrated by c) a highly compact panicle and d) a diffuse panicle.

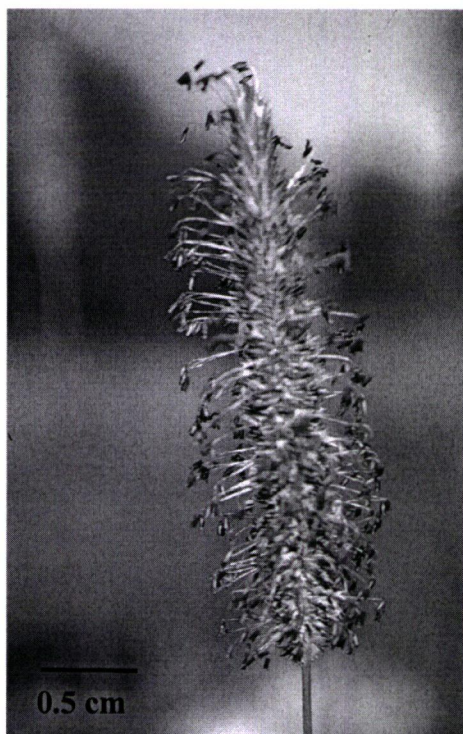
a)



b)



c)



d)



aspects of inflorescence architecture are subject to natural selection. However the functional significance of this convergence remains to be explained (Clayton 1990).

The diversity of inflorescence architecture, coupled with extensive homoplasy in the distribution of inflorescence types in the grass phylogeny, has obscured the developmental mechanisms by which such diversity has arisen (Doust & Kellogg 2002). The most primitive inflorescence is proposed to have only primary and secondary branches (Stebbins 1982). Transitions between different architectures may involve few developmental changes (Stebbins 1973; Doebley & Lukens 1998; Kellogg 2000a), and developmental changes can be combined in a variety of ways leading to diverse morphologies (Doust & Kellogg 2002). For example, a raceme can be produced from the original panicle by simplification of the primary branches followed by a reduction of branches. If the basal internode is reduced, the raceme can be transformed into a spike (Vegetti & Anton 1995). Kellogg (2000a) described a series of developmental patterns relying on binary genetic “switches” that can produce many inflorescence forms with few genetic changes.

Although inflorescence architecture has long been of interest to taxonomists, there has been a paucity of studies investigating the functional role of architecture (although see summary in Niklas 1985). The inflorescence functions primarily in reproduction, thus the vast diversity in structure and design of inflorescences likely reflects selection for increased pollen release, capture and outcrossing success in response to contrasting reproductive environments. Furthermore, the similarity of inflorescence architectures in unrelated species likely represents evolutionary convergence, suggesting the influence of similar reproductive environments. The aerodynamics of wind pollination generates an intimate relationship between form and function in anemophilous plants. Surprisingly, the adaptive significance of this relationship has never been explored.

1.3 Objectives

In this thesis I address two main questions:

- 1) Do associations exist between inflorescence architecture, floret and stem characteristics and can these be related to the aerodynamics of pollination?
- 2) What is the functional significance of specific features of the grass inflorescence for anemophily?

In Chapter 2, I discuss the relationship between the reproductive morphology of several grass species growing in Kananaskis, Alberta, by quantifying associations between floret, stem and inflorescence characteristics. In Chapter 3, I quantify the pollen-transfer efficiency for six wind-pollinated grass species with contrasting inflorescence architectures, and I assess the functional significance of two features of inflorescence architecture through manipulative studies. These studies were designed to assess the impacts of changes to inflorescence mobility (through manipulations to limit the motion of the inflorescence in the wind) and inflorescence architecture (through manipulations to convert a diffuse panicle to a more compact panicle). I assess the impact of these manipulations on pollination by comparing components of male (pollen removal) and female (pollen receipt) function in flowers that have and have not been manipulated.

In Chapter 4, I summarize and synthesize the results and conclusions from Chapters 2 and 3. This chapter provides an overview of the complexity of the relation between inflorescence architecture and the wind in the success of wind pollination, and provides some insight on future directions that will help elucidate the details of this relation.

2. ASSOCIATIONS BETWEEN INFLORESCENCE ARCHITECTURE AND OTHER ASPECTS OF REPRODUCTIVE MORPHOLOGY

2.1. Introduction

The aerodynamics of wind pollination selects for an intimate relation between form and function in anemophilous plants. Niklas (1985; 1987; 1988) has shown that the efficiency of pollen capture varies with the fluid-flow patterns created by the receptive structure and the physical properties of pollen, and he proposed that inflorescence architecture and pollen morphology are co-adapted to enhance pollination efficiency. Thus, morphological differences among anemophilous plants, and within the grass family in particular, presumably resulted from natural selection, and may reveal alternate mechanisms for modifying aerodynamics to facilitate pollination.

Grasses display a remarkable diversity in the architecture of their inflorescences and structure of their spikelets. Differences in panicle geometry and its capability and range of harmonic motion affect the aerodynamics of pollen capture (Niklas 1987). Based on wind-tunnel experiments of two grass species, Niklas (1987) identified a dichotomy between compact and diffuse panicles, based on the different airflow patterns that they created. The size of an obstruction to airflow affects the aerodynamic environment surrounding it. Larger objects have proportionally thicker boundary layers and therefore deflect more streamlines, requiring larger pollen with more inertia to pass through and collide. A compact panicle acts as a bluff-bodied obstruction to airflow and creates leeward eddies. In contrast, a diffuse panicle undergoes a series of complex harmonic movements whereby individual spikelets move somewhat independently and sample a large volume of airspace by direct inertial collision (Niklas 1987). Thus, the size and morphology of the obstruction to airflow affect aerodynamic and pollen capture characteristics. Although no studies have investigated the aerodynamics of pollen removal from anthers, it is likely that the patterns of streamline deflections created by the

inflorescence also affect the ambient wind velocity necessary to dislodge pollen and its subsequent trajectory.

The distinction between compact and diffuse panicles represents the ends of a continuum of reproductive morphologies within the Poaceae (Clayton 1990). Grass inflorescences range from a spike-like panicle with highly contracted branches (e.g., *Phleum pratense*), to a single raceme with branches on one, or both sides of the rachis (e.g., *Elymus repens*), to a narrow (e.g., *Phalaris arundinaceae*) or broad panicle (e.g., *Deschampsia cespitosa*; Clayton & Renvoize 1986). Furthermore, the spikelets comprising the inflorescence can vary in size and morphology. For example, the number of flowers per spikelet ranges from one (e.g. *Stipa* and *Agrostis*) to at least 40 in *Eragrostis oxylepsis* (Clifford 1986; Hitchcock 1950), with corresponding diversity in size. Thus the simple distinction between compact and diffuse panicles that Niklas (1987) identified based on the aerodynamics of pollen capture by two species may be incomplete, and may obscure other relevant morphological features.

In addition to inflorescence architecture, the efficiency of wind-pollination may depend on the position of inflorescences and the characteristics of their supporting stems. Average horizontal wind speed increases logarithmically with height above ground level, due to the presence of the boundary layer of still air near objects. The rate of increase in wind speed through the boundary layer depends on surface roughness, which is influenced by the spacing among plants and average plant height (Pasquill & Smith 1983; Vogel 1983a). Therefore, taller plants generally experience higher wind speeds than shorter grasses in the same habitat. Bickel & Freeman (1993) suggested that spherical plants do not benefit from added height, due to their low ratio of surface area to volume and the associated thicker boundary layer, which limits pollen dispersal. Given this influence of plant form, it is not surprising that grasses generally display their flowers above the boundary layer of their foliage and the apical location of the inflorescence

maximizes both the height of the structures involved in pollen removal and capture (and seed dispersal), and the airspace through which the inflorescence sweeps. Indeed, the rate at which paternal fitness saturates (and thus the allocation of reproductive resources to male reproduction) may be determined by plant height (Burd & Allen 1988), as pollen released from greater heights remains entrained in the airstream longer and disperses farther (Okubo & Levin 1989).

The apical location of grass inflorescences imposes structural demands on grass stems. Zebrowski (1999) noted that the apical location of the inflorescence is sub-optimal with regard to the mechanical stability of the stem, and high-yielding grasses may suffer from stem breakage. Not surprisingly, grasses have an impressive capacity to cope with strong winds without damage, especially considering the relatively heavy apical load of the inflorescence. One of the most striking features of grasses moving in wind is the so-called “Honami” phenomenon – the regular ocean-like waves which appear in grass canopies on windy days (Finnigan & Mulhearn 1978). During the passage of waves, individual stalks oscillate at the same frequency as when they are isolated, and resonate at a well-defined natural frequency, even when forced into motion by turbulent airflow (Finnigan & Mulhearn 1978). Four features influence the oscillatory motion of panicles: the length of the subtending stem, the drag created by the panicles, the mass of the inflorescence and the flexural rigidity of the stem (Niklas 1987). Plants with different panicle arrangements (which create different drag, see eq. 1.10) may have stems that differ in their flexural rigidity. Furthermore, the flexural rigidity of the stem influences the range of wind speeds that can be tolerated before stem breakage (Baker 1995; Dunn & Dabney 1996).

The size of an obstruction to airflow and the settling velocity of pollen can be expected to vary in a manner that optimizes pollen capture. Paw U and Hotton (1989) presented mathematical models describing an optimal relation between the sizes of pollen

grains and “female receptors”, which maximize pollen receipt (see eq. 1.8 and 1.9).

Although Paw U and Hotton referred to “female receptors” as the structure that interacts with the airstream to influence pollen impact with stigmas, stigmas can represent a small fraction of their “receptor”. To avoid confusion, I will refer to this structure as the “interactor”. The interactor is either the inflorescence (for compact architectures) or the flower (including subtending bracts, for diffuse architectures). Paw U and Hotton ignored the details of interactor morphology to focus on the possibility of pollen even reaching the structure.

The feather-like structure of grass stigmas has been proposed as an adaptation that increases the efficiency of pollen capture (Faegri & van der Pijl 1979; Whitehead 1983). A larger surface area enhances total particle capture (Niklas 1985). However, an increase in stigma area thickens the boundary layer, which decreases the probability of pollen capture, especially at low Reynolds numbers (i.e. low wind velocity). Furthermore, the collection efficiency of an object is related to the radius of the filtering element (see eq. 1.7). In grasses, the feather-like structure of the stigma maximizes surface area, while minimizing diameter, and thus ameliorates the boundary-layer problem and increases its collection efficiency (Crane 1986).

The importance of the morphology of the receptive structure, its movement in wind, and pollen morphology for abiotic pollination implies that traits that function together to influence pollination should be highly correlated in wind-pollinated plants. Although differences in inflorescence architecture can be characteristic of certain tribes of grasses, analysis of the phylogenetic distribution of inflorescence architecture suggests that different designs have arisen repeatedly and independently (Stebbins 1982; Kellogg 2000a). The repeated evolution of similar architectures may reflect common selective environments, and suggests that functional aspects of inflorescence architecture are subject to natural selection. In particular, selection on the functional correlation of traits

can occur when certain trait combinations confer higher fitness than other combinations, given adequate genetic variance (Conner & Sterling 1995). Because the conditions for anemophily require interaction and coordination between various floral, inflorescence and stem characteristics, strong correlations among these traits could reveal associations that have evolved to optimize pollination. However, the presence of correlated traits does not necessarily mean that the correlation is selectively advantageous, as trait associations can result from developmental allometry (Gould 1966; Pigliucci et al. 1996).

In this chapter, I quantify associations between floret, stem and inflorescence characteristics for 25 grass species. I explore correlations that may be related to the aerodynamics of wind pollination, and specifically consider whether different architectures possess unique mechanisms to aid pollination. I also determine whether species that occupy dissimilar habitats, with different aerodynamic environments, exhibit consistently different morphological characteristics.

2.2. Methods

2.2.1. Study site and data collection

I studied the grass species in three habitat types in Kananaskis, Alberta (51° 02'N, 115° 03'W), which differed in their expected average wind speeds: understory of a mixed aspen forest, open meadow, and exposed windward hill. Two sites of each habitat were sampled. During the 2001 flowering season, the six sites were sampled on a rotational basis during a 45-day period, with each habitat sampled at least 30 times. Twice daily during the flowering period, I recorded the air velocity for 10 min using a hot-wire Airflow anemometer, at three heights above ground level (15 cm, 30 cm, and 45 cm).

At each site, I characterized the grass flora by recording the grass species in 10 1m x 1m plots, separated by 1 m along two 20-m transects. The origins and directions of the two transects were selected randomly. Within each quadrat, I recorded the percent

cover and number of flowering culms for each species. I measured the elevation of the bottom and top of the inflorescence for 10 individuals per species. I then cut the flowering culms at ground level and placed them in vials with water. The plants were transported immediately to the laboratory (no more than 15 min) where they were measured.

2.2.2. Analysis of morphological characteristics

2.2.2.1. Inflorescence architecture

Inflorescence architecture was measured using several techniques. The volume of inflorescences was determined using a magnetic Polhemus 3Space digitizer. The digitizer creates a $1.8 \times 1.8 \times 1.8$ m magnetic field, and measures the x , y , z coordinates at the tip of a clicker-pen. For each plant, I recorded the positions of nodes, lengths of branches and the positions of the tips of the terminal florets on branches. The data were read directly by a laptop computer. Qhull software (The National Science and Technology Research Center for Computation and Visualization of Geometric Structures, version 2.6), which implements the Quickhull algorithm (Barber et al. 1996), was used to compute the convex hull containing the set of points measured for an inflorescence. For each individual, the volume of the convex hull was computed using the same software. The length of the inflorescence was calculated from the co-ordinates of the most basal node producing flowers to the tip of the most distal flower using the Pythagorean theorem. The total number of florets on the inflorescence was counted and divided by the inflorescence volume to calculate inflorescence density (Weller et al. 1998). The width of the inflorescence was measured on dried specimens collected from the field sites, except for three species, which were measured from herbarium specimens.

2.2.2.2. Culm properties

In addition to the drag created by the panicle and its mass, the oscillatory motion of grass inflorescences depends on two characteristics of the supporting stem; its length and flexural rigidity (Niklas 1987). Flexural rigidity equals the product of the modulus of elasticity (E) and the second moment of area of the stem (I). I calculated culm elasticity based on measurements of load and displacement taken with a simple tensometer (for details see Dunn & Dabney 1996) for the internode closest to the inflorescence. Leaf blades and the inflorescence were removed, and the length of the stem was measured (b). Previous work has demonstrated that the nodes of grass species are at least as rigid and strong as the internodes (Dunn & Dabney 1996). The dimensions of the stem were measured by cutting a small cross section of the stem using a razor blade, and measuring its dimensions at 10X with a dissecting microscope. Because hollow stems are usually non-circular, both the largest and smallest dimensions were measured and averaged separately. Second moments of area for bending parallel to the major axis (I_{maj}) and the minor axis (I_{min}) were calculated as follows:

$$I_{maj} = \frac{\pi}{64} (d_1^3 d_2 - d_a^3 d_b) \quad (2.1)$$

$$I_{min} = \frac{\pi}{64} (d_2^3 d_1 - d_b^3 d_a), \quad (2.2)$$

where d_1 and d_2 are the average outside diameters of the major and minor axes, respectively, and d_a and d_b are the average inside diameters of major and minor axes (Niklas 1992).

Modulus of elasticity (E) was determined by loading horizontal grass stems. Each stem was placed in a protective plastic sleeve in a vise that acted as the cantilever attachment point. The initial position of the stem was recorded. A small load (1.1g; P) was attached at the end of the internode segment. Deflection of the stem (Δ) was read

from a grid attached to the board behind the stem. Modulus of elasticity was calculated according to

$$E = \frac{Pb^3}{3I\Delta} \quad (2.3)$$

(Finnigan & Mulhearn 1978; Dunn & Dabney 1996).

2.2.2.3. Floret characteristics

To quantify pollen size and pollen production, I collected freshly dehiscent anthers from 10 individuals per species, for all species in each habitat. Anthers were stored in micro-centrifuge tubes with 1.5 mL of 70% ethanol. I sonicated the tubes with anthers in an ultrasonic bath to dislodge the pollen, and measured the size and number of pollen grains using an Elzone 5380 particle analyzer (Micromeritics). If too few pollen grains were present to use the particle analyzer (<4000 grains), I hand-counted the pollen grains using an inverted microscope at 100X magnification. I measured pollen grain diameter of 20 randomly selected grains using an ocular micrometer.

I measured the characteristics of stigmas collected from 10 individuals per species, which were stored individually in micro-centrifuge tubes with 1.5 mL of 70% ethanol. Each stigma was placed on a microscope slide, stained with 1% basic fuchsin solution and photographed with a digital camera (Nikon Coolpix 4500) at 100X magnitude under a dissecting microscope. For each photograph I recorded the focal distance for later calculation of an image's magnification using a calibration curve derived from photographs of a micrometer scale taken at different focal lengths. Adobe Photoshop 6.0 was used to increase the contrast between background and object. I then used Scion Image software to measure stigma length and area, and the length of a stigmatic branch. If the stigma had overlapping branches, I estimated the amount of overlap and recalculated the area.

The length and width of florets and spikelets were measured for plants with digital calipers. Measurements were taken from dried specimens collected from the field sites, except for three species, which were measured from herbarium specimens.

2.2.3. Data Analysis

I classified each species' inflorescence type according to criteria of Hitchcock (1950) and Moss (1983) (see Table 2.1). Compact inflorescences lack branches and are spike-like, whereas both narrow and diffuse inflorescences bear their spikelets on branches. The branches of narrow inflorescences are appressed to the inflorescence axis, resulting in a high density of florets. Diffuse inflorescences have spreading branches and a corresponding low density of florets.

Variation in characters and species was analysed using both univariate and multivariate techniques. All variables were log transformed due to non-normality.

The correlation matrix of the variables is large and intractable, so I used two approaches to summarize correlations among traits. I used a variable clustering procedure (varclus procedure with centroid option, SAS, release 8.2, SAS Institute Inc., 1999) to explore the relationships among variables. Varclus is a type of oblique component analysis related to multiple-group factor analysis (Harman 1976). This procedure divides a set of numeric variables into clusters, to maximize the sum across clusters of the variance of the original variables that is explained by the centroid components. I also used canonical correlation (cancorr procedure of SAS, release 8.2, SAS Institute Inc., 1999) to determine whether inflorescence-level characteristics, as a group, correlate with floret-level characteristics, as a group. Canonical correlation provides a statistical analysis of the linear relationship between two sets of variables (Tabachnick & Fidell 1989).

Table 2.1. Grass species found in three habitats in Kananaskis, Alberta during the summers of 2001 and 2002. Nomenclature and tribal classifications are according to Soreng et al. (2003) and Watson & Dallwitz (2003). Panicle architecture is assigned according to criteria established by Hitchcock (1950) and Moss (1983).

Species	Synonym	Tribe	Subtribe	Native	Habitat	Architecture
<i>Achnatherum lemmonii</i> (Vasey) Barkworth	<i>Stipa columbiana</i> Macoun	Stipeae	Stipinae	yes	Hill, Meadow	Narrow
<i>Agropyron cristatum</i> (L.) Gaertn.		Triticeae	Hordeinae	no (Asia)	Hill	Compact
<i>Agrostis scabra</i> Willd.		Poeae	Agrostidinae	yes	Meadow	Diffuse
<i>Agrostis stolonifera</i> L.		Poeae	Agrostidinae	no (Eur)	Meadow	Diffuse
<i>Anthoxanthum nitens</i> (Weber) Y. Schouten & Veldkamp	<i>Hierochloe odorata</i> (L.) P. Beauv.	Poeae	Phalaridinae	yes	Meadow	Diffuse
<i>Avenula hookeri</i> (Scribn.) Holub	<i>Helictotrichon hookeri</i> (Scribn.) Henrard	Poeae		yes	Meadow	Narrow
<i>Bromus carinatus</i> Hook. & Arn.		Bromeae		yes	Hill, Meadow	Diffuse
<i>Bromus inermis</i> Leyss.		Bromeae		no (Eur)	Hill, Meadow	Diffuse
<i>Calamagrostis rubescens</i> Buckley		Poeae	Agrostidinae	yes	Forest	Compact

Species	Synonym	Tribe	Subtribe	Native	Habitat	Architecture
<i>Deschampsia cespitosa</i> (L.) P. Beauv.		Poeae	Agrostidinae	no (Eur)	Forest	Diffuse
<i>Elymus lanceolatus</i> (Scribn. & J.G. Sm.) Gould subsp. <i>lanceolatus</i>	<i>Agropyron dasystachyum</i> (Hook.) Scribn. var. <i>dasystachyum</i>	Triticeae	Hordeinae	yes	Meadow	Compact
<i>Elymus repens</i> (L.) Gould	<i>Agropyron repens</i> (L.) P. Beauv.	Triticeae	Hordeinae	no (Eur)	Meadow	Compact
<i>Elymus trachycaulus</i> (Link) Gould ex Shinnars subsp. <i>trachycaulus</i>	<i>Agropyron trachycaulum</i> (Link) Malte ex H.F. Lewis var. <i>trachycaulum</i>	Triticeae	Hordeinae	yes	Hill	Compact
<i>Elymus trachycaulus</i> subsp. <i>glaucus</i> (Pease & A.H. Moore) Cody	<i>Agropyron trachycaulum</i> var. <i>glaucum</i> (Pease & A.H. Moore) Malte	Triticeae	Hordeinae	yes	Hill	Compact
<i>Festuca campestris</i> Rydb.		Poeae	Loliinae	yes	Meadow	Diffuse
<i>Festuca rubra</i> L.		Poeae	Loliinae	no (Eur)	Hill, Meadow	Diffuse
<i>Koeleria cristata</i> Pers.		Poeae	Aveninae	yes	Meadow	Compact
<i>Leymus innovatus</i> (Beal) Pilg.	<i>Elymus innovatus</i> Beal.	Triticeae	Hordeinae	yes	Forest	Compact
<i>Muhlenbergia richardsonis</i> (Trin.) Rydb.		Cynodonteae	Muhlenbergiinae	yes	Meadow	Compact
<i>Phalaris arundinacea</i> L.		Poeae	Phalaridinae	no (Eur)	Hill, Meadow	Narrow

Species	Synonym	Tribe	Subtribe	Native	Habitat	Architecture
<i>Phleum pratense</i> L.		Poeae	Alopecurinae	no (Eur)	Hill, Meadow	Compact
<i>Poa pratensis</i> L.		Poeae	Poinae	no (Eur)	Hill, Meadow	Diffuse
<i>Poa secunda</i> J. Presl subsp. <i>secunda</i>	<i>Poa sandbergii</i> Vasey	Poeae	Poinae	yes	Hill	Narrow
<i>Poa secunda</i> subsp. <i>juncifolia</i> (Scribn.) Soreng	<i>Poa juncifolia</i> Scribn.	Poeae	Poinae	yes	Meadow	Narrow
<i>Schedonorus pratensis</i> (Huds.) P. Beauv.	<i>Festuca pratensis</i> Huds.	Poeae	Loliinae	no (Eur)	Meadow	Narrow

I identified groups of species with similar reproductive morphology with cluster analysis (cluster procedure of SAS, release 8.2, SAS Institute Inc., 1999). This analysis considered 18 floral, inflorescence and stem characters for 20 taxa for which all characters had been measured. The data were range-standardized and the mean Euclidean distance was used as the distance measurement (Sneath & Sokal 1973). Clusters were identified by Ward's minimum-variance method (Ward 1963), which combines subgroups (initially consisting of one species) at each iteration so as to minimize the within-cluster ANOVA sum of squares. The number of significant clusters in the data was determined using the pseudo F and pseudo t^2 statistics (Milligan & Cooper 1985). The contribution of each character to the formation of the clusters was determined using stepwise discriminant function analysis (Tabachnick & Fidell 1989). To illustrate the ability of the discriminant functions to separate groups, I re-classified each species using a discriminant function computed from the observations for all other species (discrim procedure of SAS, release 8.2, SAS Institute Inc., 1999). Chi-square tests of independence were used to assess whether clusters associate with either architecture or habitat type.

