Aerobiology of *Pinus taeda* pollen clouds

Claire G. Williams

Abstract: Better knowledge of aerobiological properties of pollen clouds is needed for pines and other woody perennials because these properties shape the predictive accuracy of spatially explicit pollen dispersal models. Four properties were experimentally measured in this study using processed *Pinus taeda* L. (loblolly pine) pollen as well as pollen concentration and viability data collected from an 18-year-old *P. taeda* plantation. Results showed that *P. taeda* pollen had a settling or terminal velocity value of 2.1 $cm \cdot s^{-1}$; that daytime pollen count from a plantation was sparse, reaching a maximum of 1480 $grains \cdot m^{-3}$ at peak pollen shed; and that pollen concentration showed no vertical gradient above or below the plantation's canopy. Pollen sampled above and within the canopy had comparable germination rates. Surprisingly, a low concentration of viable *Pinus* spp. pollen was present during nighttime. Study results added further to the idea that *P. taeda* pollen has a higher nuisance value than either *Zea mays* L. (maize) or *Agrostis stolonifera* L. (creeping bentgrass) pollen because of its low terminal velocity value, persistent viability, and perennial production.

Résumé : Une meilleure connaissance des propriétés aérobiologiques des nuages de pollen est nécessaire dans le cas des pins et des autres plantes ligneuses pérennes parce que ces propriétés déterminent la précision des prédictions des modèles spatialement explicites de dispersion du pollen. Quatre propriétés ont été mesurées expérimentalement dans cette étude à l'aide de pollen traité de *Pinus taeda* L. (pin à encens) et de données de concentration et de viabilité du pollen collectées dans une plantation de *P. taeda* âgée de 18 ans. Les résultats montrent que le pollen de *P. taeda* a une valeur de vélocité terminale ou de sédimentation de 2,1 $cm \cdot s^{-1}$, que la quantité de pollen comptée durant le jour dans une plantation est faible, atteignant un maximum de 1 480 grains $par \cdot m^{-3}$ au plus fort de la libération du pollen et que la concentration de pollen ne montre aucun gradient vertical au-dessus ou au-dessous de la canopée de la plantation. Le pollen échantillonné au-dessus de la canopée avait des taux de germination comparables au pollen échantillonné à l'intérieur de la canopée. Étonnamment, une faible concentration de pollen viable de *Pinus* spp. était présente durant la nuit. Les résultats de cette étude viennent renforcer la notion que le pollen de *P. taeda* a une valeur de nuisance plus élevée que le pollen du *Zea mays* L. (maïs) ou de l'*Agrostis stolonifera* L. (agrostide) à cause de sa faible valeur de vélocité terminale, de sa viabilité persistante et de sa longue période de production.

[Traduit par la Rédaction]

Introduction

Little is known about conifer pollen release and transport (Jackson and Lyford 1999), but this is vital knowledge for understanding pollen dispersal and gene migration (DiGiovanni and Kevan 1991). Applications include pinpointing rare gene flow events for fragmented forest populations (Robledo-Arnuncio and Gil 2005), reconstructing forest range shifts during the Holocene (Clark et al. 1998; Jackson and Lyford 1999), and predicting escape of genetically modified pollen (van Franfenhuyzen and Beardmore 2004; Kuparinen 2006; Kuparinen and Schurr 2007). In all of these applications, measuring pollen cloud properties can lead to greater accuracy and precision for spatially explicit pollen dispersal predictions.

Pollen clouds are integral to the highly synchronized wind-pollination system in the Pinaceae. Separate male and female reproductive structures on the same plant do show coordinated development, but turbulence and other meteorological processes also shape the pollen cloud: timing of pollen release, modes of dispersal, chances of pollen capture, and even germination.

An overview of these processes is shown in Fig. 1. The majority of released pollen is deposited close to its source, but a small fraction of pollen is uplifted above the forest canopy then transported via horizontal winds far from its source. Horizontal wind speeds are higher for a taller, mature forest canopy because wind speed increases with distance from the earth's surface (Fig. 1). Canopy attributes (leaf area index or LAI) can slow movement of released pollen grains, pollen can be rereleased via precipitation scrubbing or washout where airborne pollen is washed downward by rainfall or other heavy precipitation (McDonald 1962). Such pollen can be resuspended after drying then rereleased for further aerial transport (McDonald 1962). For *Pinus taeda* L. (loblolly pine), pollen release peaks during daytime hours between 1000 to 1300 with a minor peak from 1400 to 1700 (Blush 1986). Nighttime pollen concentrations for this species are low or even absent (Blush 1986). The likely explanation is that wind speeds at night close to the

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C.G. Williams.[1] Department of Biology, Duke University, Durham, NC 27708, USA (e-mail: claire-williams@fulbrightweb.org).

[1]Present address: Nicholas Institute of Environmental Policy Solutions, Duke University, Durham, NC 27708, USA.