As described below (section 2.3.3), the cluster analysis largely grouped species according to inflorescence type (compact or diffuse) and flower size (small or large). I therefore used analysis of variance (glm procedure of SAS, release 8.2, SAS Institute Inc., 1999) to identify how the 18 variables were associated with these categories. I also used multivariate-analysis of variance (Tabachnick & Fidell 1989: glm procedure of SAS, release 8.2, SAS Institute Inc., 1999) to test for overall morphological differences between inflorescence and flower categories.

I assessed whether my observations for 21 grass species conformed to Paw U & Hotton's (1989) predictions for the optimum pollen and interactor size (see eq. 1.8 and 1.9). Paw U and Hotton (1989) calculated the optimum pollen-interactor sizes for 28

species (of which only one is a grass), for wind speeds between 2 and 5m/s (the average range they expected plants to encounter in nature). According to Paw U and Hotton's criteria, the width of the spikelet represents the aerodynamic unit for diffuse and narrow architectures, whereas the width of the entire inflorescence represents the interactor for compact panicles. I calculated settling velocity according to Stokes' law (see eq. 1.5). I also calculated the optimum interactor diameter for each species, given the measured range of wind speeds it experienced and its pollen size.

I used discriminant function analysis (Tabachnick & Fidell 1989) to determine which morphological traits associate significantly with the habitat type in which a species is found. If species occupied more than one habitat, I assigned them to the habitat in which they were more abundant.

2.3. Results

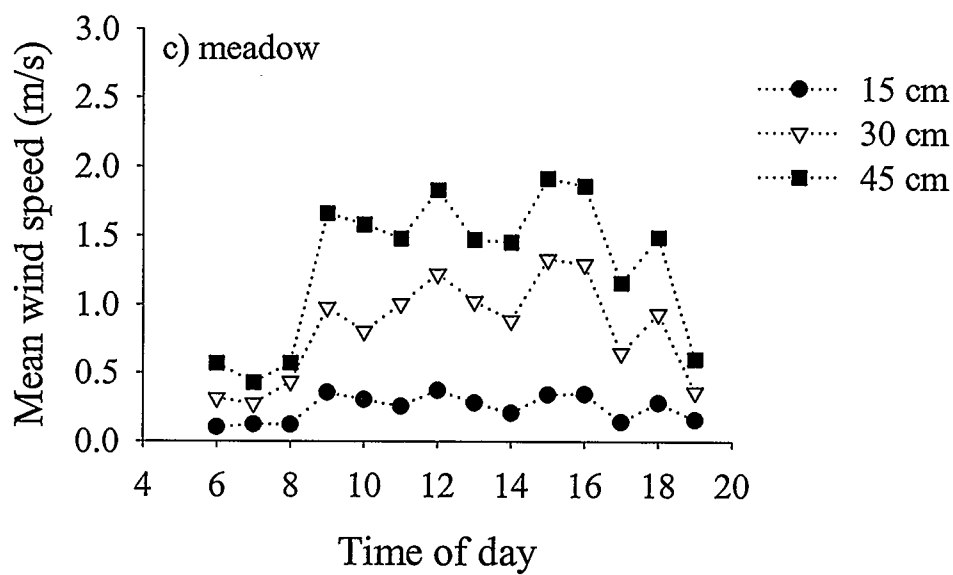
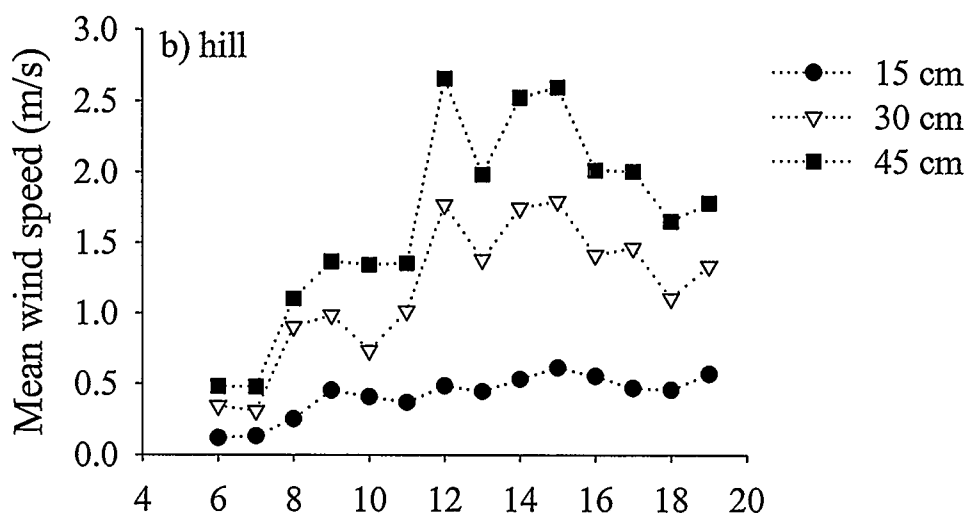
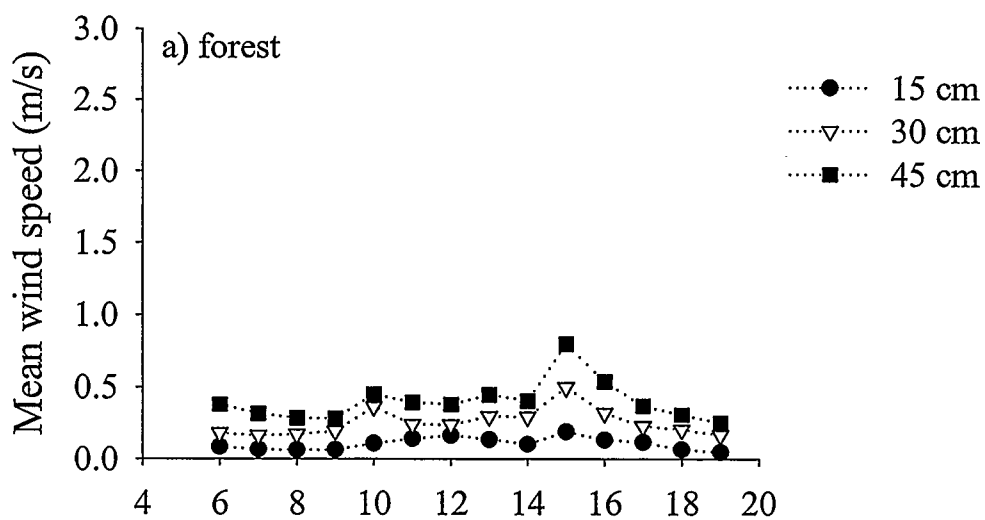
2.3.1. Environmental measurements

Wind speed varied between habitats, vertically within habitats and with time of day (Fig. 2.1). Forests experienced the lowest wind speeds, then the meadows, and finally the hills experienced the highest wind speeds (Fig. 2.1). The effect of the earth's boundary layer is clearly evident in the vertical wind profile, so that tall species experienced faster airflow than short species in the same habitat. Finally, winds were lightest during early morning and late evening. Therefore, from the plant's perspective, three factors can modify the wind speed experienced during flowering – habitat, height, and time of flowering.

2.3.2. Associations between inflorescence architecture and other characters

A total of 25 species were found in the six sites (2 replicates each of forest understory, hillside and meadow; Table 2.1). Pooling all species, the attributes measured during this study showed coefficients of variation among species that range from 20%

Figure 2.1. Daily variation in mean wind speed for three habitat types in Kananaskis, Alberta during June, July and the first two weeks of August 2001. Wind speeds were measured at three heights above ground level for 10 min.



(pollen size) to 192% (inflorescence volume) (see Table 2.2). Cluster analysis of all the characters measured revealed a major dichotomy between inflorescence-level and floret-level characters (Fig. 2.2). A two-cluster solution explained over 50% of the total variation in the variables. Within the cluster of floret traits, the correlations between pollen characteristics and stigma characteristics all exceed $r=0.61$. The correlations within the cluster including floret length, spikelet length, inflorescence mass and stem density are all at least $r=0.64$. Furthermore, the splitting of these two clusters is the final step in the clustering procedure, and represents only a small increase in the proportion of variance explained by the clusters. Therefore these “floret” characters are all closely associated across the species. In contrast, the “inflorescence” traits associate less strongly with each other, but are more highly correlated among themselves than with floret characters. Interestingly, measures of vertical dimensions (plant and inflorescence length) and flexural rigidity associate more closely with floral traits than with inflorescence traits.

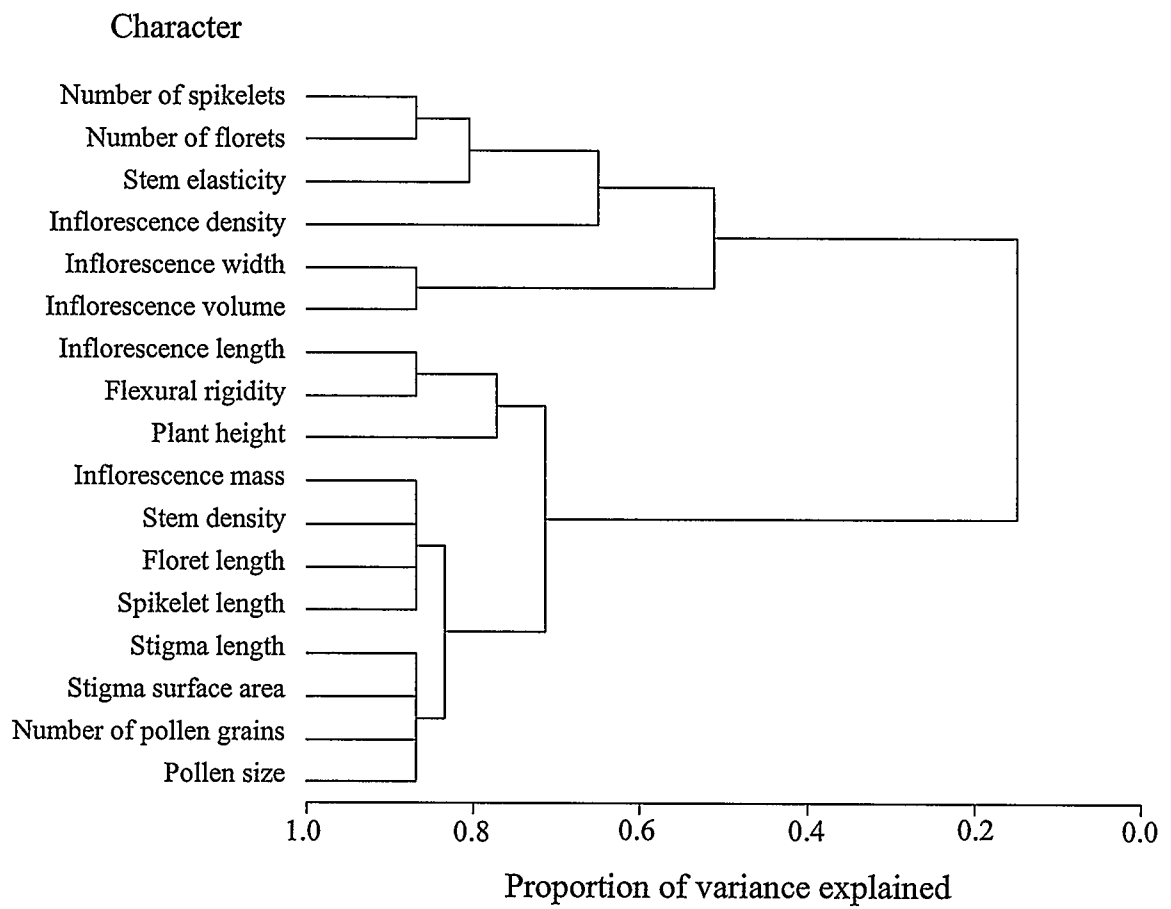
Canonical correlation found a significant overall correlation between inflorescence-level characters (height, length, width, volume, number of florets, inflorescence density, inflorescence mass, flexural rigidity and stem density) and floret-level characters (length of florets, stigma length, stigma branch length, stigma surface area, pollen production and pollen size). Two of the six possible canonical variates differed significantly from 0 and explained 95% of the variation (first variate $R_c=0.99$ [85% of variance]; second variate $R_c=0.95$ [10% of variance]). Variables in the inflorescence set that exhibited strong correlations ($r>0.3$) with the first canonical variate included number of florets (-0.76), inflorescence mass (0.72), stem density (0.72), flexural rigidity (0.69), inflorescence length (0.69), and height (0.66). Among the floret variables, length of the floret (0.96), stigma branch length (0.71), stigma surface area (0.68), and pollen grain size (0.65) correlated with the first canonical variate. Therefore,

Table 2.2. Descriptive statistics of floral characteristics for 25 grass (Poaceae) species in Kananaskis, Alberta, based on species means.

Variable	Mean	<i>n</i>	Range	Standard Deviation	Coefficient of Variation
Height (cm)	57.07	25	73.62	21.30	37.3
Inflorescence mass (g)	0.27	23	0.56	0.17	65.7
Inflorescence length (cm)	9.91	25	17.12	4.25	42.9
Inflorescence width (cm)	3.06	25	9.70	2.74	89.5
Inflorescence volume (cm ³)	59.74	25	538.12	114.60	191.8
Elasticity (N m ⁻²)	1.96×10 ⁸	24	6.25×10 ⁸	1.36×10 ⁸	69.4
Flexural rigidity (N m ²)	1.89×10 ⁻⁴	24	6.87×10 ⁻⁴	1.66×10 ⁻⁴	87.8
Stem density (g cm ⁻¹)	0.01	23	0.02	0.01	44.4
Number of spikelets	73.29	25	317.50	86.23	117.7
Number of florets	162.37	25	393.40	109.08	67.2
Inflorescence density (fl cm ⁻³)	96.08	25	642.42	176.64	183.8
Length of florets (mm)	6.30	25	11.07	3.50	55.5
Length of spikelets (mm)	9.94	25	27.96	7.28	73.2
Stigma length (mm)	1.86	19	2.73	0.67	36.3
Stigma branch length (mm)	0.62	19	0.83	0.26	41.7
Stigma surface area (mm ²)	4.32	19	8.65	2.87	66.4
Number of pollen grains	9853.51	21	20373.77	6378.55	64.7
Pollen diameter (µm)	28.57	21	18.44	5.59	19.6

Figure 2.2. Cluster analysis of the correlations between the 17 variables measured.

Variables clustered together have higher correlations with each other than with variables in more distant clusters.



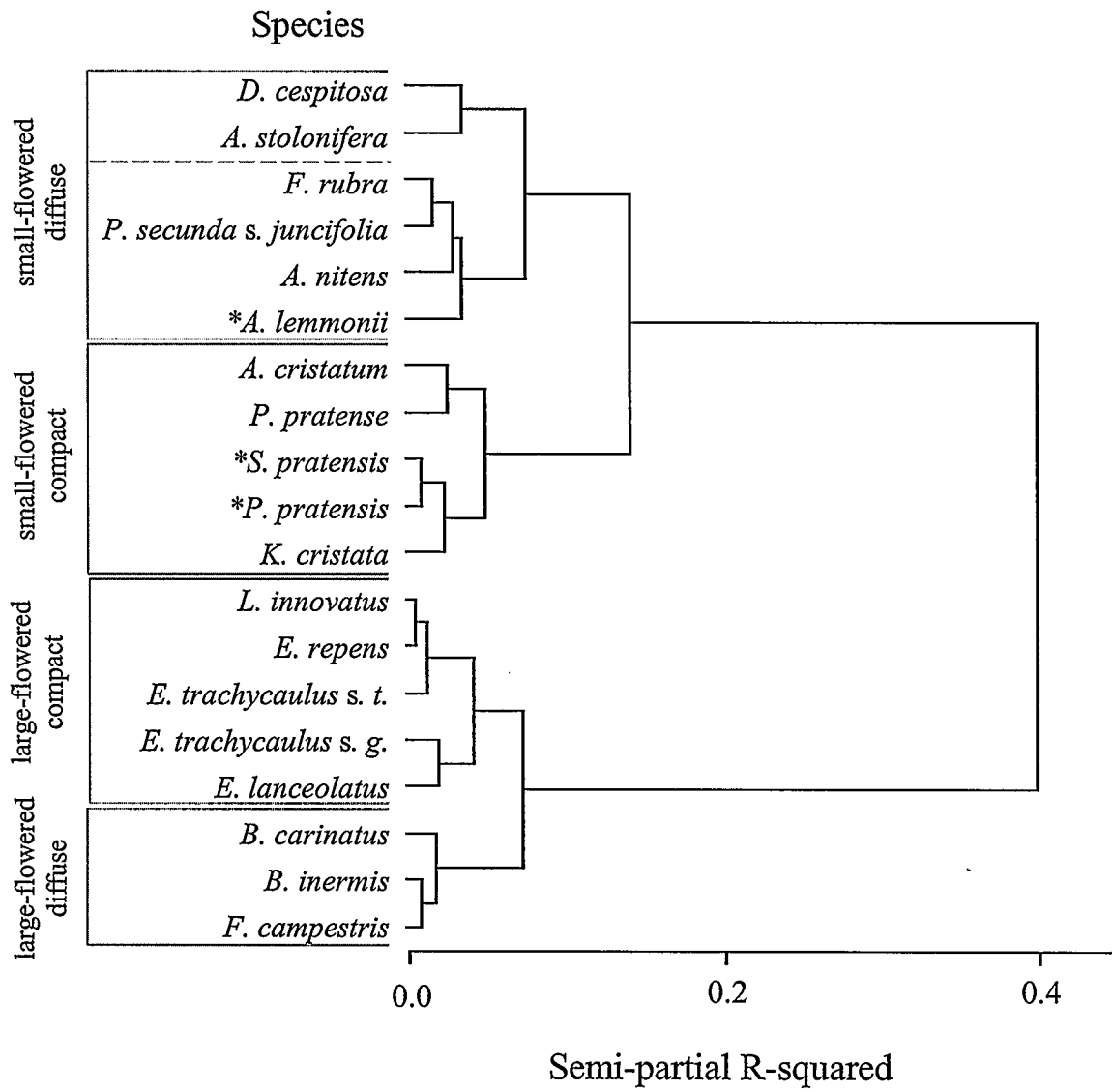
this canonical variate largely reflects a size-number tradeoff in flower production. The second canonical variate largely involved inflorescence mass (0.52), stem density (0.42) and flexural rigidity (0.37) for the inflorescence variables; stigma surface area (0.43) and number of pollen grains (0.46) for the floret variables.

2.3.3. Associations between species

Cluster analysis of 20 species detected five significant clusters (Fig. 2.3). Based on stepwise discriminant function analysis, the variables that contributed to the formation of the two major clusters included floret length, stigma branch length, number of florets and number of pollen grains, so that these clusters differed in flower size. The cluster of small-flowered species was further divided in two based primarily on inflorescence density, number of florets, inflorescence volume, stigma branch length and length of florets, producing clusters differing in architecture type. The cluster of small-flowered diffuse species was split based on inflorescence density, inflorescence volume, length of florets, and number of florets. This grouping represents a distinction between small-flowered diffuse species with many flowers, and those with fewer flowers. Finally, the large-flowered cluster split in two based on inflorescence width, inflorescence density, number of florets, stigma branch length and length of florets. Thus, each flower-size cluster divided into groups of species with either compact or diffuse inflorescences. Indeed, the 20 species fall into distinct groups in a simple plot of inflorescence width and floret length (Fig. 2.4). A significant association between the clusters and architecture type ($\chi^2 = 19.47$, $df = 8$, $P = 0.01$) verifies this composition.

When I used the discriminant functions to reclassify each species into these categories, only two species were misclassified – *Agropyron cristatum* was placed in the large-flowered compact group, and *Poa secunda subsp juncifolia* was placed in large-flowered diffuse group. Comparison of the cluster analysis and phylogenies of Poaceae

Figure 2.3. Cluster analysis (Ward's method) of 20 grass species in Kananaskis, Alberta. The semi-partial R^2 scale represents the decrease in the proportion of overall variation accounted for by joining two clusters. The five significant clusters correspond largely to four classes: small-flowered, diffuse inflorescences; small-flowered, compact inflorescences; large-flowered, compact inflorescences; and large-flowered, diffuse inflorescences. * indicates exceptions to this categorization.



indicate that the clustering does not conform to phylogenetic relationships of species (Catalan et al. 1997; Mathews et al. 2000; Grass Phylogeny Working Group 2001).

Three species did not fall clearly into the four categories based on flower size and inflorescence type: *Achnatherum lemmonii*, *Schedonorus pratensis*, and *Poa pratensis*. *Achnatherum lemmonii* is classified as small-flowered because it has very small stigmas and pollen (and is selfing: Smith 1944); however, based on floret length it may be better classified as large-flowered. *Schedonorus pratensis* is classified as a small-flowered species with a compact inflorescence, whereas it has a branched inflorescence. However, the inflorescence branches are appressed against the central axis of the inflorescence, resulting in a high density of florets. *Poa pratensis* was placed in a cluster of species with compact inflorescence, whereas I would classify it as diffuse. The architecture of *P. pratensis* inflorescences varies considerably; however, all plants tend to have a high density of florets.

Two-factor analyses of variance found contrasting patterns of variation for the 18 measured variables between classes of flower size and inflorescence type (Table 2.3). Only three variables (inflorescence width, volume and density) did not exhibit significant differences between small- and large-flowered species. In contrast, 11 variables did not differ among inflorescence types, including some unexpected traits (plant height, inflorescence mass, and stem density). Only the number of florets exhibited a significant interaction between flower size and inflorescence type. Similarly, multivariate analysis of variance found significant overall differences between flower-size classes ($F_{12,4} = 14.9$; $P < 0.01$) and inflorescence types ($F_{12,4} = 186.5$; $P < 0.0001$), but no significant interaction ($F_{12,4} = 0.62$; $P > 0.5$). Therefore, these two factors seem to have largely independent influences on the reproductive characteristics of grasses.

Figure 2.4. Ordination of 24 grass species sampled in Kananaskis, Alberta with respect to inflorescence width and floret size. Different symbols illustrate the four categories of flower-inflorescence types identified by the cluster analysis. Abbreviations indicate the first letter of the genus and the species names of the exceptions to the categorization (see Fig. 2.3).

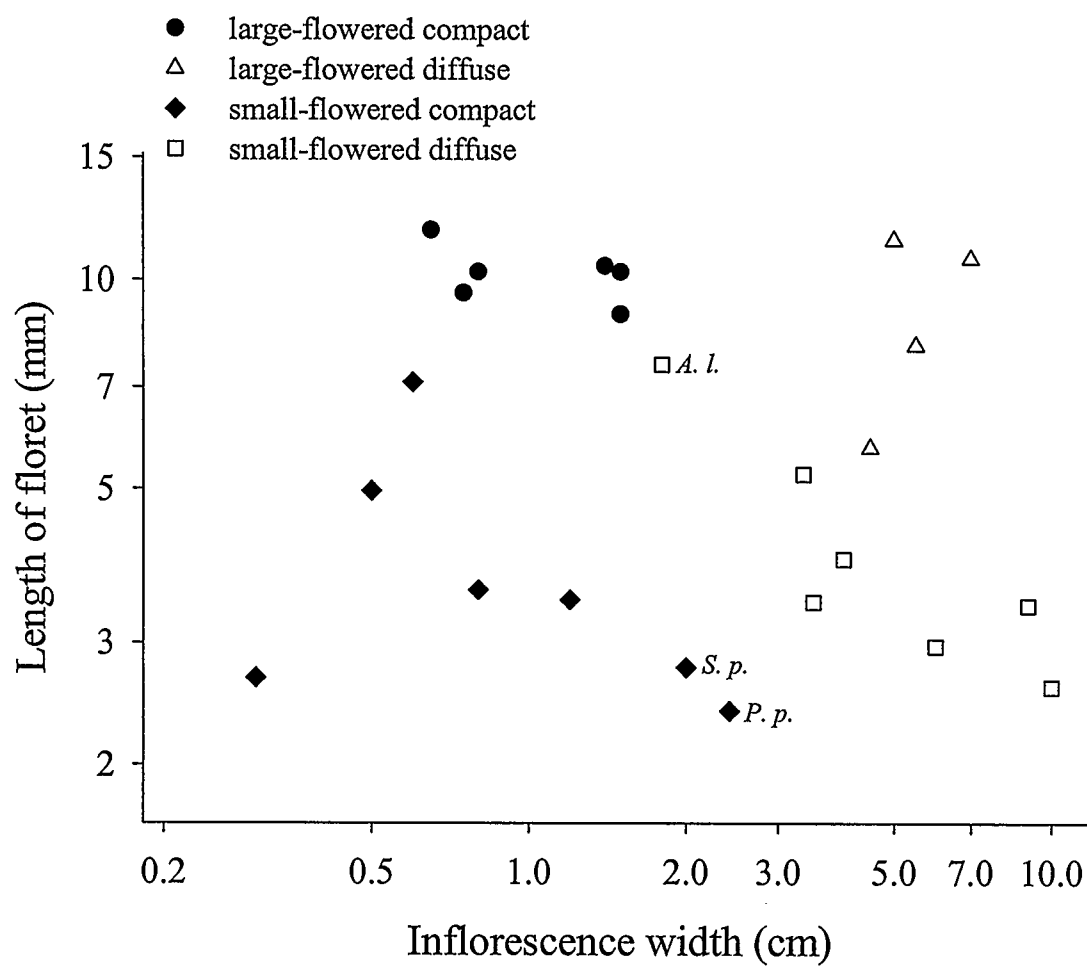


Table 2.3. Analyses of variance of species means for each variable considering the effects of flower type, inflorescence type, and their interaction. Superscripts following a test statistic for a main effect indicate significant differences, where ^L, ^S, ^C, or ^D indicate whether large- or small-flowered, compact or diffuse inflorescences, respectively, had the larger value for that trait and one, two, or three symbols indicated $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. The interaction between flower type and inflorescence type for floret number resulted because small-flowered compact inflorescences produced more florets than large-flowered compact inflorescences ($t_{18} = -3.97$; $P < 0.005$), but floret production did not differ significantly with flower size for diffuse inflorescences ($t_{18} = -0.45$; $P > 0.5$).

Source of variation	Flower type	Inflorescence type	Flower \times Inflor. type
Height (cm)	$F_{1,19}=8.12^{LL}$	$F_{1,19}=0.81$	$F_{1,19}=2.12$
Inflorescence mass (g)	$F_{1,19}=10.52^{LL}$	$F_{1,19}=1.59$	$F_{1,19}=0.19$
Inflorescence length (cm)	$F_{1,19}=9.40^{LL}$	$F_{1,19}=14.94^{DDD}$	$F_{1,19}=1.32$
Inflorescence width (cm)	$F_{1,19}=0.05$	$F_{1,19}=41.62^{DDD}$	$F_{1,19}=1.00$
Inflorescence volume (cm ³)	$F_{1,19}=0.78$	$F_{1,19}=50.27^{DDD}$	$F_{1,19}=0.12$
Elasticity (N m ⁻²)	$F_{1,17}=19.43^{SSS}$	$F_{1,17}=1.02$	$F_{1,17}=0.75$
Flexural rigidity (N m ²)	$F_{1,17}=8.88^{LL}$	$F_{1,17}=4.27^C$	$F_{1,17}=2.39$
Stem density (g cm ⁻¹)	$F_{1,19}=12.15^{LL}$	$F_{1,19}=2.92$	$F_{1,19}=0.64$
Number of spikelets	$F_{1,18}=19.41^{SSS}$	$F_{1,18}=0.13$	$F_{1,18}=0.40$
Number of florets	$F_{1,18}=9.77^{SS}$	$F_{1,18}=0.64$	$F_{1,18}=5.99^*$
Inflorescence density (fl cm ⁻³)	$F_{1,19}=4.07$	$F_{1,19}=71.66^{CCC}$	$F_{1,19}=0.69$
Length of florets (mm)	$F_{1,19}=57.32^{LLL}$	$F_{1,19}=3.78$	$F_{1,19}=0.30$
Length of spikelets (mm)	$F_{1,19}=35.36^{LLL}$	$F_{1,19}=3.89$	$F_{1,19}=0.03$

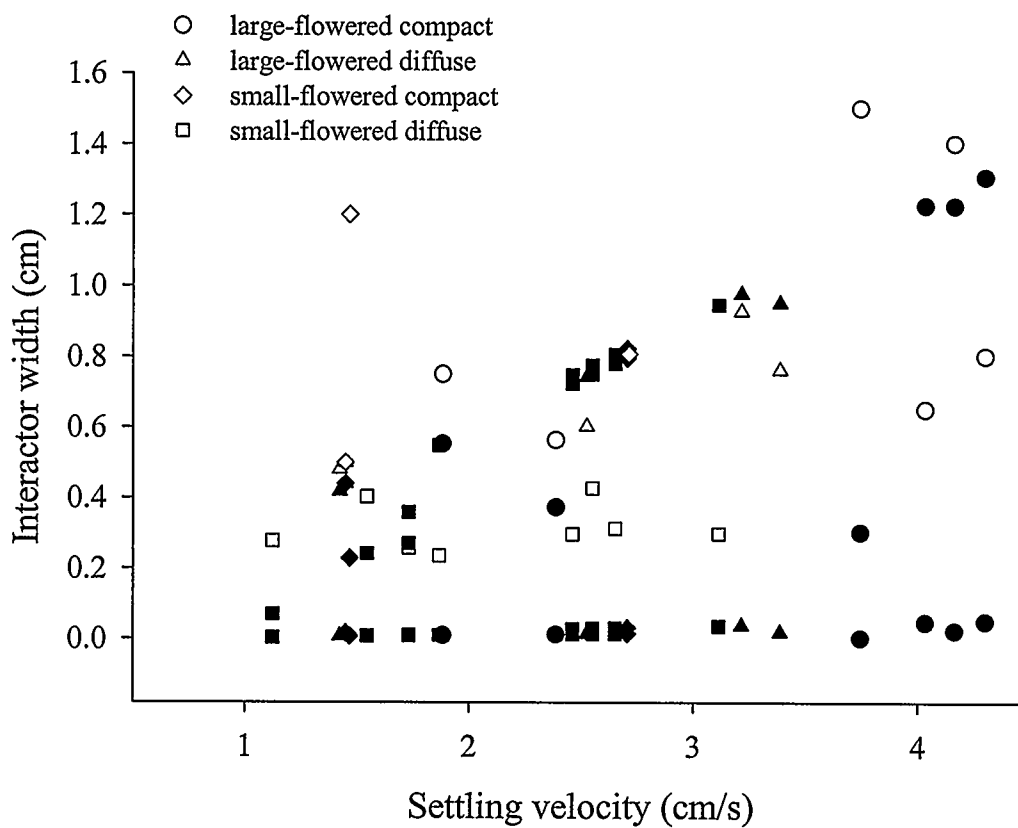
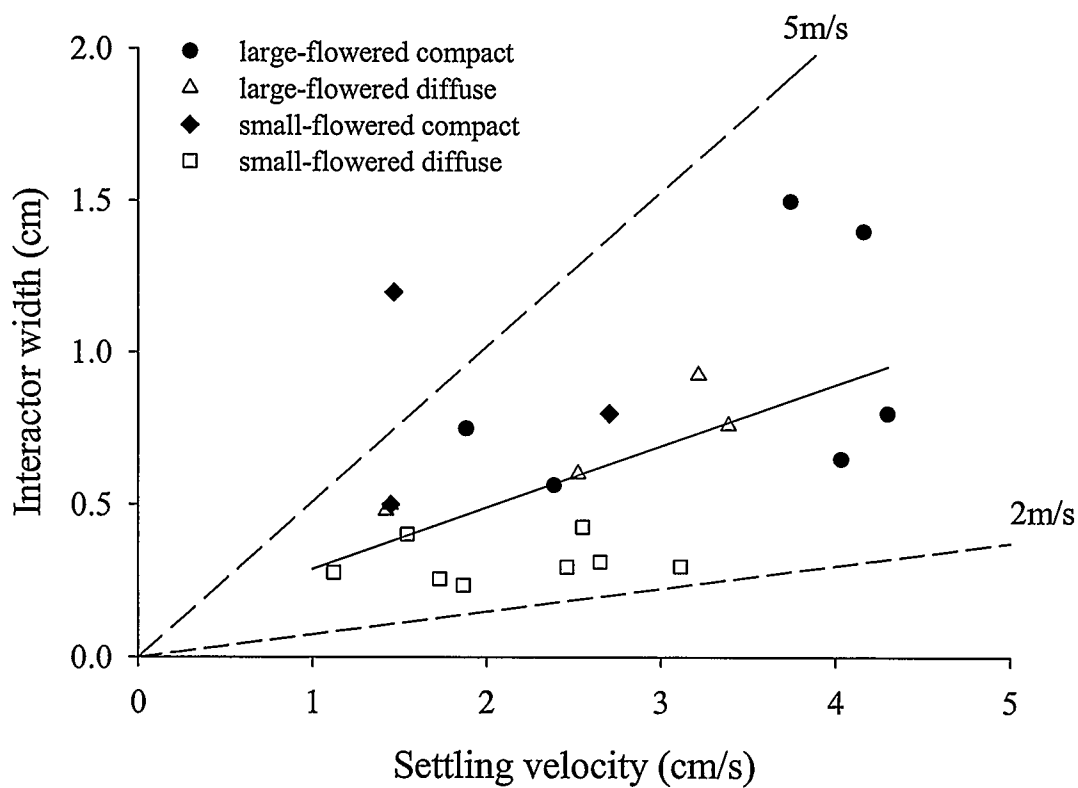
Source of variation	Flower type	Inflorescence type	Flower \times Inflor. type
Stigma length (mm)	$F_{1,15}=14.23^{LL}$	$F_{1,15}=0.43$	$F_{1,15}=0.18$
Stigma branch length (mm)	$F_{1,15}=42.44^{LLL}$	$F_{1,15}=0.01$	$F_{1,15}=0.43$
Stigma surface area (mm ²)	$F_{1,15}=31.51^{LLL}$	$F_{1,15}=0.00$	$F_{1,15}=0.05$
Number of pollen grains	$F_{1,16}=18.76^{LLL}$	$F_{1,16}=0.02$	$F_{1,16}=1.50$
Pollen diameter (μ m)	$F_{1,16}=5.56^L$	$F_{1,16}=0.05$	$F_{1,16}=0.06$

Nearly all of the 21 species for which I measured pollen characteristics have combinations of pollen and interactor size within the optimal range identified by Paw U and Hotton (1989) for wind speeds ranging from 2-5 m/s (Fig. 2.5a). Linear regression of interactor size (D) on estimated settling velocity (V_{set}) found the following relation, $D = 0.20V_{set} + 0.09$ ($F_{1,24} = 8.34$; $P < 0.01$), with a slope within the range for the theoretical optimum S_o . However, similar calculations based on the wind speeds experienced by each species during flowering resulted in a poorer fit (Fig. 2.5b). All species that fall outside the predicted combination of pollen size and interactor size have larger interactors than expected given their pollen size.

2.3.4. Distribution of species in habitat types

Discriminant function analysis found no variables that significantly distinguished species based on the type of habitat in which they were found. Therefore, there is no association between the morphological measures I made and habitat type. Furthermore, the categories identified by cluster analysis associate independently with habitat ($\chi^2 = 9.78$, $df = 8$, $P > 0.25$).

Figure 2.5. Relationship between interactor size and pollen settling velocity for 21 grass species sampled in Kananaskis, Alberta. a) Observations plotted with reference to the predicted limits (dotted lines) of the optimum relationship for mean wind speeds of 2-5m/s, based on Paw U & Hotton (1989). The solid line represents the best-fit linear regression. Different symbols illustrate the four categories of flower-inflorescence types identified by the cluster analysis. b) Observations (open symbols) plotted with reference to predicted limits (closed symbols) of the optimum relationship for the range of wind speeds experienced by a particular species. Symbols (\circ , \triangle , \diamond , \square) represent species in each flower-size, inflorescence category (large-flowered compact, large-flowered diffuse, small-flowered compact, small-flowered diffuse, respectively).



2.4. Discussion

The most striking finding of this study is the distinction between floret-size classes within architecture types (Fig. 2.3). Most notably, plants are exploring all possible combinations of architecture and floret size (see Fig. 2.4). Niklas (1987) demonstrated that the aerodynamic environment created around a plant, and the associated details of pollen removal and capture differ between plants with compact versus diffuse inflorescences. My finding that species within these inflorescence types fall into distinct flower-size categories suggests that flower size may also influence the aerodynamic role of plant morphology in pollination.

Inflorescence architecture and flower size will affect the nature of the aerodynamic environment surrounding a plant. The size of an obstruction to airflow will partially affect whether vortices are shed by the obstruction, and whether leeward turbulence is created. Based on Reynolds number, for a given kinematic viscosity, a change in wind speed and/or the characteristic length (usually the greatest diameter in the direction of flow) causes a proportional change in the Reynolds number (see eq. 1.1). As the Re increases, vortices are shed and turbulence and eddies are created in the leeward region of the obstruction. Large spikelets in grasses with diffuse panicles may be large enough to shed vortices, and pollen may recirculate within the region of turbulence. von Karman vortices are shed at Re between 40 and 100000 (Vogel 1983a). For species with large-flowers, the Re , based on spikelet width, ranges from approximately 45 to 2000, depending on the wind speed. Also, larger objects have proportionally thicker boundary layers and therefore deflect more streamlines. Local boundary layer thickness depends on the local Reynolds number (see eq. 1.2). A thicker boundary layer requires larger pollen with more inertia to pass through and collide. Also, recall that the oscillatory motion of inflorescences depends on the length of the subtending stem, its flexural rigidity and the inflorescence's drag and mass (Vogel 1983a; Finnigan & Mulhearn

1978). These factors differ for species with compact and diffuse architectures, and for species with large and small flowers, suggesting that patterns of harmonic motion are likely to differ between the four groups.

Therefore, pollen removal and capture may occur differently in the four classes of architecture-flower designs, because of differences in their harmonic motion and streamline deflections created by the size and shape of the obstructions to airflow. I now discuss the possible aerodynamic environment and influences for each type.

A small-flowered compact panicle functions as one aerodynamic unit and oscillates in a single motion. A single boundary layer is likely to form around the entire inflorescence, and downstream eddies will be created, as Niklas (1987) demonstrated for *Setaria geniculata*. Pollen grains may circle within these eddies and can deposit onto stigmas. Because of the relatively large boundary layer around the whole inflorescence, effective pollen release may require specific mechanisms. For example, in *Phleum pratense*, the species in this study with the smallest florets and most compact panicle, the filaments supporting the anthers project horizontally and perpendicular to the inflorescence axis, maximizing the distance of the anthers from the inflorescence (see Fig. 1.1.c). In species with diffuse inflorescences and those with large-flowered compact inflorescences, the anthers hang on filaments that typically project out and down at angles ranging from 45° to 0° (see Fig. 1.1.d). Small-flowered species are shorter than large-flowered species, and have higher elasticity (Table 2.3). These two factors should result in higher frequencies of oscillation and less displacement for species with small-flowered compact inflorescences, than for species with large-flowered compact inflorescences.

A large-flowered compact panicle also oscillates in a single motion. However, rather than the inflorescence as a whole modifying the airstream, individual spikelets may be large enough to create local airstream deflections and downstream eddies. In most of the *Elymus* species and the one *Leymus* species that I measured (all the large-flowered

compact inflorescences), the lemma and palea spread wide open during flowering, with the stigma projecting upward between them. The bracts may create turbulent eddies and act as scoops to direct and entrain pollen from the airstream. Linder (1991 *in* Honig et al. 1992) suggested that the bracts in *Staberoha banksii* (Restionaceae) may act in this manner, as has been observed for *Simmondsia chinensis* (Buxaceae) by Niklas and Buchmann (1985).

Unlike the spikelets of a compact inflorescence, which modify airflow communally, spikelets in a diffuse inflorescence function more independently, behaving like boats moored separately in a three-dimensional stream. In diffuse panicles, spikelets oscillate relatively independently and have the potential to sweep through a large volume of air. In species with large-flowered diffuse inflorescences, the large spikelets (and stigmas) may create appreciable eddies, and may channel some pollen towards stigmas. Thus some pollen may be captured by sedimentation, as well as by impaction. However, sedimentation of pollen onto stigmas may be limited by erratic motion of spikelets. Large flowers, with large stigmas, will have a thicker boundary layer surrounding them, and require larger pollen with more inertia to pass through, than small-flowered species. Pollen size and stigma size are highly correlated ($r=0.72$) and so are pollen size and floret size ($r=0.63$), which may indicate that this association is related to selection for pollen capture.

The drag created by a panicle depends on its projected surface area. For compact panicles this area varies with the inflorescence's width and length. However, diffuse panicles allow considerable air movement through the panicle, and re-orient themselves depending on wind speed, which complicates estimation of drag (Alben et al. 2002; Steinberg 2002). However, it seems likely that diffuse panicles with large-flowers create more drag than those with small flowers, resulting in greater harmonic motion.

In a small-flowered diffuse panicle, branches also oscillate relatively independently. Flowers (and stigmas) may be too small to create significant downstream eddies, therefore they would capture pollen almost entirely by inertial collision. Floret size correlated negatively with the number of florets ($r=-0.79$), and small-flowered species tend to have many crowded flowers. Such crowding could result in interference between spikelets and the creation of very complex patterns of airflow surrounding the plant. Interestingly, the diffuse species studied by Niklas (*Agrostis hiemalis*: 1987) has very small flowers, but also has very few flowers (spikelets are one-flowered), clustered at the very tips of branches (Hitchcock 1950). Therefore, the aerodynamic effects that he observed for this species may not be representative of all grasses with diffuse architectures.

The negative correlation between the number and size of flowers ($r=-0.79$), likely represents a size-number tradeoff associated from the allocation of limited reproductive resources. Even species with few, large flowers produce only one ovule per flower, so they probably produce fewer seeds than small-flowered plants. It is likely that they produce larger seeds, given their larger floret size, which may have greater viability than small seeds. Also, large-flowered plants have larger stigmas and produce more pollen (Table 2.3), and thus have the potential to receive larger stigmatic pollen loads. Thus, although they have fewer flowers, large-flowered plants may produce comparable numbers of seeds, or more viable seeds than small-flowered plants.

In species with diffuse panicles, spikelets are attached by pedicels and can rotate freely in the wind and orient themselves to minimize the diameter of the obstructing surface. To some degree, the use of spikelet width as the aerodynamic unit in Paw U and Hotton's (1989) model should reflect this. Their model fit my data adequately well (Fig. 2.5a); however, more discrepancies arose when I use a more refined approach to include the measured range of wind speeds (Fig. 2.5b). Although the model was intended to

reflect large-scale patterns of fluid flow (Paw U & Hotton 1989), it may be too crude to explain the relationship between pollen morphology and capture adequately. The partial failure of Paw U and Hotton's model at observed windspeeds suggests that the interacting unit may not simply be a function of architecture (one type of measure each for compact and diffuse inflorescences), but may also be intricately related to floret size. The spreading of the lemma and palea during flowering can be particularly pronounced for large-flowered species. Thus, the measure of spikelet width as the obstruction to airflow becomes distorted, because air can be channeled through this space to facilitate pollen capture.