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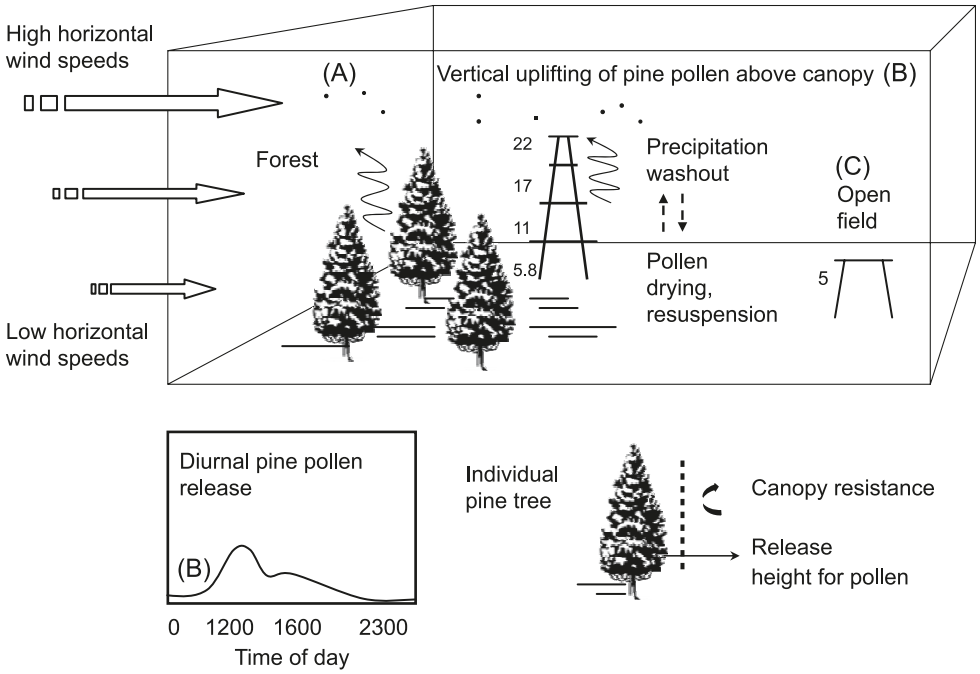
Fig. 1. Schematic diagram illustrating some aerodynamic processes for *Pinus taeda* pollen in a plantation setting as follows: (A) measuring terminal velocity, (B) determining pollen concentration and viability along a vertical gradient during diurnal release peaks (sample heights 5.8, 11, 17, and 22 m) occurred above the canopy so this sampled uplifted pollen available for long-distance dispersal, and (C) measuring pollen concentration and viability at night using an open-field platform (height 5 m) near the plantation pollen source.



earth's surface rarely exceeds 0.5 $m \cdot s^{-1}$, which is the minimum necessary to dislodge pollen (Blush 1986; Parker and Blush 1996).

Pollen form and function

A closer look at the pine pollen grain shows several unusual features relative to angiosperm plant reproduction and these also affect properties of the pollen cloud. Pollen-filled sacs are located on the underside of the microsporophylls or scales of each male strobilus. Here pollen grains develop until they are released by dehiscence. Each pollen grain is a haploid male gametophyte, a mobile multicellular organism. The five-celled pine pollen grain does not produce *de novo* RNA or protein during flight (Pettitt 1985) unlike angiosperm pollen.

Pine pollen is released in vast quantities over a short period of time lasting from 10 days to 4 weeks (Blush 1986). It retains its viability for weeks or even months or years after release if moisture content is kept below 10% before freezing (Jett et al. 1993). Increasing age and crown size translates into heavier pollen production over time (see review in Di-Giovanni and Kevan 1991). Consider that an open-grown *P. taeda* tree can release 1134 g of pollen over a 14-day pollen shed or approximately 81 $g \cdot tree^{-1} \cdot day^{-1}$ (Parker and Blush 1996). Pine pollen has an unusual degree of buoyancy relative to other seed plants. This has been demonstrated using a simple ballistics model where pine pollen is transported as far as 47–60 km within 3 h (Di-Giovanni and Kevan 1991), and these long transport distances for conifer pollen have reported for over 100 years (Table 1).

Ecologists have long recognized that pollen dispersal is bimodal (Hengeveld 1989; Nichols and Hewitt 1994; Nathan et al. 2002). The first mode is local neighborhood dispersal (LND), which accounts for 99% of pollen or seeds, and this means that most pollen grains remain within the periphery of the source (Fig. 1). The second mode is long-distance dispersal (LDD), which by definition accounts for 1% of pollen or seeds (Hengeveld 1989). As shown in Fig. 1, this mode occurs when seeds and pollen are vertically uplifted above the plant canopy by vertical eddies, which act as turbulent ejection events. Once above the canopy, the seeds or pollen grains move horizontally from source (Nathan et al. 2002).

These rare LDD dispersal events account for the many reports showing pine pollen moves on micro- or meso-scale distances of 30–1000 km from its source (Table 1). It is not yet clear whether LDD pollen retains its viability (Smouse et al. 2001; Katul et al. 2006). Only one study has shown that *Pinus sylvestris* L. (Scots pine) pollen in northern Sweden can germinate after long-distance transport (Lindgren et al. 1975) and whether this result extrapolates to a wider range of conditions has yet to determined. No doubt long-distance pine pollen is subjected to high levels of abiotic stress such as extreme cold, ultraviolet radiation, and high moisture levels but pine pollen has persistent viability.