The elaborate, highly branching feathery structure of grass stigmas suggests they are the result of selection (Whitehead 1983; Linder 1998). These stigmas bear a striking resemblance to silkmoth antennae, for which Vogel (1983b) found that the structure exerts appreciable drag and creates a region of downstream eddies. Also, up to 18% of the airstream flow passes through the antenna (at a flow speed of 3 m/s). This result is not directly analogous to grass species for several reasons. First, silkmoth antennae are quite a bit larger (Vogel [1983b] reported averages of 1.35 cm length, and 0.6 cm width, but see Loudon & Koehl [2000] who report an average width of 0.2 cm), which would result in a larger Reynolds number (see eq. 1.1). At higher Re the effects of viscosity will be diminished, allowing more flow through the antenna. Also, the antennae were mounted in isolation, and therefore experienced none of the interference that stigmas may encounter from surrounding floral parts. Nonetheless, this study indicates that the branching-structure of stigmas may not only increase its capture efficiency (see eq. 1.7), but may also enable air to flow through the stigma and thus filter pollen directly from the air. This may be especially true for stigmas of large-flowered plants, which tend to be larger and project further away from floret bracts. Furthermore, studies of small-scale fluid dynamics around moth antennae show that the spacing and "leakiness" of the

branches affect how the antennae sample their fluid environments (Koehl 1996). Clearly, the inertial properties of pollen differ to the pheromone molecules picked up by antennae. However, the branching pattern of stigmas shows considerable interspecific variation (pers obs.) and a mechanistic explanation for this is lacking.

Pollen in anemophilous plants is constrained by a trade-off between small pollen that travels farther and large pollen with more inertia to facilitate capture (Whitehead 1968; Niklas 1985). Also, the higher the point of release, the farther pollen travels (see eq. 1.6). Thus the positive correlation between plant height and pollen size ($r = 0.51$) may reflect slightly different solutions to constraints on pollen size. Taller plants may experience conditions sufficient to favour slightly larger pollen which optimizes capture, because dispersal distance is adequate given their height. Also, taller plants experience higher wind speeds (see Fig 2.1), which increases dispersal distance, and also facilitates removal of larger pollen grains from the anther (see eq. 1.4). The model developed by Paw U and Hotton (1989) implies that optimal interactor size is less constrained for species with large pollen (or pollen size is less constrained for species with large interactors). I found that species with pollen with the largest settling velocity always fall into the large-flowered category (see Fig. 2.5a). Although these associations may be related to developmental allometry, the relation between form and function in anemophilous plants suggests they would promote effective pollen release and capture.

Contrary to my expectations, I did not find any associations between habitat and architecture-type or other morphological traits. There are several possible explanations for this. First, different architectures may be equally competent at pollination under a variety of wind environments, so that inflorescence characters have little influence on the assembly of grass communities. Alternatively, given a specific architecture and the disposition for a habitat type based on ecological requirements, other traits (such as height, floret size, pollen size, stigma size) may be selected to compensate for particular

wind speeds of that habitat. Because there are numerous possibilities for these combinations, I could not detect the differences. Finally, only three grass species grew in the forest sites that I sampled, so that my ability to detect differences between habitats may be compromised by limited statistical power.

The range of panicle morphologies and spikelet and floret constructions within the Poaceae suggests adaptations that have increased the efficiency of pollen removal and receipt. However, enhanced performance is possible only if different morphologies experience particular features in panicle and pollen motion that affect pollen removal and capture. Many of the observed correlations within architecture types and within flower-size types are consistent with the aerodynamic requirements of wind pollination. However, the correlations between characters, and the presence of significant differences between the four categories, seem to contradict the exploration of all flower size-inflorescence diffusion options (see Fig. 2.4). Therefore, each species may have a particular combination of inflorescence type and flower size that function aerodynamically in a specific manner to facilitate pollination.

3 INFLORESCENCE ARCHITECTURE AND THE EFFICIENCY OF POLLEN TRANSFER FOR WIND-POLLINATED GRASSES

3.1 Introduction

3.1.1 Pollen-transfer efficiency in anemophilous plants

Traditionally, abiotic pollen vectors are viewed as random and relatively poor modes of pollen dispersal. To assure successful pollination, it is widely believed that anemophilous plants must produce copious pollen (Faegri & van der Pijl 1979; Whitehead 1983). Faegri & van der Pijl (1979) reported pollen-to-ovule ratios for wind-pollinated plants exceeding $10^6:1$; however, Cruden (2000) reported a median pollen-ovule ratio of 22150:1 for hermaphroditic, wind-pollinated plants. These ratios are much higher than those for out-crossing animal-pollinated plants, which usually are between 1200:1 and 8000:1 (Cruden 1977; 2000). Therefore, even if all ovules in a population are fertilized, much more pollen fails to sire seeds for abiotically pollinated species, than for those pollinated by animals.

During pollen dispersal, pollen can experience several fates, which determine a pollen grain's siring success (Harder 2000). If pollen vectors are not sufficiently abundant or active, a proportion of pollen can remain in anthers with no opportunity to interact with ovules (pollen removal failure). In addition, pollen lost during transport contributes to unsuccessful male function. Wind-dispersed pollen could leave anthers and be lost before reaching stigmas of conspecific plants for three reasons. Pollen could impact other vegetation or floral structures, it can settle out below the level of stigmas as a result of gravity, or it can disperse beyond the population due to turbulence. Successful pollination occurs only when pollen lands on stigmas, either on the same or a different plant. Siring success additionally requires that a dispersed pollen gametophyte out-competes other pollen during pollen-tube germination and growth and successfully

fertilizes an ovule. Because the proportion of pollen that can compete for access to ovules in pistils depends on the interaction between the plant and its pollen vector (in this case, wind), the relative incidence of these pollination fates will differ among species with dissimilar floral and inflorescence morphologies. However, Cruden (2000) was unable to find any published observations to support the expectation that pollination efficiency of wind-pollinated plants varies as a function of reproductive morphology.

Harder (2000) reported measures of pollen removal failure (proportion of pollen remaining in anthers of wilted flowers), and pollen transfer efficiency (proportion of removed pollen deposited on stigmas) for 24 monocot species. Although only two wind-pollinated species were included (both grasses), their removal failure and pollination efficiency fell well within the range observed for animal-pollinated species with granular pollen. Thus, despite the recurring claim, wind pollination may not be less efficient than animal pollination.

3.1.2 Functional aspects of inflorescence architecture

Grasses are almost entirely wind pollinated, so that inflorescence architecture should be subject to selection for efficient pollination. Morphological modifications that capitalize on the aerodynamics of pollen release and capture should be favoured. The interaction between inflorescence structure and wind may influence the success of pollen release and capture. Wind-tunnel studies, combined with an understanding of fluid dynamic principles, show that grasses with contrasting architectures capture pollen differently. Niklas (1987; 1988) found that compact and diffuse grass panicles create different aerodynamic environments around the florets, and that the harmonic motion of the two panicle morphologies determines the mode of pollen capture. Within a range of low airflow speeds, (10cm/s to 50cm/s) the compact panicle that he studied (*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 recirculate. In contrast, the diffuse panicle that he studied (*Agrostis hiemalis*) captured pollen only by direct inertial collision. Furthermore, the diffuse panicle sweeps a large volume of airspace with spikelets out of phase with each other. The compact panicle sweeps a lesser volume and oscillates with a single motion.

Niklas' studies seem to be the only mechanistic tests of the function of inflorescence architecture for wind-pollinated plants, although Ackerman (1997a; 1997b) has conducted similar studies for water-pollinated plants. Both authors interpreted their detailed observations of wind- and water-pollination in the context of fluid-flow theory and suggested that inflorescence architecture and pollen morphology are co-adapted to increase pollination efficiency. However, these studies neither measured pollen removal and the proportion of successful pollen transfer, nor tested the functioning of inflorescence architectures in natural conditions. Paw U & Hotton (1989) presented a mathematical model relating the size of the unit interacting with the airstream to the settling velocity of pollen, and found high agreement between the model and published data for wind-pollinated species, but found no association for animal-pollinated plants.

The interaction of fluid dynamics and biological structures influence the effectiveness of wind pollination (Ackerman 2000). Specifically, the efficiency of pollen capture is influenced by the airflow patterns created by the female receptive structure and the physical properties of pollen. However, Paw U & Hotton (1989) argued that more than the stigma is involved in modifying the airstream, and that the interacting structures are the surfaces which block airflow. They argued that in certain species the entire inflorescence serves as the interactor, whereas in others it is the flower plus subtending bracts. Thus, in grasses, spikelets and/or the inflorescence may be the airflow control units.

In contrast to pollen receipt, pollen removal from anthers has not been studied mechanistically and the role of architecture in pollen removal remains unexplored. Anthers generally avoid the boundary layer surrounding the plant by hanging below the floret on thin filaments. There is some variety in the angle of projection of the anthers, ranging from completely vertical (for example in *Bromus inermis*), to angles greater than 90° from the inflorescence (for example in *Phleum pratense*) (pers. obs.). These differences may represent distinct strategies for effective pollen removal.

The developmental association among floral traits complicates an understanding of causality in the relationship between form and function in the inflorescence (Conner & Sterling 1995). Experimental manipulations can help elucidate the functional components affecting pollination efficiency by disrupting this association. This approach has been used to alter the mechanical fit between flowers and animal pollinators (e.g. Wilson 1995; Campbell et al. 1996; Nilsson 1998; Cresswell 2000; Walker-Larsen & Harder 2001) and to modify various aspects of inflorescence display (e.g. Willson & Rathcke 1974; Barrett et al. 1994; Harder et al. 2000); however, it has not been used to investigate the role of reproductive morphology for wind-pollinated plants.

In this study, I quantify the pollen-transfer efficiency for six wind-pollinated grass species with contrasting inflorescence architectures and report the effects of two manipulations on pollen release and capture for these species in otherwise natural conditions. For one manipulation, I tied the inflorescences to stakes just below the inflorescence to limit their motion in the wind. This experiment involved five species, three with compact panicles (*Elymus repens* (L.) Gould., *Leymus innovatus* (Beal) Pilg. and *Phleum pratense* L.), and two with diffuse panicles (*Bromus inermis* Leyss. and *Festuca campestris* Rydb.). This manipulation should particularly affect pollination of species with diffuse panicles, as they probably capture most of their pollen by inertial collision, and maximize this by sweeping through a large volume of air. In contrast, the

manipulation should have little effect on the ability of compact panicles to create extensive downwind turbulence and capture pollen by sedimentation. Although less is known about the mechanics of pollen removal, I expect that removal will not be adversely affected, because anthers are suspended on thin filaments beyond the boundary layer and can move freely (Whitehead 1968).

For the second manipulation, I tied branches of the diffuse panicles of two species (*Bromus inermis* and *Anthoxanthum nitens* (Weber) Y. Schouten & Veldkamp) together to create a more compact inflorescence. 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. Furthermore, the aggregated panicle may function as a bluff-bodied obstruction 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.

3.2 Methods

3.2.1 Study design and species

I conducted manipulative studies near the Barrier Lake Field Station of the Kananaskis Field Stations, Alberta (51° 02'N, 115° 03'W), during June, July and August of 2001 and 2002. In total, seven trials were performed, and are outlined below. For each trial, a population was selected with at least 500 flowering plants. I manipulated all experimental plants before the onset of flowering (1-2 days prior to first floret opening), and care was taken to minimize disturbance of the surrounding vegetation. Although the previous chapter introduces four classes of architecture-flower types, these experiments

were designed prior to this finding, and thus explore only differences in the two architecture types.

The species involved are described in Table 3.1 and below:

Elymus repens (= *Agropyron repens* (L.) P. Beauv.) is an introduced perennial grass of open areas. It is virtually self-incompatible, with high levels of genetic variability (Taylor & Aarssen 1988). This species propagates by both rhizomatous growth and seed, although the relative importance of seeds versus rhizomes for recruitment is unknown (Taylor & Aarssen 1988). Flowers open during morning.

Bromus inermis is an introduced perennial grass of disturbed, open areas. Almost all seeds are outcrossed, and McKone (1985) reported *B. inermis* to be self-incompatible. Flowers open during early evening.

Leymus innovatus (= *Elymus innovatus* Beal.) is a native perennial with rhizomes. High outcrossing has been reported (Sadasivaiah & Weijer 1981; Plourde et al. 1989). Plants grow in open woods, and flowers open during early evening.

Festuca campestris is a native, tussock-forming perennial. Plants grow in mesic grasslands, where they are often a dominant component in Alberta (Moss 1983). Flowering occurs diurnally – most flowers open during morning, but some open during late afternoon.

Anthoxanthum nitens (= *Hierochloe odorata* (L.) P. Beauv.) is a native, rhizomatous perennial. Plants are andromonoecious – the upper florets within a spikelet are perfect with two anthers, whereas lower florets are staminate, with three anthers. Spikelets are three-flowered. Apomixis has been reported in this species, but other studies report high fertility and outcrossing (Norstog 1963; Ferris et al. 1992). Flowers open during early morning in early June. Plants grow in moist, open meadows (Moss 1983).

Table 3.1. Summary of morphological measurements for each species involved in the staking and inflorescence aggregation experiments (mean \pm S.D).

Species	Architecture	Height (cm)	Inflorescence volume (cm ³)	Pollen size (μ m)	Stigma surface area (mm ³)	Flexural rigidity (N m)	Distance to neighbours (cm)
<i>Elymus repens</i>	Compact	109.4 \pm 6.2	4.2 \pm 3.5	37.56 \pm 1.38	4.2 \pm 0.5	5.3E-04 \pm 1.3E-04	20.3 \pm 7.7
<i>Leymus innovatus</i>	Compact	92.2 \pm 13.3	6.0 \pm 2.9	34.42 \pm 1.26	4.6 \pm 0.8	2.3E-04 \pm 1.3E-04	58.1 \pm 27.6
<i>Phleum pratense</i>	Compact	63.5 \pm 9.1	0.3 \pm 0.2	22.19 \pm 1.76	1.0 \pm 0.3	5.5E-04 \pm 2.2E-05	21.5 \pm 10.1
<i>Anthoxanthum nitens</i>	Diffuse	25.1 \pm 3.2	66.0 \pm 14.6	22.63 \pm 1.89	1.6 \pm 0.5	1.1E-04 \pm 4.6E-05	16.2 \pm 8.1
<i>Bromus inermis</i>	Diffuse	78.8 \pm 11.0	204.3 \pm 60.6	33.40 \pm 2.40	3.6 \pm 0.8	2.0E-04 \pm 5.9E-05	25.3 \pm 12.7
<i>Festuca campestris</i>	Diffuse	51.7 \pm 6.5	79.5 \pm 31.3	28.52 \pm 1.46	2.6 \pm 0.6	1.8E-04 \pm 4.3E-05	35.5 \pm 28.6

Phleum pratense is a native perennial grass of open, often disturbed habitats. The most common mode of fertilization is by cross-pollination, although selfing is possible (Clarke 1927, Beddows 1931). The inflorescence is spike-like and spikelets are one-flowered. Flowers open during early morning.

3.2.1.1 Restriction of inflorescence movement

For three species with compact panicles (*E. repens*, *L. innovatus* and *P. pratense*), and two with diffuse panicles (*B. inermis* and *F. campestris*), I tied the inflorescence to two stakes placed on opposite sides of the plant, to limit the movement of the inflorescence in the wind. Thirty plants were manipulated, 20 plants were unaltered, and 20 plants controlled for the effects of the presence of the stakes on local airflow. The plants for each treatment were selected randomly. For each manipulated plant, two wooden stakes (smooth doweling, 7 mm diameter) were pushed into the ground 5 cm on either side of the flowering stem. The middle of the inflorescence was tied to the stakes with 6-lb test, nylon fishing line. The control plants had stakes placed on either side, like manipulated plants, but they were not tied to the stakes with the nylon line. Plants in the natural treatment were unaltered. The flowering culm of each plant was labelled with a jeweller's tag at the base of the stem within the surrounding vegetation.

3.2.1.2 Altered inflorescence morphology

For two species with open panicles (*B. inermis* and *A. nitens*) I tied branches of the panicle together to create a more compact inflorescence. Thirty plants were manipulated, 20 plants were unaltered, and 20 plants controlled for effects of mass added to the inflorescence. For each manipulated plant, the branches of the inflorescence 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 in three places, but the branches of the inflorescence were not gathered up. Plants in the natural treatment were unaltered. 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. To quantify the mass of nylon line added to inflorescences, I 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 (\pm S.D.) mass of $1.00 \text{ g} \pm 0.40$. For control plants, the nylon line represented $0.94\% \pm 0.29$ of the total mass, and for manipulated plants it represented $1.46\% \pm 0.99$ (not significantly different: $F_{1,18} = 2.37$, $P > 0.1$). *Anthoxanthum nitens* inflorescences weighed $0.24 \text{ g} \pm 0.06$. For control plants the nylon line represented $1.80\% \pm 0.55$ of the inflorescence mass, and for manipulated plants it represented $2.32\% \pm 0.76$ (not significantly different: $F_{1,18} = 3.10$, $P = 0.1$).

3.2.2 Measurements

During each experiment, I measured wind velocity and direction, temperature and relative humidity at anther dehiscence, and once an hour for the remaining five hours. All measurements were taken at inflorescence height, at a randomly selected location within the study site. Wind velocity was measured with an Airflow hot-wire anemometer, and relative humidity was measured with a Hygros hygrometer.

Inflorescence height and spacing between plants strongly influence pollen capture (Bateman 1947; Levin & Kerster 1974; Griffiths 1950; Whitehead 1983). Therefore, for each plant, I recorded the heights of the top and bottom of the inflorescence, and the plant's distance and direction to its 5 nearest conspecific neighbours.

I measured pollen release by comparing samples of pollen production with the pollen remaining in anthers after 3 or 5 h. I collected freshly dehiscent anthers from at least 20 plants not involved in the experiments to provide a population estimate of pollen production. Each day during an experiment, I observed plants to determine each species'

flowering time. For most species (except *F. campestris*), flowering is tightly synchronized, and florets opened within 15-30 min of each other.

Few published data detail how soon after anthesis the pollination process ends. Dowding (1987) reported a flowering duration of 1-10 h for various grass species, although it is unclear whether this refers to wilting of anthers or stigmas. Gregor (1928) stated that 3 h elapsed between the opening and closing of *Lolium perenne* and *L. italicum* flowers. Beddows (1931) reported on flowering in various grasses, and in all cases anthers wilted within a few hours of anthesis, but stigmas remained exerted (however, no information is provided on whether they were receptive). I chose to collect anthers and stigmas 5 hours after anthesis to ensure enough time had passed to get a reliable measure of pollen removal failure and pollen receipt. However, for *F. campestris* I collected anthers and stigmas at both 3 and 5 h intervals to see whether removal and receipt differed.

Five hours after anthesis, I collected anthers to count the remaining pollen (except for *F. campestris*, for which anthers were collected both 3 h and 5 h post-dehiscence). Anthers were collected from two flowers at three levels within an inflorescence (lower, middle and upper), for a total of six flowers per plant. For any day during the experiment, at least one set of anthers was collected from each plant with open florets. Anthers were stored in micro-centrifuge tubes containing 1.5 mL of 70% ethanol, until the pollen grains could be counted. I sonicated the micro-centrifuge tubes with anthers in an ultrasonic bath to dislodge the pollen, and counted the pollen grains using an Elzone 5380 particle counter (Micromeritics Inc. Atlanta, GA). If too few pollen grains were present to use the particle counter (<4000 grains), I hand-counted the pollen grains using a Wild Heerbrugg inverted microscope at 100X magnification.

To assess the effect of the inflorescence manipulation on pollen capture, I counted pollen on stigmas that had been exposed for 3 or 5 h. Each day, I observed the plants to

determine flowering time. Stigmas were collected 5 h after emergence (except for *F. campestris*, for which stigmas were collected both 3 h and 5 h post-emergence). Stigmas were collected from flowers at three levels within an inflorescence (lower, middle and upper), for a total of 6 per plant, to determine whether position of the floret within the inflorescence affected pollen removal or capture. For any day during an experiment, at least one set of stigmas was collected for each plant with open florets. I stored stigmas individually in micro-centrifuge tubes containing 1.5 mL of 70% ethanol. In the laboratory, I stained stigmas with 1% basic fuchsin, and counted the pollen grains that had been deposited under a compound microscope (20X). It is very difficult to differentiate pollen from different grass species (Driessen et al. 1989; Salih et al. 1997), so my counts may include heterospecific pollen.

3.2.3 Data analysis

I 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. 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 independent variables. The inflorescence aggregation experiment considered species, treatment and floret position 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 capture by stigmas also included the effect of the number of experimental plants in flower as a covariate. Flowering in grasses tends to be tightly synchronized, and begins with few florets open on the first days, and then

proceeds with many florets open on each plant. Therefore, the number of experimental plants in flower reflects 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 ($\alpha=0.05$) 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 species within architecture and treatment, were always retained in the model to reflect the experimental design.

Because I measured plants repeatedly, I used restricted maximum likelihood (general linear models: Jennrich & Schluchter, 1986) to characterize the covariance between responses for the same plant. In all cases, a model of either compound symmetry or heterogeneous compound symmetry was more appropriate than one of independent responses ($P<0.05$). Denominator degrees of freedom for F -tests of the general linear models were calculated by Kenward and Roger's (1997) approximation.

I 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 can each be tested with a Type I error rate of 0.05 (Kirk 1995). Because I am interested in only these specific contrasts, I do not present the overall test of treatment effects, as it includes comparisons that are not of interest (e.g., control versus manipulated plants). I 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.

For *F. campestris* I compared the amount of pollen remaining in anthers and the amount captured by stigmas at 3 h after anthesis to the amounts at 5 h after anthesis. This analysis was carried out with a repeated-measures general linear model (Neter et al. 1996: mixed procedure of SAS, release 8.2, SAS Institute Inc., 1999). The model included the effect of exposure duration, as well as all the covariates listed above. I am primarily interested in the effects of time and any interactions in which it is involved, therefore I report only these effects (other effects are described in the overall analyses of staking experiments which include *F. campestris* [5 h after anthesis]).

3.3 Results

3.3.1 Comparison of species with compact and diffuse architectures

Species with compact and diffuse architectures differ significantly in several traits (see Table 2.3). The traits most relevant to this analysis are the higher flexural rigidity and the higher inflorescence density for compact species. The significant differences between inflorescence volume and inflorescence width support the categorization of compact and diffuse inflorescences.

3.3.2 Staked inflorescences

3.3.2.1 Pollen production and removal failure

Overall, the three species with compact panicles produced significantly less pollen per flower than the two species with diffuse panicles ($F_{1,107} = 14.70$, $P < 0.001$; Fig. 3.1a). 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 the anthers (Fig. 3.1b). The amount of pollen remaining varied significantly among inflorescence architectures and among species within architectures (Table 3.2). Overall, more pollen

Figure 3.1. Average (\pm SE) a) pollen production per flower and b) pollen remaining in anthers for the five species in the staking experiment. In both panels, closed symbols represent species with compact panicles and open symbols depict those with diffuse panicles. In panel b, the three lines represent 1% (dotted line), 5% (short-dashed line), and 10% (long-dashed line) of pollen remaining. See Table 3.2 for the statistical details for pollen remaining.

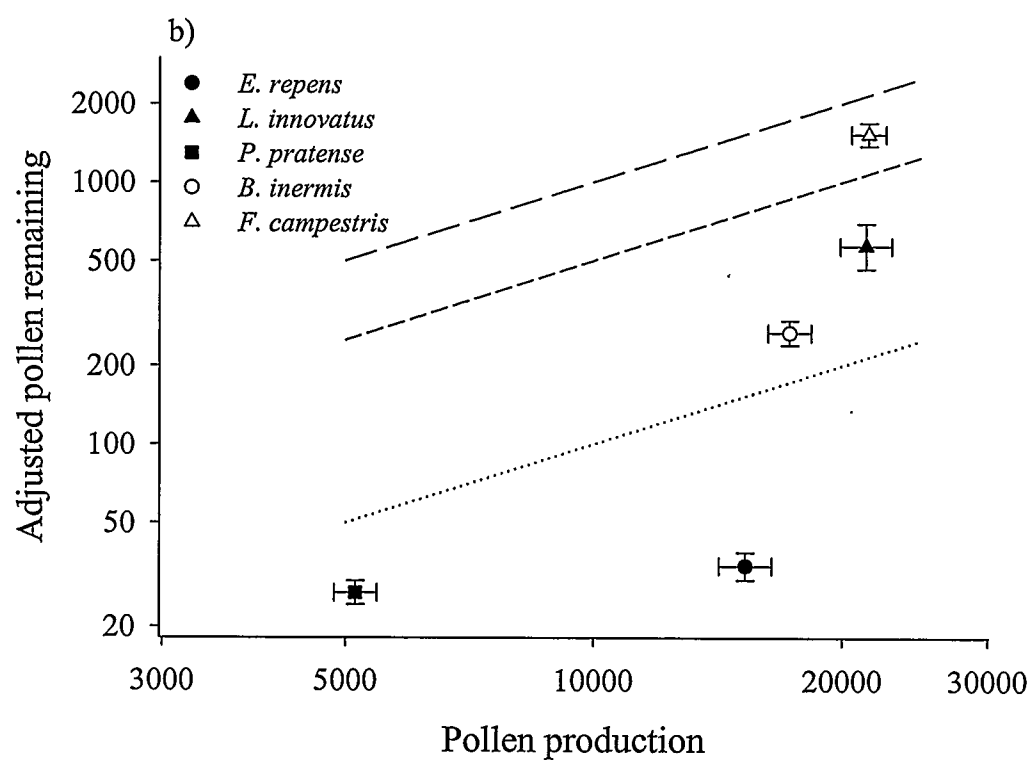
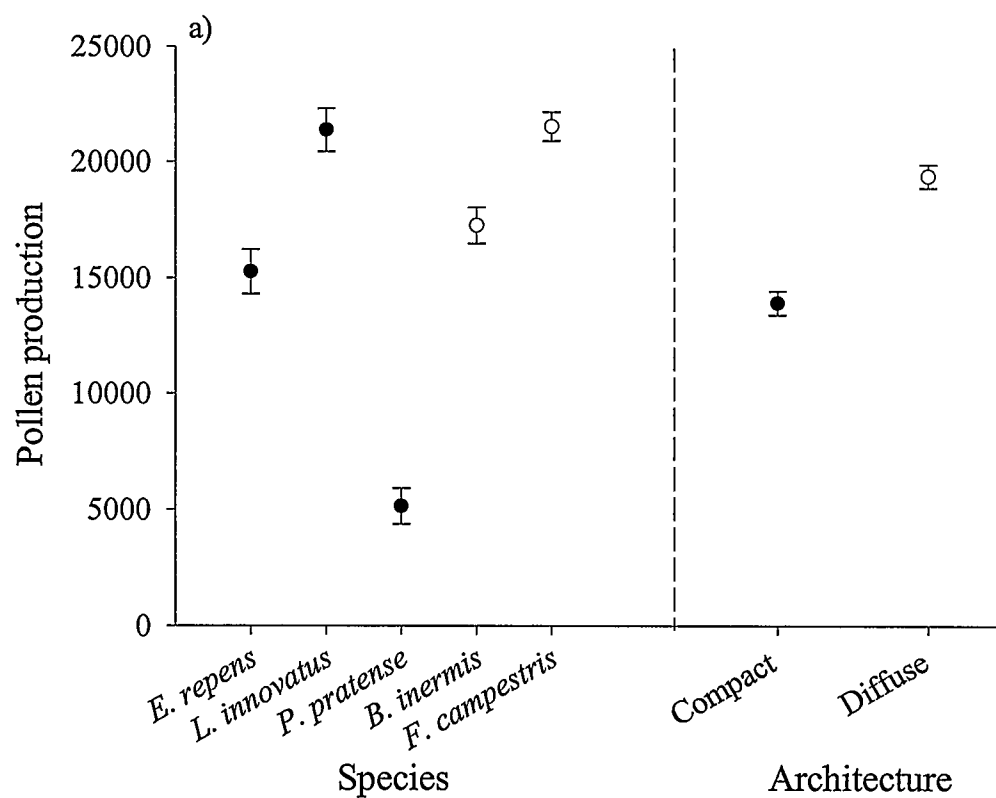


Table 3.2. Summary of general linear models of the influences on pollen remaining in anthers and pollen receipt during the staking experiment.

Source of variation		Pollen remaining in anthers	Pollen receipt by stigmas
Architecture		$F_{1,826}=351.9$ ***	$F_{1,1310}=8.7$ **
Species (Architecture)		$F_{3,608}=47.8$ ***	$F_{3,528}=50.1$ ***
Treatment	Natural v. Control	$F_{1,313}=0.22$	$F_{1,311}=0.04$
	N+C v. Manipulated	$F_{1,305}=3.73$	$F_{1,306}=20.98$ ***
Treatment x	N+C v. M Compact	$F_{1,294}=3.88$ *	$F_{1,292}=2.99$
Architecture	N+C v. M Diffuse	$F_{1,315}=0.64$	$F_{1,313}=21.43$ ***
Treatment x Species (Architecture)		$F_{6,306}=0.9$	$F_{6,291}=0.5$
Mean wind speed		$F_{1,1787}=14.7$ ***	$F_{1,1544}=0.01$
Mean wind speed x Architecture		$F_{1,1791}=15.3$ ***	
Mean wind speed x Species (Architecture)		$F_{3,1766}=19.7$ ***	$F_{4,1676}=4.6$ **
Position		$F_{2,1683}=0.7$	$F_{2,1664}=0.8$
Position x Architecture		$F_{2,1683}=4.8$ **	$F_{2,1664}=5.0$ **
Count			$F_{1,1674}=8.3$ **
Count x Architecture			$F_{1,1318}=16.26$ ***
Count ²			$F_{1,1692}=6.8$ **

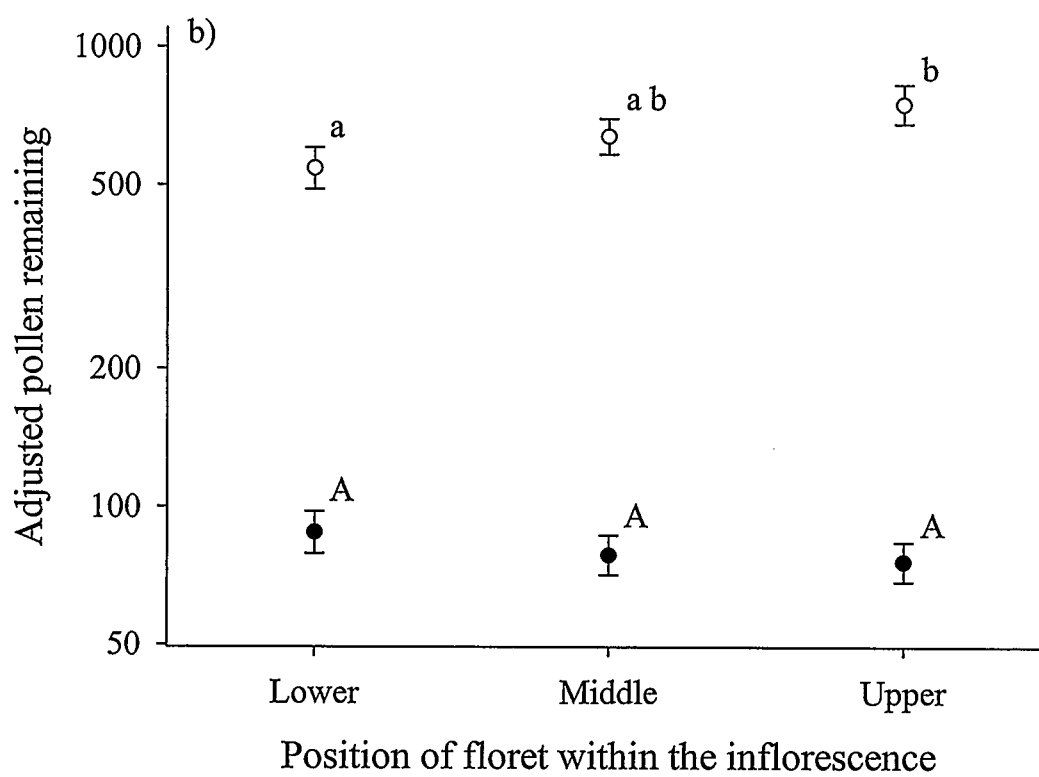
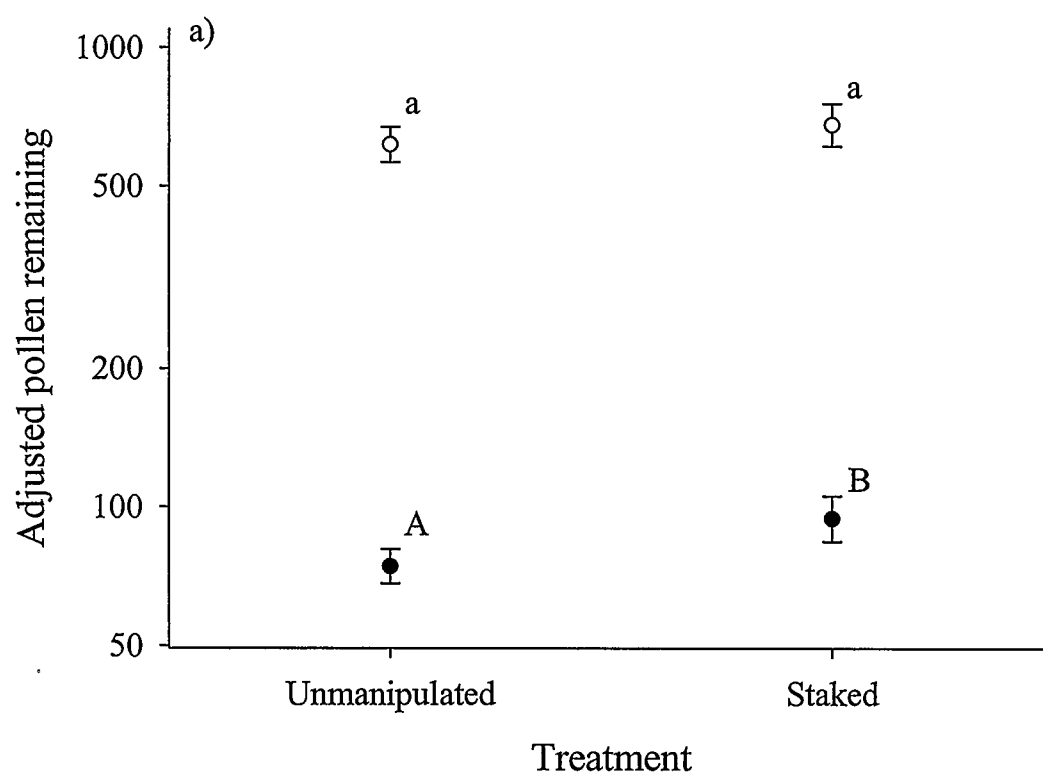
* $P<0.05$, ** $P<0.01$, *** $P<0.001$

remained in anthers of diffuse panicles than of compact panicles, even after accounting for differences in production (Fig. 3.1b). 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). Within each inflorescence type, all species differed significantly in the amount of pollen remaining, except *E. repens* and *P. pratense*.

Inflorescence staking had mixed effects on pollen removal (Table 3.2, Fig. 3.2a). The quantity of pollen remaining in the anthers did not differ significantly between control and natural plants (Table 3.2), 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 3.2); however, this result obscures differential responses by species with compact versus diffuse inflorescences. In the three compact species, staked plants had significantly more pollen remaining in anthers than unmanipulated plants, but staking did not affect removal from diffuse species (treatment x architecture contrast, Table 3.2; Fig 3.2a). These differences were consistent features of compact and diffuse inflorescences, as pollen remaining in anthers did not differ significantly between species within architectures (Table 3.2).

A floret's position within the inflorescence affected pollen removal for diffuse inflorescences, but not for compact inflorescences (position x architecture, Table 3.2; Fig 3.2b). Within diffuse panicles, significantly more pollen remained in anthers of upper florets than in lower florets (upper vs. lower: $t_{1727} = -2.74$, $P < 0.05$), but middle florets did not differ significantly from these extremes (middle vs. lower: $t_{1718} = -1.44$, $P > 0.5$; upper vs. middle: $t_{1693} = -1.56$, $P > 0.5$).

Figure 3.2. Influences of a) treatment in the staking experiment and b) floret position on the (mean \pm SE) pollen remaining in anthers for 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 3.2 for statistical details.



Daily mean wind speed affected pollen removal failure differently for the species within each architecture, and generated different overall responses for the two architectures (Table 3.2, Fig. 3.3). The pollen remaining in anthers varied significantly with mean wind speed for only two species, decreasing for *L. innovatus* ($b \pm SE = -0.0816 \pm 0.222$, $t_{1843} = -4.87$, $P < 0.0001$, Fig. 3.3a), and increasing for *B. inermis* ($b \pm SE = 2.9276 \pm 0.5585$, $t_{1792} = 5.27$, $P < 0.0001$, Fig. 3.3b). 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 my ability to detect the effect of wind speed for these species.

3.3.2.2 Pollen receipt

Pollen capture by stigmas varied significantly among inflorescence architectures, among species within architectures, and among flowers at different positions within inflorescences (Table 3.2). In general, stigmas on compact panicles captured more pollen than stigmas on diffuse panicles (Fig. 3.4). Within each inflorescence type, all species differed significantly in the amount of pollen captured, except *E. repens* and *L. innovatus*. Pollen capture varied with floret position within the inflorescence for species with diffuse panicles, but not for those with compact inflorescences (position \times architecture, Table 3.2; Fig. 3.4b). For diffuse panicles, stigmas at upper positions captured significantly more pollen than stigmas at lower positions ($t_{1785} = -2.78$, $P < 0.05$), but middle florets did not differ from upper or lower florets (middle vs. lower, $t_{1602} = -1.47$, $P > 0.5$; upper vs. middle, $t_{1653} = -1.44$, $P > 0.5$).

Staking strongly affected pollen capture (Table 3.2, Fig. 3.4a). In contrast to control and natural plants, which captured equivalent amounts of pollen, manipulated plants captured significantly less pollen. This effect was strongest for the two species with diffuse panicles, for which staking significantly reduced pollen receipt (Table 3.2,

Figure 3.3. Interacting effects of mean wind speed during the staking experiment on the mean (\pm SE) pollen remaining in anthers for grass species with a) compact or b) diffuse panicles. Each mean represents a different sampling day. The fitted lines are based on a repeated-measures analysis (see Table 3.2 for statistical details).

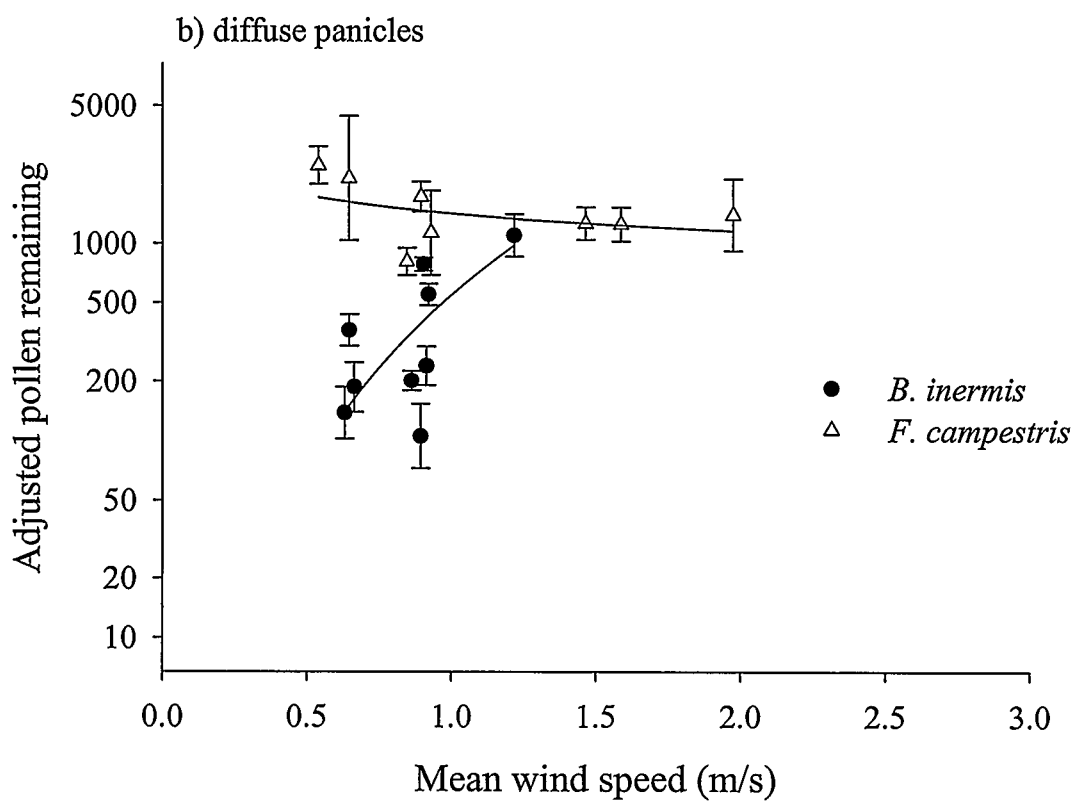
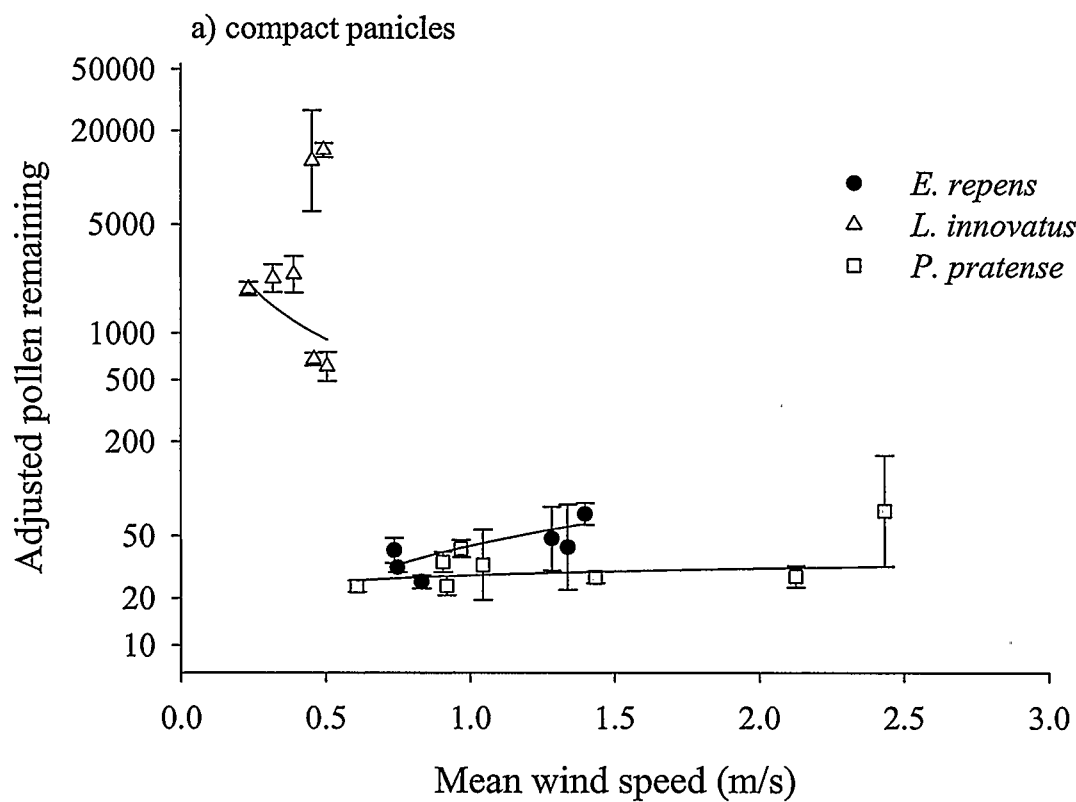


Figure 3.4. Influences of a) treatment in the staking experiment and b) floret position on mean (\pm SE) pollen receipt by stigmas on compact panicles (●) and diffuse panicles (○). Letters indicate the outcomes of Dunn-Šidák multiple comparisons among position for each architecture, with capital letters for compact panicles and lower-case letters for diffuse panicles. See Table 3.2 for statistical details.

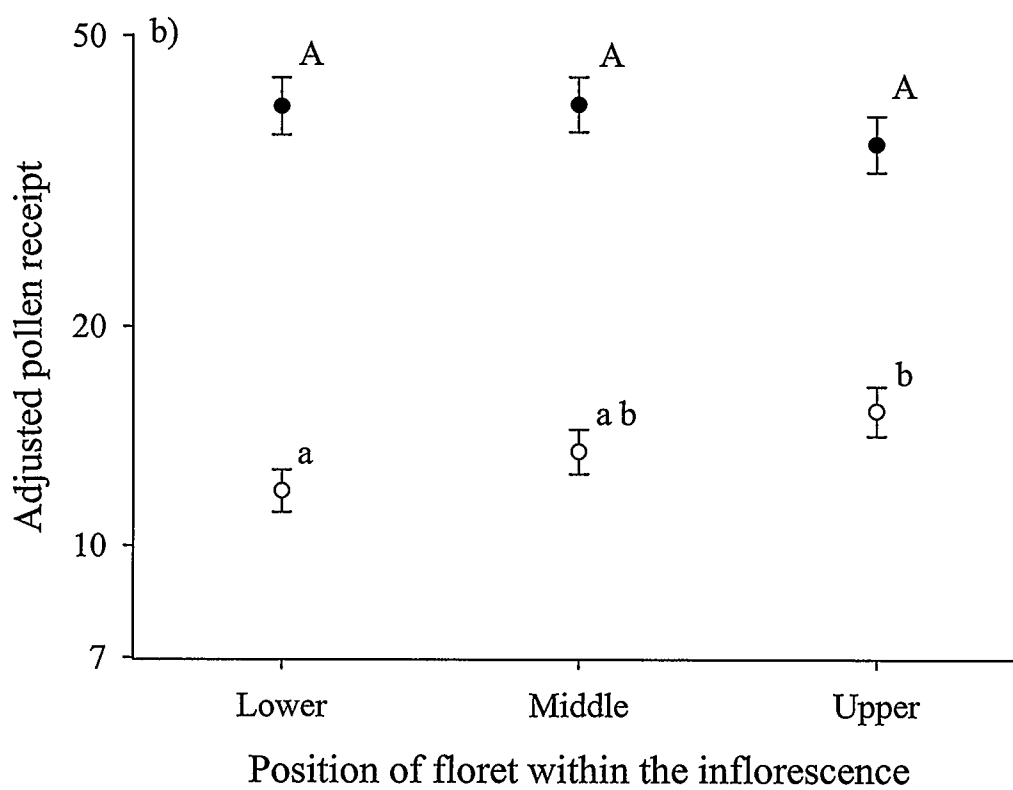
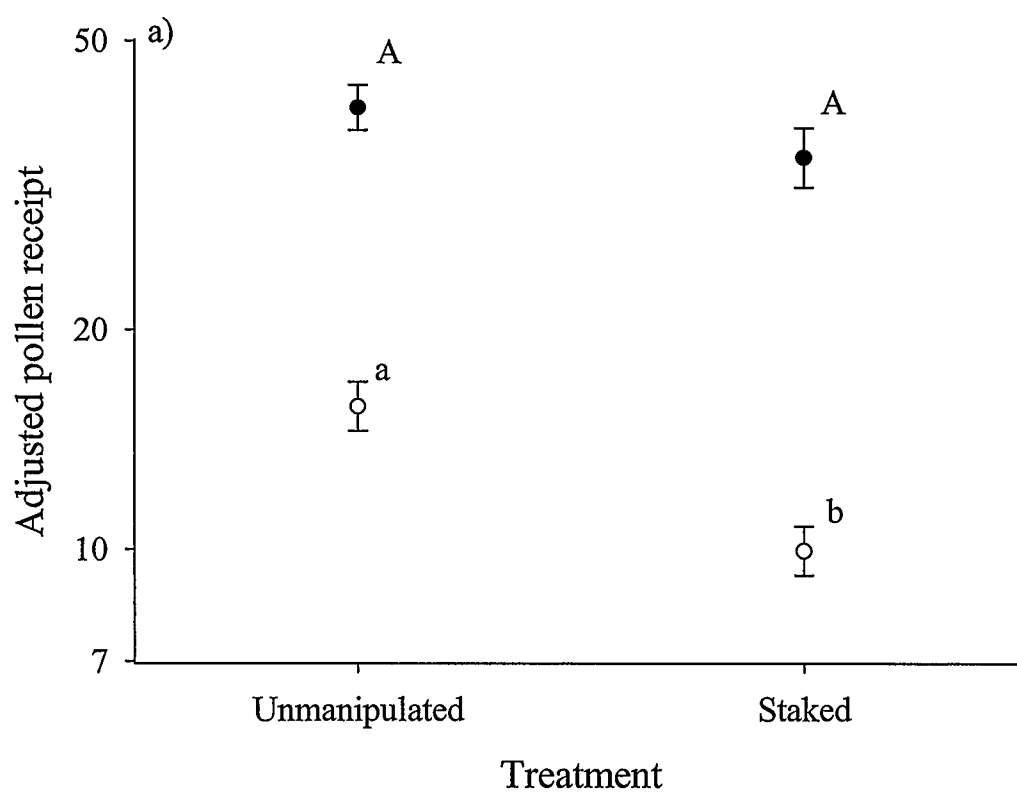


Fig. 3.4a), whereas staking did not significantly affect pollen receipt for compact species (Table 3.2; Fig. 3.4a). These differences were consistent features of compact and diffuse inflorescences, as pollen capture did not differ significantly between species within architectures (Table 3.2).

Daily mean wind speed affected pollen capture differently for the species within each architecture (Table 3.2; Fig. 3.5). Although none of the partial regression coefficients for the effect of wind speed differ significantly from zero, they differ significantly between species pairs within each architecture. Of the diffuse species, pollen capture in *B. inermis* varied negatively with mean wind speed, whereas pollen capture varies positively with mean wind speed for *F. campestris*, generating a significant difference between the partial regression coefficients ($t_{1355} = -2.91, P < 0.05$). For the compact species, a negative association for *L. innovatus* and a positive association for *E. repens* generates the interaction ($t_{1749} = 2.89, P < 0.05$).

The measure of pollen availability (the proportion of experimental plants in flower), and its interaction with architecture both significantly affected pollen capture (Table 3.2; Fig 3.6). In particular, pollen capture varied significantly with the proportion of plants flowering for the compact species ($F_{1, 1733} = 13.76, P < 0.001$); but not for the diffuse species ($F_{1, 1579} = 4.00, P > 0.05$). For both architecture types, the square of the proportion of plants flowering significantly affected capture (Table 3.2), indicating saturating benefits of increased pollen availability. Mean distance to neighbours, and interactions involving it, never significantly influenced pollen receipt, and were subsequently dropped from the analyses.

3.3.3 Exposure duration

The staking experiment for *F. campestris* included a comparison between two exposure durations for anthers and stigmas. However, exposure for 3 or 5 h did not affect

Figure 3.5. Interacting effects of mean wind speed during the staking experiment on mean (\pm SE) pollen receipt for grass species with a) compact or b) diffuse panicles. Each mean represents a different sampling day. The fitted lines are based on a repeated-measures analysis (see Table 3.2 for statistical details).

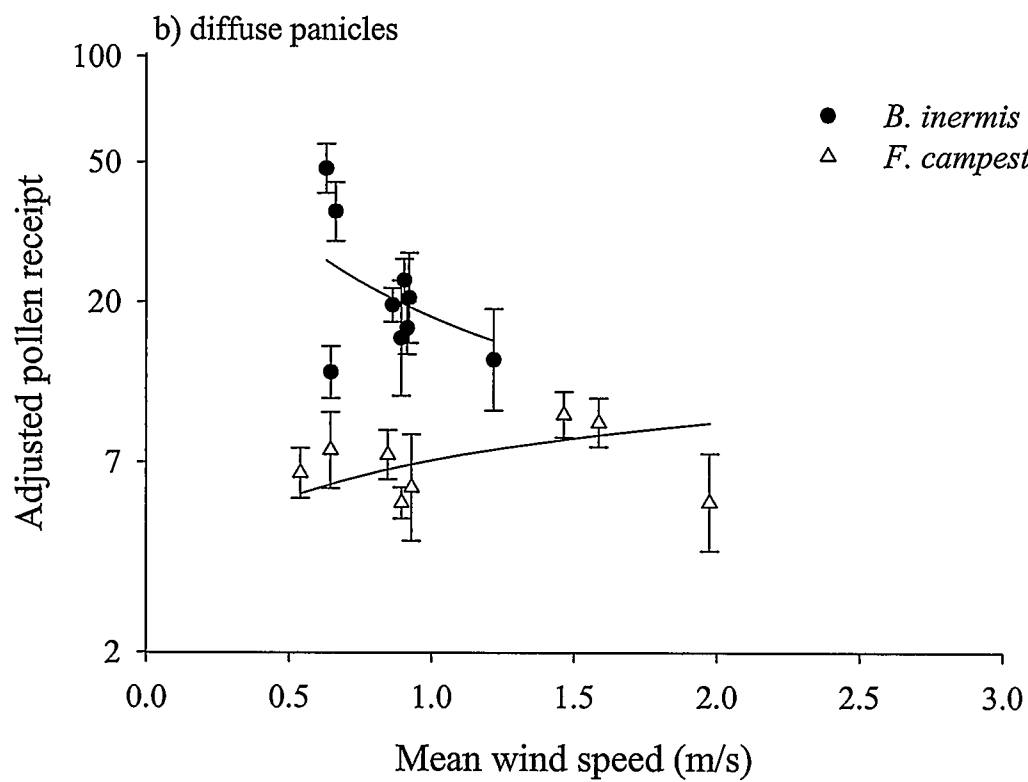
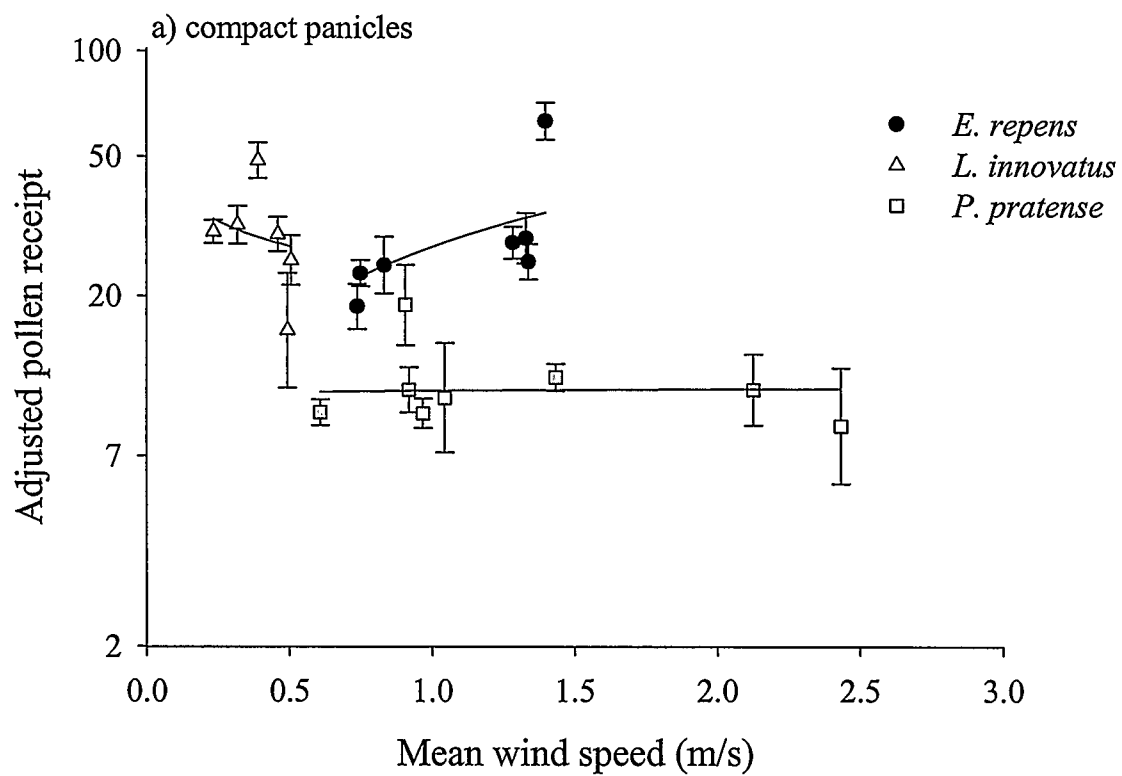
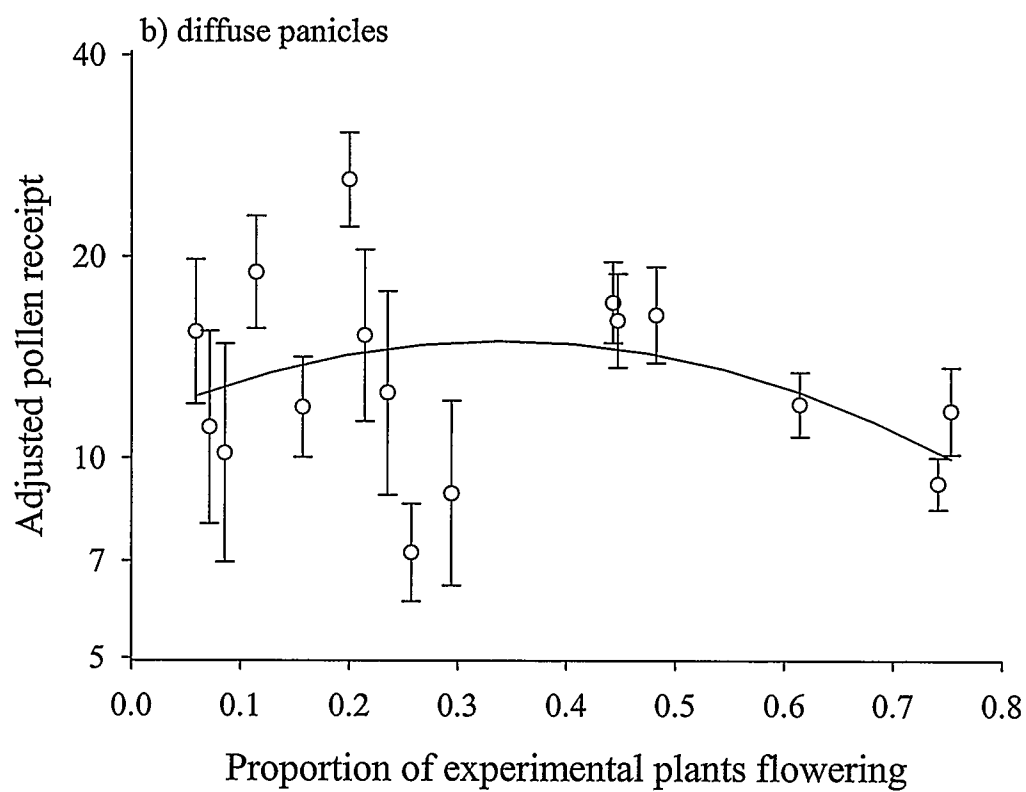
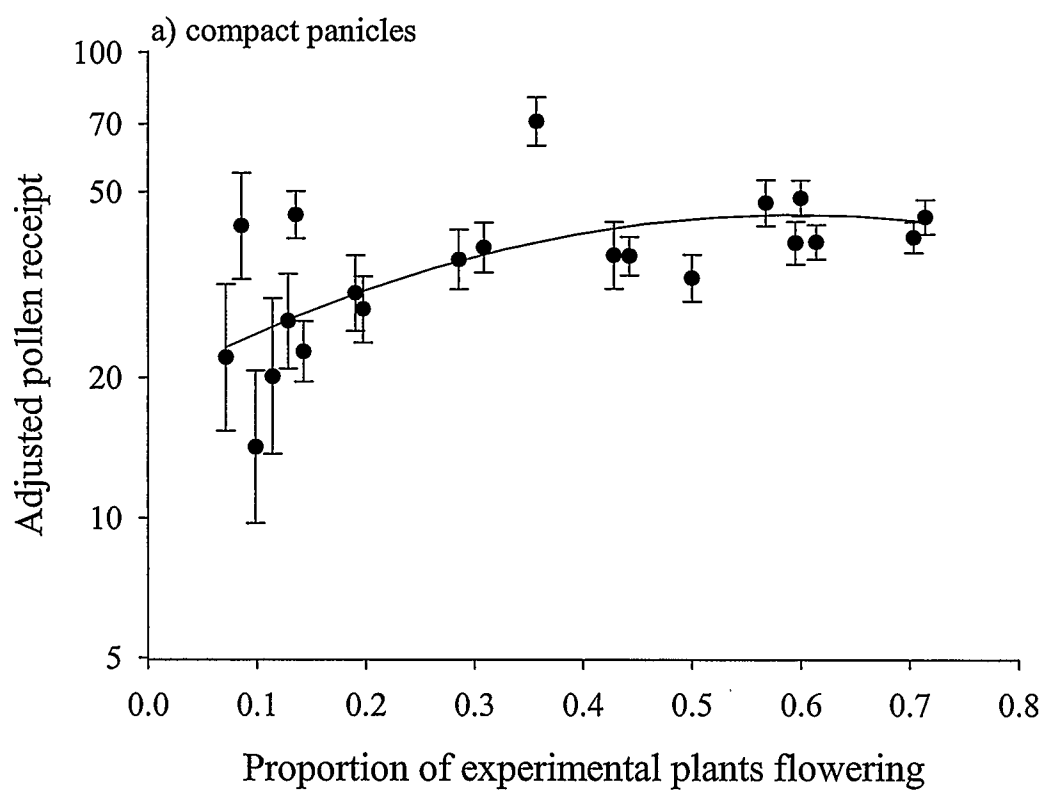


Figure 3.6. The relation of the pollen captured by stigmas of grass species with a) compact and b) diffuse panicles to the proportion of conspecific plants in flower during the staking experiment. The fitted lines depict quadratic regressions based on a repeated-measures analysis (compact, $y = 2.95 + 2.85x - 2.39x^2$; diffuse, $y = 2.43 + 1.63x - 2.39x^2$). See Table 3.2 for statistical details.



either the amount of pollen remaining in anthers ($F_{1, 698} = 0.47, P > 0.4$), or the number of pollen grains captured by stigmas ($F_{1, 690} = 2.41, P > 0.1$). This result implies that, at least for this species, pollination occurred within the first three hours of floret opening.

3.3.4 Aggregated inflorescences

3.3.4.1 Pollen production and removal failure

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 ($t_{25} = -3.26, P < 0.005$), so that staminate flowers produce about twice as much pollen per flower (Fig. 3.7a). *Bromus inermis* flowers produce significantly more pollen than both perfect and staminate flowers of *A. nitens* (Fig. 3.7a; *B. inermis* vs. *A. nitens* perfect: $t_{68} = 13.26, P < 0.0001$; *B. inermis* vs. *A. nitens* staminate: $t_{68} = 10.40, P < 0.0001$).

Bromus inermis and *A. nitens* had different amounts of pollen remaining in anthers (Table 3.3, Fig. 3.7b). Despite its greater pollen production, much less pollen remained in the anthers of *B. inermis* 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 significantly affected pollen removal consistently for both species (Table 3.3, Fig. 3.8). 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 than control and natural plants.

Figure 3.7. Average (\pm SE) a) pollen production per flower and b) pollen remaining in anthers for the two species used in the inflorescence aggregation experiment. In both panels, circles represent *B. inermis* and triangles depict *A. nitens*. *Anthoxanthum nitens* flowers are either perfect or staminate, whereas *B. inermis* flowers are perfect. In panel b, the three lines represent 1% (dotted line), 10% (short-dashed line), and 20% (long-dashed line) of pollen remaining. See Table 3.2 for the statistical details for pollen remaining.

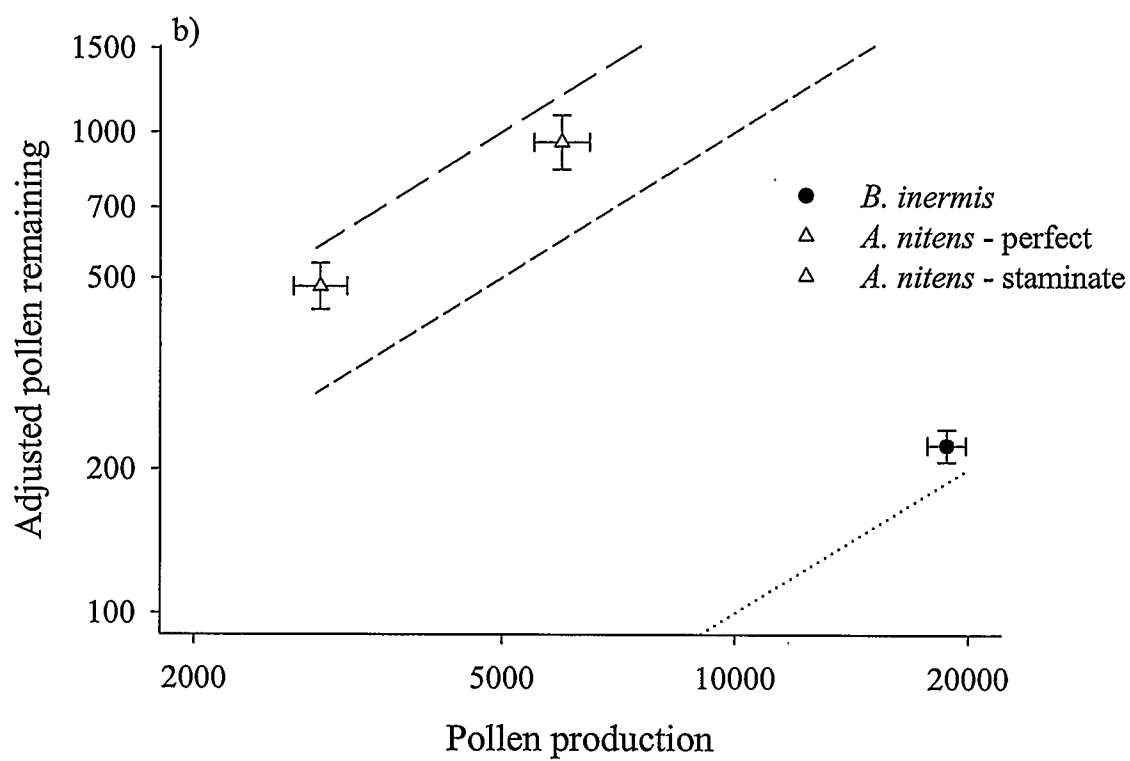
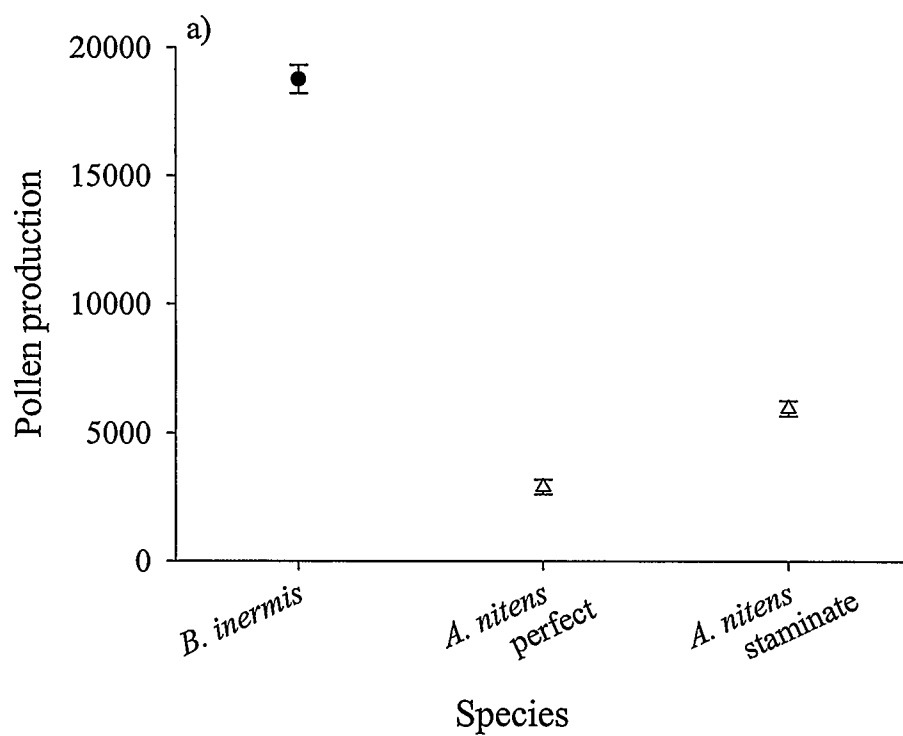
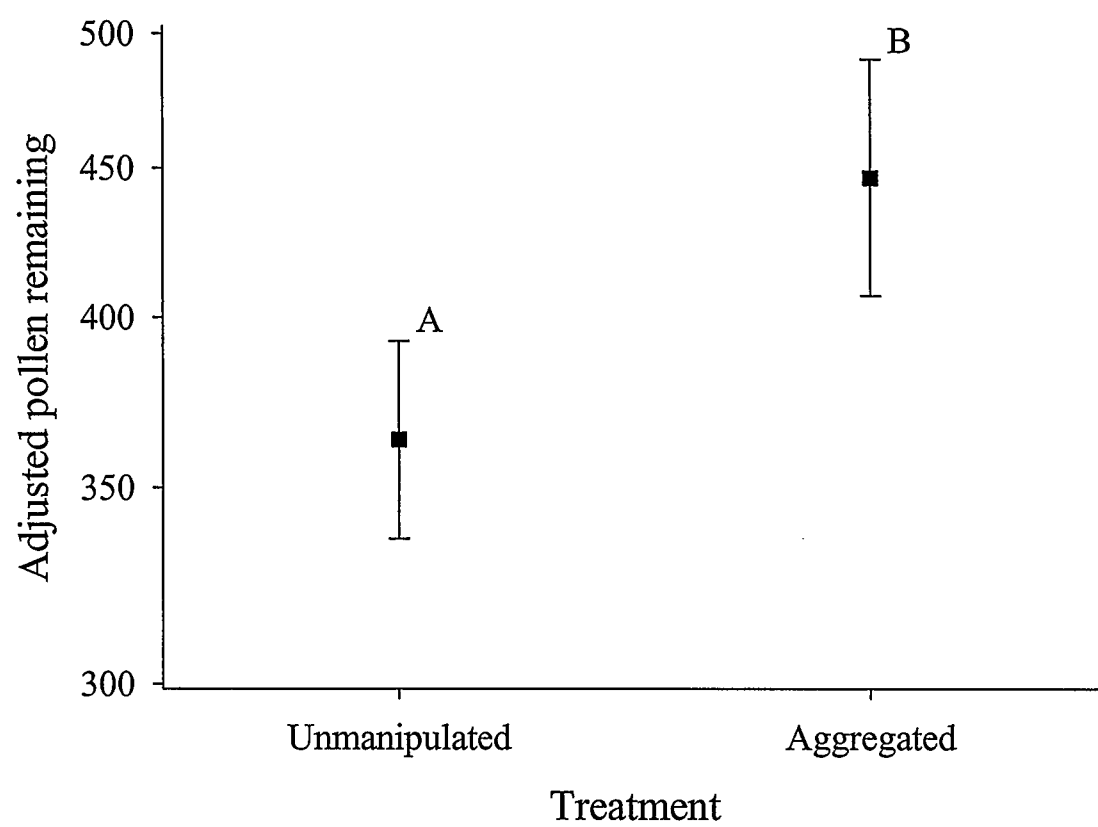


Table 3.3. Summary of general linear models of the influences on pollen remaining in anthers and pollen receipt during the inflorescence aggregation experiment.

Source of variation		Pollen remaining in anthers	Pollen receipt by stigmas
Species		$F_{1,204}=63.5$ ***	$F_{1,217}=874.8$ ***
Treatment	Natural v. Control	$F_{1,776}=0.32$	$F_{1,138}=0.26$
	N+C v. Manipulated	$F_{1,773}=9.3$ **	$F_{1,67}=8.94$ **
Treatment x Species		$F_{2,203}=0.4$	$F_{2,108}=19.8$ ***
Mean wind speed		$F_{1,693}=10.1$ **	$F_{1,563}=12.2$ ***
Mean wind speed x Treatment		$F_{2,693}=4.0$ *	
Flower type (Species)		$F_{1,721}=32.15$ ***	
Position			$F_{2,455}=7.0$ ***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Figure 3.8. Influences of treatment during inflorescence aggregation experiment on the (mean \pm SE) pollen remaining in anthers for both species combined. Letters indicate the outcomes of Dunn-Šidák multiple comparisons. See Table 3.2 for statistical details.



For both species, fewer pollen grains remained in anthers as mean wind speed increased (Table 3.3; Fig. 3.9a). This effect primarily involved plants with aggregated inflorescences ($b \pm \text{SE}$: -1.13 ± 0.27 , $t_{704} = -4.23$; $P < 0.0001$; Fig. 3.9b), as pollen removal did not vary significantly with mean wind speed for unmanipulated plants (control plants: -0.33 ± 0.28 , $t_{677} = -1.16$, $P > 0.5$; natural plants: -0.08 ± 0.28 , $t_{699} = -0.28$, $P > 0.5$; Fig. 3.9b). Thus low wind speeds seem least favorable for pollen removal in manipulated plants.

3.3.4.2 Pollen receipt

Pollen receipt by *B. inermis* and *A. nitens* responded differently to inflorescence aggregation (Table 3.3, Fig. 3.10a). Stigmas on plants in control and natural treatments captured equivalent amounts of pollen for both species (*B. inermis*: $t_{125} = 0.95$, $P > 0.5$; *A. nitens*: $t_{144} = -0.05$, $P > 0.5$). For *B. inermis*, stigmas on aggregated plants captured significantly more pollen than stigmas on unmanipulated plants ($t_{124} = 6.93$, $P < 0.0001$; Fig 3.10a). In contrast, stigmas on aggregated plants of *A. nitens* captured significantly less pollen than those on unmanipulated plants ($t_{28.4} = -2.28$, $P < 0.05$; Fig 3.10a).

Pollen capture varied significantly with both a floret's position within the inflorescence (Table 3.3, Fig. 3.10b) and mean wind speed (Table 3.3, Fig. 3.11). Lower and middle florets received significantly less pollen than upper florets (middle vs. lower: $t_{377} = -1.07$, $P > 0.5$; upper vs. middle: $t_{660} = -2.45$, $P < 0.05$; upper vs. lower: $t_{421} = -3.70$, $P < 0.001$). For both species, pollen capture varied positively with mean daily wind speed.

Figure 3.9. Interacting effects of mean wind speed during the inflorescence aggregation experiment on the mean (\pm SE) pollen remaining in anthers for a) the two species and b) the three treatment groups. Each mean represents a different sampling day. The fitted lines are based on a repeated-measures analysis (see Table 3.2 for statistical details), In panel b the fitted lines represent manipulated plants (long-dashed line), control plants (short-dashed line), and natural plants (dotted line).

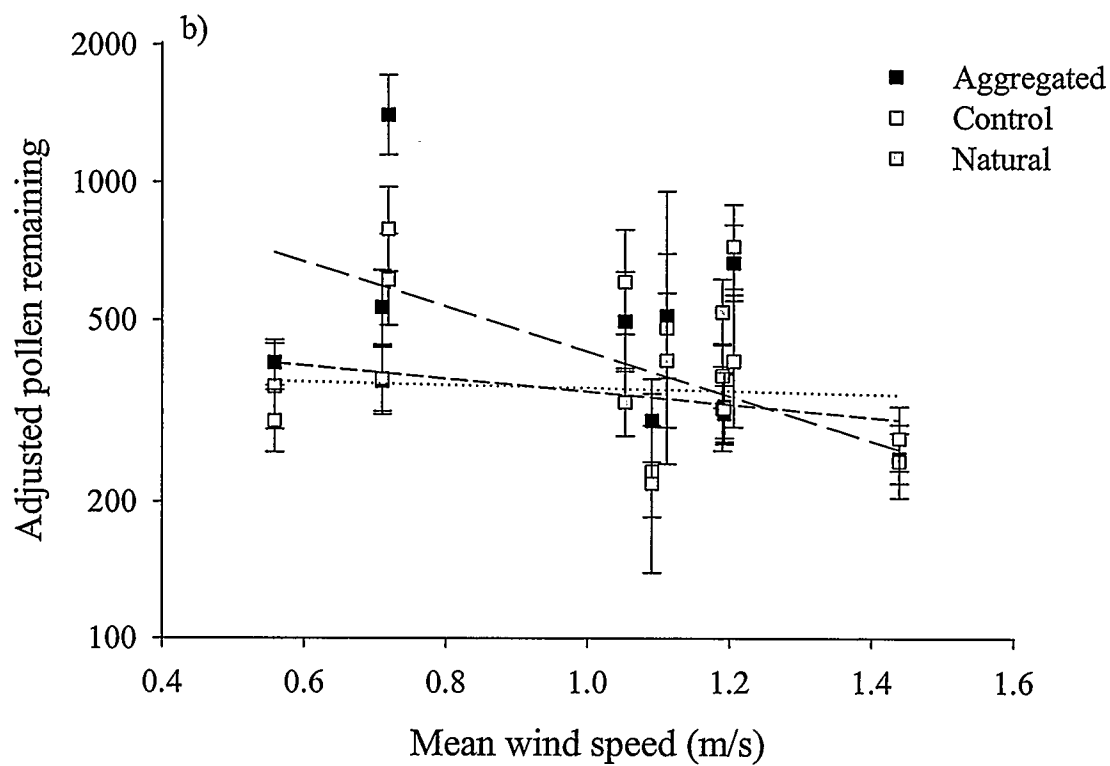
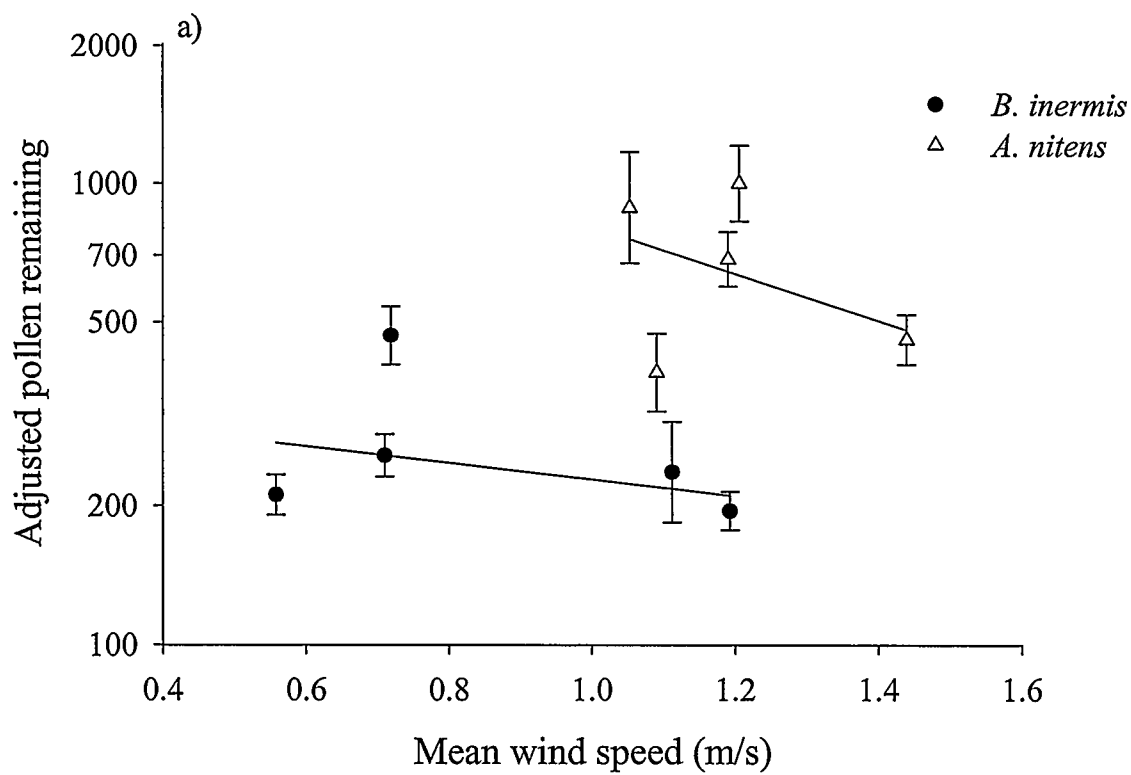


Figure 3.10. Influences of a) treatment during the inflorescence aggregation experiment and b) floret position on mean (\pm SE) pollen receipt by stigmas for *B. inermis* (●) and *A. nitens* (△). Letters indicate the outcomes of Dunn-Šidák multiple comparisons among position for each species, with capital letters for *B. inermis* and lower-case letters for *A. nitens*. See Table 3.2 for statistical details.

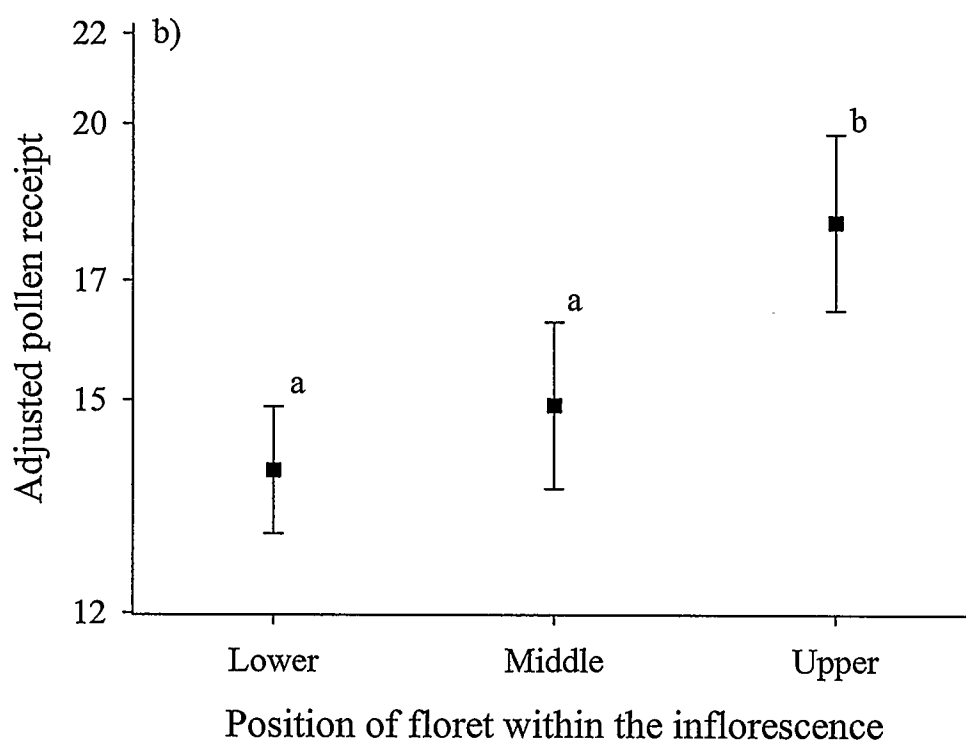
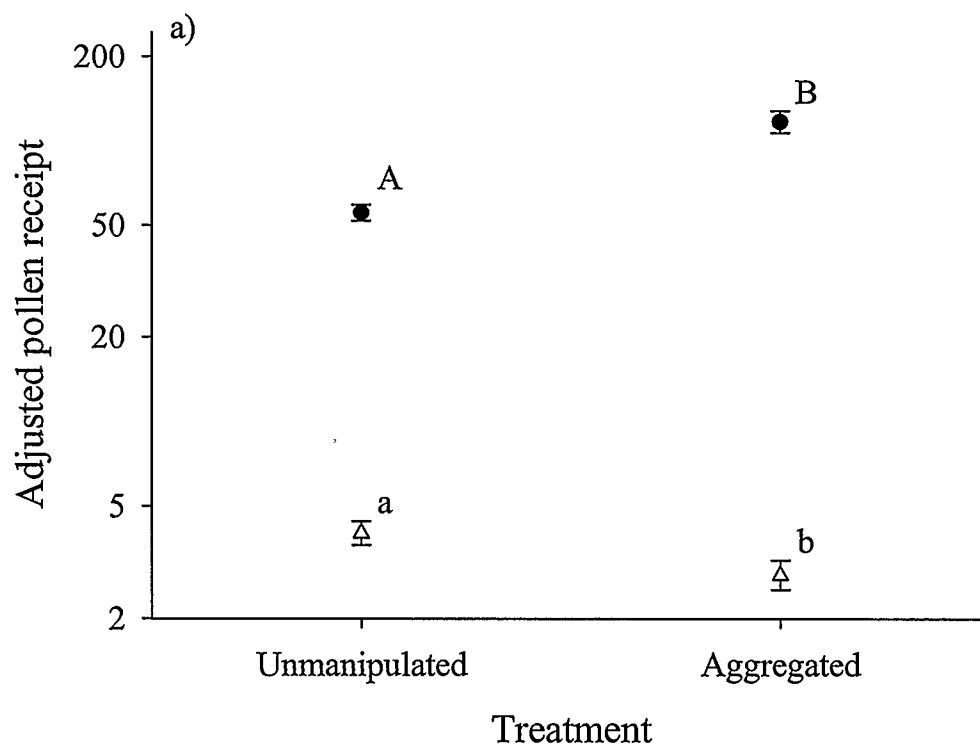
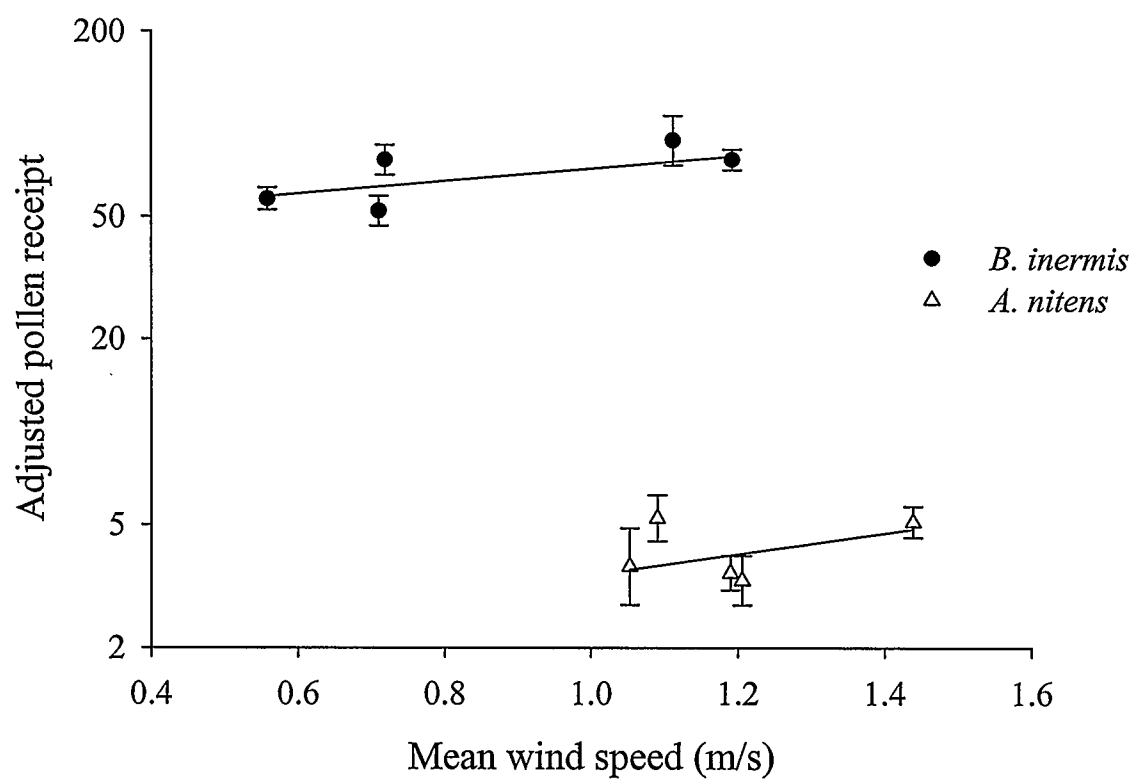


Figure 3.11. Interacting effects of mean wind speed during the inflorescence aggregation experiment on mean (\pm SE) pollen receipt for *B. inermis* (●) and *A. nitens* (\triangle). Each mean represents a different sampling day. The fitted lines are based on a repeated-measures analysis (see Table 3.2 for statistical details).



3.4 Discussion

My experiments examined the influence of two features of inflorescence structure on the pollen-transfer efficiency of grasses. The staking experiment limited oscillation of the entire inflorescence and revealed that compact and diffuse architectures were affected differently by the manipulation, and the response differed for pollen removal and pollen receipt. The inflorescence aggregation experiment disrupted associations between architecture and floret, pollen and stem characteristics, which increased pollen remaining in anthers, but had inconsistent effects on pollen receipt, perhaps due to differences in flower size. These results are generally consistent with current understanding of the aerodynamics of wind pollination. Overall, I found compact species had less pollen remaining in their anthers than diffuse species, and they captured more pollen than diffuse species.

3.4.1 Effects of constrained motion of inflorescences

The staking experiment constrained the motion of the entire inflorescence, by preventing oscillation. Niklas (1987) showed that compact and diffuse species have different natural frequencies and displacements of oscillation between 10 and 120 cm/s (frequency range: compact species: $0.57 - 1.27 \text{ s}^{-1}$, diffuse species: $0.33 - 0.66 \text{ s}^{-1}$; displacement range: compact species: 2.3 - 0.8 cm; diffuse species: 10 - 3.3 cm), and therefore the consequences of constrained mobility may be expected to vary for species with the two types of architectures. In particular, limited motion of the inflorescence isolates the effect of the streamline deflections created around the panicle from the effect of harmonic motion on pollination.

The staking experiment revealed that different inflorescence architectures do employ oscillatory motion in contrasting ways to affect pollen removal and capture under natural conditions. Staking reduced pollen removal in compact species, because anthers

may have experienced the wind velocity needed to dislodge pollen less frequently. In contrast, pollen removal from diffuse species was not affected by staking. This lack of influence probably resulted because only the central rhachis of the inflorescence was tied to the stakes, so that secondary branches could still move in the wind. Such secondary mobility may enable enough motion and adequate wind velocity for pollen grains to be removed from the anthers of diffuse species.

In contrast to pollen removal, pollen receipt by species with diffuse architectures was reduced by the lack of oscillatory motion, whereas that of compact species was unaffected. Diffuse plants capture pollen by inertial collision, and maximize the amount captured by sweeping through and sampling much of the surrounding airstream (Niklas 1987). By being unable to oscillate, staked plants with diffuse panicles could sample much less air, therefore reducing the potential for inertial collision with pollen. Pollen capture by compact species was not adversely affected by staking. The inflorescence dimensions for all three compact species (Table 3.1) are large enough to create vortices at the wind speeds that they experienced (Vogel 1983a). Given such leeward turbulence, pollen grains probably could recirculate within this region, and settle onto stigmas. However, the staked plants could not be thrust into this region by oscillatory motion, and so pollen capture may be reduced slightly.

The staking experiment revealed that different inflorescence architectures employ alternate methods of facilitating pollen removal and pollen import. Pollen removal from species with diffuse inflorescences was unaffected by the lack of oscillatory motion, but pollen capture was adversely affected. Thus, the lower flexural rigidity of diffuse species (Table 3.1) may allow them to oscillate with a lower frequency, to maximize pollen capture. However, reduced flexural rigidity may come at a cost to the mechanical stability of the plant. In the compact species, pollen removal decreased slightly with the loss of mobility, but capture was unchanged. Thus, these plants may maintain more rigid

stems, and compromise some pollen removal. This concession may be negligible, as I found almost all the pollen removed from two of the compact species. Interestingly, the one compact species that has much higher removal failure (*L. innovatus*) also has the least rigid stem of the compact species (see Table 3.1).

3.4.2 Effects of inflorescence aggregation

The experiment involving inflorescence aggregation assessed whether the architectures of diffuse inflorescences are associated with pollen characteristics in a way that maximizes pollen removal and receipt. Aggregated inflorescences may create a single large boundary layer around the entire inflorescence, whereas in plants with diffuse architectures each spikelet may cause its own, thinner, boundary layer. Also, the constrained motion of branches increases the correlation of spikelet position and reduces the independent nature of spikelets in plants with diffuse architectures.

Plants with aggregated inflorescences had more pollen remaining in their anthers (see Fig. 3.8). In species with diffuse inflorescences, anthers generally hang below the florets, probably avoiding the boundary layer surrounding the floret. The reduction in pollen removal from manipulated plants may have resulted from spikelets interfering with each other, retaining anthers within the boundary layer and restricting their movement in wind. Such interference is supported by the interacting effect of mean wind speed on the treatment groups (see Fig. 3.9). Low wind speeds were least favorable for pollen removal in manipulated plants, probably because of proportionally thicker boundary layers. Species with compact inflorescences may avoid this problem with alternate mechanisms for getting anthers beyond the boundary layer of the plant. For example, in *Phleum pratense*, the filaments supporting the anthers project horizontally from the inflorescence.

Inflorescence aggregation increased pollen capture by *B. inermis*, but decreased capture by *A. nitens*. The larger boundary layer created around aggregated inflorescences allows only larger pollen with more inertia to pass through and collide with stigmas. *Bromus inermis* has relatively large pollen, whereas *A. nitens* has very small pollen (see Table 3.1). This difference may explain why manipulated *A. nitens* plants captured less pollen than unmanipulated plants. Aggregated inflorescences may have been more prone to geitonogamous pollination as the anthers hang in close proximity to neighbouring flowers. Geitonogamy may be particularly pronounced for *B. inermis*, which has larger spikelets, anthers and stigmas than the small-flowered *A. nitens*.

3.4.3 Effects of plant height and floret position within the inflorescence

In wind-pollinated species, male function has been predicted to increase with plant height, as taller plants experience greater wind speeds for pollen removal, and pollen released at a height travels farther (Burd & Allen 1988; Okubo & Levin 1989; Young & Schmitt 1995). However, stigmas below the height of the pollen source may be more effective for pollen capture because of the gravitational effect on airborne pollen (Whitehead 1983). Given this expectation, the absence of a significant effect of plant height on pollen removal and capture in any of the experiments is surprising (as a consequence this variable was removed from the analyses). The variation in height within a given population may not be sufficient to affect pollination significantly, or height may be correlated with other traits that obscure its effect.

In contrast to plant height, the position of the floret within the inflorescence significantly affected pollination of diffuse species in both experiments, but had no influence on compact species (staking experiment only). In diffuse species, upper florets captured more pollen (see Fig. 3.4b, and 3.10b). In unconstrained plants, upper florets may capture more pollen because they move through a larger arc during oscillation.

However, upper florets in staked plants also capture more pollen. The increased elevation of upper florets may result in greater wind speed, which increases the inertia of pollen and decreases the thickness of the boundary layer surrounding the plant, increasing the collection efficiency of the stigma (see eq. 1.7).

These explanations do not clarify why pollen deposition did not vary with a floret's position within compact inflorescences. In wind-tunnel studies of an *S. geniculata* panicle (a compact species) subject to an airflow of 50 cm/s, Niklas (1987) found pollen grains moved over and around a vertical panicle (inclination angle of 90°) and were deflected backwards toward leeward surfaces where they moved downward over floral structures. When the panicle was inclined at 45°, pollen grains tended to travel towards a distal region, and another midway along the length of the leeward side of the panicle. Therefore, the pattern of pollen movement within the eddies around the leeward side of the plant would explain why upper florets in compact species do not capture more pollen than lower florets, and also why capture did not differ significantly between positions.

3.4.4 Effect of pollination environment

The role of mean daily wind speed in affecting pollen removal and receipt at first seems obscure. In the inflorescence aggregation experiment, more pollen was removed during days with stronger winds, as expected. Similarly, more pollen was captured on these days, although it is unclear whether this occurred because of direct effects of increased wind speeds or indirect effects of greater pollen availability due to increased removal. During the staking experiments, mean wind speed affected *L. innovatus* differently than the other compact species (Fig. 3.4 and 3.5). *Leymus innovatus* inhabits woods, where wind speeds are lower than in open habitats (see Fig. 2.1). For this species, higher wind speeds were more favorable for pollen removal. Thus, anthers may

experience velocities sufficient to dislodge pollen grains only periodically. In contrast, the pollen remaining in *B. inermis* varied positively with mean wind velocity during the staking experiment. Because this pattern was evident for only one set of experiments involving *B. inermis*, and one day of higher wind speeds determines the association (see Fig 3.3b and 3.5b), I am inclined to believe that factors other than mean wind speed have increased removal failure and decreased capture. Specifically, few plants flowered on this day, and coincidentally almost all of them were staked plants. Because manipulated plants have more pollen remaining and lower pollen capture (see Fig. 3.2a and 3.4a), they may be causing this anomalous association with wind speed for *B. inermis*.

The relation between the proportion of plants flowering and pollen receipt during the staking experiment suggests that successful pollination depends on pollen availability, but in a complex manner. When few plants flowered, pollen receipt varied extensively (see Fig. 3.6). The variation may be related to wind directionality when a floret flowered and the specific location of neighboring flowering plants. Plants downwind of flowering individuals probably experience relatively high pollen capture compared to plants with no upwind neighbours in flower. The proportional benefit of increased flowering density was greater for compact species than for diffuse species, perhaps because diffuse species sweep a large volume of air and sample a broader range of stratified pollen sources (Niklas 1987). Thus, the difference between minimal pollen availability and slightly more pollen availability is less pronounced. The decelerating relations of pollen removal and receipt to flowering density imply that the benefit attained from greater pollen availability saturates. Interestingly, a plant's distance to its five nearest neighbours did not affect pollination significantly (and was subsequently dropped from the analyses). Therefore, the proportion of plants flowering seems to provide a more relevant measure of pollen availability. Also, these experiments were conducted in populations with small

interplant distances (see Table 3.1), so that the pollen available in the airstream around a plant need not vary directly with its proximity to its five nearest neighbours.

3.4.5 Pollination efficiency

My observations of pollen production, pollen remaining in anthers and pollen deposition on stigmas for unmanipulated plants allow me to calculate pollen-removal failure (percentage of pollen remaining in anthers) and pollen-transfer efficiency (percentage of removed pollen reaching stigmas) for the six study species (see Table 3.4). The proportion of pollen remaining in anthers is lower for compact species than for diffuse species, except for *L. innovatus*, which experienced pollen-removal failure comparable to the diffuse species (Table 3.4). *Leymus innovatus* grows in woods, where wind speeds are typically much lower than in open areas (see Figure 2.1); therefore it may experience the conditions necessary for pollen removal less often than species in open habitats. It is not immediately clear why the diffuse species have higher pollen removal failure. *Anthoxanthum nitens* is a short grass (see Table 3.1), and thus also experienced much lower wind speeds than the other species in this experiment (see Figure 2.1). *Festuca campestris* was the only species in these experiments whose flowering was not tightly synchronized. Most *F. campestris* flowers tend to open during morning, but some flowers open during early evening. Perhaps pollen adheres more strongly to anthers to stagger its removal. Also, periodically I observed one anther in a *F. campestris* floret that did not drop down and was possibly “trapped” on the bracts. In this situation, the anther typically retained its deep colouration, indicating that it had not dried out and dehisced. This may contribute to the large amount of undispersed pollen in *F. campestris*.

Table 3.4. Mean (95% C.I.) pollen-removal failure (percentage of pollen remaining in anthers) and pollen-transfer efficiency (percentage of removed pollen reaching stigmas) for six grass species. Only unmanipulated plants were included, and confidence intervals were calculated by 10000 bootstrap resamplings of the observations.

Species	Architecture	Percent removal failure	Percent pollen- transfer efficiency
<i>Elymus repens</i>	Compact	0.62 0.33 - 0.97	0.57 0.51 - 0.63
<i>Leymus innovatus</i>	Compact	13.86 10.60 - 17.31	0.57 0.47 - 0.68
<i>Phleum pratense</i>	Compact	1.76 1.08 - 2.49	0.74 0.62 - 0.86
<i>Anthoxanthum nitens</i>	Diffuse	22.53 18.77 - 26.61	0.21 0.16 - 0.26
<i>Bromus inermis</i>	Diffuse	4.49 3.71 - 5.33	0.39 0.32 - 0.46
<i>Festuca campestris</i>	Diffuse	21.22 18.33 - 24.22	0.13 0.11 - 0.15

Pollen-transfer efficiency measures the proportion of removed pollen that actually reaches stigmas on the same or other plants, and ranged from 0.13% for *F. campestris* to 0.74% for *P. pratense* (see Table 3.4). The three compact species realized higher pollen-transfer efficiency than the three diffuse species, perhaps because of higher self-pollination. 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, I could not distinguish self-pollen from outcross-pollen.

It appears that compact species have less removal failure and greater pollen transfer efficiency than diffuse species. No studies have investigated the mechanics of pollen removal from anthers, and clearly we need to learn more about the biology and aerodynamics of pollen removal. As mentioned previously, higher pollen transfer in compact species may be related to the frequency of self-pollination, and appeals for studies investigating the mating systems of grasses with different architectures.

My results contradict several published assertions about the reproduction of wind-pollinated species (also see Harder 2000). Grass flowers have been presumed to produce only single ovules because of the very small chance of multiple pollen grains landing on each stigma (Dowding 1987). Pohl (1929 cited in Linder 1998) suggested that pollen of wind-dispersed species is usually dispersed as single units and that stigmas capture insufficient pollen to fertilize many ovules. Although I found some stigmas that had no pollen on them, the mean number of pollen grains received across all six species was 54.7. Therefore, it seems unlikely that seed-set is pollen-limited in the six species I studied (except possibly *A. nitens*). Honig et al. (1992) found comparable pollen load sizes on stigmas of *Staberoha banksii*, a wind-pollinated species of Restionaceae, and Linder & Midgley (1996) reported large pollen loads on the stigmas of a *Staberoha*, *Restio*, *Leucadendron* and *Cliffortia* species. Those findings point to the need for a more

comprehensive examination of the functional advantage of uni-ovulate carpels and stigmatic pollen load sizes in grasses and other wind-pollinated plants.

The pollen-transfer efficiencies of the six grass species that I studied fall within the range observed for wind- and animal-pollinated plants with granular pollen (see Harder 2000 for data on efficiency in animal-pollinated plants), even though pollen-to-ovule ratios of wind- and animal-pollinated species differ considerably. Cruden (2000) reported a median pollen-ovule ratio of 22150:1 for wind-pollinated plants, and a median of 3450:1 for animal-pollinated species. Furthermore, the ratio of stigmatic pollen loads-to-ovules in animal-pollinated plants is about 8:1 (e.g. Snow 1982; Galen 1992; Bosch & Waser 2001). This ratio is much lower than the 55:1 ratio that I found for grasses, and the similarly high ratios reported by Honig et al. (1992) and Linder & Midgley (1996) for Restionaceae.

What are the implications of relatively high ratios of stigmatic pollen loads to ovules for wind-pollinated plants? Poaceae (and Restionaceae) pollen is always trinucleate, containing twin sperm cells and a vegetative cell (Brewbaker 1967). Trinucleate pollen is a derived condition in the angiosperms (Brewbaker 1967), and contains fully developed mitochondria at the time of dehiscence, allowing for rapid germination on stigmas, but very brief viability (Hoekstra 1979). In wind-pollinated plants, pollen grains arrive independently and so may be drawn more evenly from potential sires than with animal pollination. The above considerations point to the possibility of intense post-pollination competition. Gametophytic competition is most intense when pollen grains arrive roughly synchronously on a stigma (Thomson 1989). I observed that anthesis is highly synchronous within grasses populations (within 20 min), and I have shown that pollination is over fairly quickly (< 5 h). Furthermore, mating is quite local in many grass species (e.g. Bateman 1947; Griffiths 1950; Copeland & Hardin 1970; Rognli et al 2000). These considerations suggest that pollen may indeed arrive

synchronously and may compete vigorously for the single ovule in each floret. Pollen-pollen competition leads to gametophytic selection and can result in greater progeny vigour (Mulcahy & Mulcahy 1987). Thus, given that wind-pollinated species have equivalent pollen-transfer efficiency to animal-pollinated species, the copious pollen production of wind-pollinated taxa probably reflects the intensity of post-pollination competition, rather than the inferiority of wind as a pollen vector.

4 CONCLUDING DISCUSSION

The morphology of angiosperm flowers and inflorescences exhibit incredible interspecific variation. Such diversity likely represents alternative mechanisms that facilitate successful pollination and fertilization. The form and function of flowers has received considerable attention, particularly studies investigating how floral morphology manipulates behaviour of animal pollinators for efficient pollination and mating success (e.g. Wyatt 1982; Bell 1985; Campbell et al. 1996; Harder & Barrett 1996). However, few studies have explicitly investigated the function of floral morphology and inflorescence architecture in wind-pollinated plants.

My studies of grasses reveal strong correlations between various aspects of floral morphology and between features of inflorescence architecture. Associations may be due to selection for efficient pollination, and may represent particular solutions to the aerodynamic requirements of wind pollination. In chapter 2, I identified four distinct categories of flower-inflorescence types, which differ significantly for a variety of morphological traits. Each species has a particular combination of inflorescence type and flower size that may function aerodynamically in a specific manner to facilitate pollination. In chapter 3, I showed that the two inflorescence-type categories (species with diffuse or compact inflorescences) employ different mechanisms to facilitate pollen removal and capture. Furthermore, aggregation of the inflorescence in two diffuse species showed that pollen removal was less effective, and that spikelet interference and the size of the boundary layer have different consequences on pollen capture for the two species, possibly due to different floret sizes.

Furthermore, I have demonstrated that wind pollination in six species is no less efficient than animal pollination. Thus, the copious production of pollen by wind-pollinated species does not seem to be related to poor pollination efficiency, but may be more related to intense post-pollination competition. In contrast to animal-pollinated

plants, anemophilous plants often occur in clumped distributions with regionally high abundance, leading to small inter-individual distances (Regal 1982). This distribution pattern, combined with the high degree of synchrony in pollen release among wind-pollinated plants, and the possibility of capturing pollen of multiple genotypes, indicate post-pollination competition may be intense. In animal-pollinated plants, post-pollination competition may be less intense because of high relatedness between deposited pollen due to the transfer of pollen in clumps (Kress 1981), or differences in the timing of pollen arrival (Snow 1986; Thompson 1989). If high pollen-to-ovule ratios in wind-pollinated plants are related to post-pollination competition, then the shape of the male fitness-gain curve will need to be re-examined. Furthermore, pollen size may not only be constrained by selection for small grains that travel farther and large grains that are captured better, but also by selection for large grains whose pollen tubes grow more rapidly and are more competitive on stigmas (see review in Sarkissian 1999).

Recent studies indicate that anemophilous plants preferentially capture conspecific pollen from the airstream. Niklas' (1985, 1987) wind-tunnel studies demonstrated that for any given condition of ambient airflow, an ovulate organ creates a characteristic airflow pattern which deflects airborne pollen toward receptive surfaces. Also, the size, shape and density of pollen dictate the particle's trajectory as it moves through an airflow pattern. Thus, the morphology of the ovulate organ and pollen grains may bias pollen receipt in favour of conspecific pollen. Niklas & Buchmann (1987) demonstrated striking pollen discrimination between pollen and ovules of two *Ephedra* species. Linder & Midgley (1996) provided the strongest evidence for pollen discrimination by anemophilous species in their field study of four sympatric species. They found the highest proportion of pollen grains on stigmas to be conspecific (a minimum of 40% and over 80% in one case), in contrast to the pollen frequencies in the air, as sampled on gel-coated slides. In nature, plants may limit their exposure to inter-

specific pollen by flowering at characteristic times of day. von Marilaun (1895 cited in Gregory 1973) found that different grasses flowered for brief periods of 15 to 20 minutes daily, and at distinctive times of day. Prairie wind-pollinated species had narrower phenological curves than insect-pollinated plants, due either to shorter flowering times or greater synchrony in flowering (Rabinowitz et al. 1981). Gene flow between two rice species, *Oryza sativa* and *O. glaberrima*, was partially reduced by diurnal differences in flowering (Sano 1989).

Together, the aforementioned findings challenge the traditional belief that wind pollination is random, chaotic and inefficient (Whitehead, 1968; Regal 1982) and that pre-zygotic barriers to interspecific pollination are unattainable for wind-pollinated species (Grant 1949).

Recently, some of the genetic and developmental causes of changes to inflorescence architecture have been explored (e.g. Dorweiler et al. 1993; Rua & Boccaloni 1996; Kellogg 2000b; Doust & Kellogg 2002), but there is no explicit evidence showing that changes are adaptive, rather than associated with other developmental effects. The diversity and repeated evolution of different architectures in grasses, and the role of the inflorescence in reproduction, strongly suggest that changes are a result of natural selection. Doebley & Lukens (1998) suggested that morphological differences between maize and its wild ancestor, teosinte, resulted from modifications of gene regulation, rather than the structural genes themselves. In addition previous work showed that morphological changes can result from changes at a single locus (Dorweiler et al. 1993). If inflorescence architecture can be modified fairly easily, then selection in response to the pollination environment could produce large morphological changes.

Grasses also produce wind-dispersed seeds, so that selection on inflorescence architecture may be constrained by traits that simultaneously confer higher pollination efficiency and benefit seed dispersal. For example, small-flowered species of grasses

typically produce small seeds. The balance between these two factors may differ between species with contrasting architecture types or flower-size types, and warrants exploration.

Differences in the mode and efficiency of pollination in plants with different architectures create the possibility for differences in mating patterns. The range of sexual systems in grasses has been well documented (Connor 1979; 1981), but the association between sexual systems and phylogenetic relations has not been explored. Correspondingly, the potential associations between sexual systems and inflorescence architectures have not been investigated. Wind-pollinated plants have a strongly bimodal distribution of outcrossing rates (Schemske & Lande 1985; Aide 1986), with species either selfing or outcrossing almost exclusively. This bimodal pattern be related to the effective and quite prevalent self-incompatibility system in grasses (Weimarck 1968; Baumann et al. 2000), or suggests that self-pollination between flowers on the same plant (geitonogamy) is rare. I do not know of any studies investigating geitonogamy in grasses, but it would be useful to explore the extent of geitonogamy and whether this varies with inflorescence architecture or flower-size.

The rich history characterizing pollination biology is largely limited to biotically-pollinated plants. Given that at least 10% of angiosperms are wind pollinated, and that this mode of pollination has evolved repeatedly and independently, the discrepancy in research intensity is surprising. We know very little about the physical attributes of wind-pollinated plants that influence pollination and mating patterns. And we know even less about the mechanisms and morphology that may have evolved in response to selection for efficient pollination. Here, I described simplified expressions of the physical features relevant to anemophily, and then showed that associations between architecture and other morphological traits can be strong. I also showed that differences exist in the behaviour of species with different architectures and their manner of pollen removal and capture,

and that this is consistent with our understanding of fluid dynamics. Future studies that combine aerodynamic theory, functional morphology, and the extensive body of theory and empirical research on animal pollination biology and mating systems, have the potential to reveal complex and fascinating details about the reproduction of wind-pollinated plants.

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