Wind-mediated pollen release and dispersal

Other useful properties of *P. taeda* pollen clouds include terminal velocity, uniformity of pollen concentration during night or day, and pollen grain viability.

Measuring terminal velocity

How far the pollen particle moves depends on many factors including velocity of sedimentation (settling or terminal velocity V_t), which is the rate at which particles

Table 1. Pollen dispersal distances reported for two genera within the Pinaceae family: *Pinus* spp. and *Picea* spp.

Pollen source	Location	Distance from nearest source (km)	Reference
Pinus spp.	Iowa, USA	600	Bessey 1883
Pinus spp.and *Picea* spp.	Gulf of Bothnia	30–55	Hesselman 1919 (in Koski 1970)
Pinus spp.and *Picea* spp.	North Atlantic Ocean	1000	Erdtman 1937
Pinus sylvestris	Southern Sweden	72	Lanner 1966
Pinus spp.	Shetland Islands	250	Tyldesley 1973
Pinus spp.and *Picea* spp.	Greenland	300	Rousseau et al. 2006

Table 2. Reported terminal velocity values for pollen among four genera within the Pinaceae: *Pinus*, *Larix*, *Picea*, and *Abies* (all values are compared against *Zea mays*).

Species	Terminal velocity ($cm \cdot s^{-1}$)	Source
Pinus banksiana	3.1	Eisenhut 1961
Pinus banksiana	2.3	Di-Giovanni et al. 1996
Pinus taeda	2.3	Niklas 1984
Pinus contorta	3.8	Eisenhut 1961
Pinus montana	3.3	Eisenhut 1961
Pinus nigra	4.5	Eisenhut 1961
Pinus parviflora	3.3	Eisenhut 1961
Pinus peuce	3.5	Eisenhut 1961
Pinus rigida	4.0	Eisenhut 1961
Pinus strobus	3.1	Eisenhut 1961
Pinus sylvestris	3.7	Eisenhut 1961
Larix deciduas	12.6	Eisenhut 1961
Larix leptolepis	13.1	Eisenhut 1961
Larix laricina	3.1	Niklas 1984
Picea abies	5.6	Eisenhut 1961
Picea glauca	2.7	Niklas 1984
Picea orientalis	6.1	Eisenhut 1961
Picea mariana	3.2	Di-Giovanni et al. 1996
Abies balsamea	9.7	Eisenhut 1961
Zea mays	26.6	Aylor 2002
Zea mays	31.0	Di-Giovanni et al. 1996

descend in still air owing to gravitational effects. When sedimentation is the only force responsible for deposition of particles, then the capture efficiency is simply the ratio between the vertical force (gravitational settling) and the horizontal force (horizontal wind speed). Precise estimates hinge on directly measuring terminal velocity of the pollen particle itself. In still air, pollen falls slowly under gravity. The rate of its fall can be predicted or measured directly.

Terminal velocity can be approximated for pollen and other spheroidal particles ranging in diameter from 1 and 70 μm using Stokes' Law, which is described by

$$V_t = \frac{2}{9} \frac{r^2 g(p - \sigma)}{\mu}$$

where V_t is terminal (or settling) velocity ($cm \cdot s^{-1}$), p is pollen density ($g \cdot cm^{-3}$), σ is the density of air ($g \cdot cm^{-3}$), r is the radius of the pollen grain (cm), g is the acceleration due to gravity ($cm \cdot s^{-2}$), and μ is dynamic viscosity of air ($g \cdot cm^{-1} \cdot s^{-1}$). For example, *Pinus banksiana* Lamb. (jack pine) pollen has a width of roughly 50 μm (Di-Giovanni et al. 1996), so it falls within the range for Stokes' Law; however, it is not perfectly spheroidal, and this deviation affects the fit between predicted versus measured V_t values (Jackson and Lyford 1999). Thus, measured V_t is consistently higher than predicted V_t based on Stokes' Law for most conifers (Jackson and Lyford 1999), so direct measurement is preferred.

Terminal velocity is often measured using fall towers. Pollen is released from the top of a cylinder then timed before it reaches the bottom of the tower. Fall towers vary in design and dimensions, and this contributes to measurement error among studies (Eisenhut 1961; Di-Giovanni et al. 1996; Jackson and Lyford 1999; Aylor 2002). Despite this source of error, V_t values for many conifer species range between 3 and 4 $cm \cdot s^{-1}$ (Table 2); the only notable exception is *P. taeda*, which has a V_t value lower than other species, i.e., a value of 2.3 $cm \cdot s^{-1}$ (Niklas 1984). This singular estimate for this species was obtained using a rectangular box rather than a falling tower, so this low value could be artifactual. If so, measuring V_t using the falling tower method as described by several authors including Aylor (2002) should yield a V_t value between 3 and 4 $cm \cdot s^{-1}$ (Table 2).

The common explanation for pine pollen buoyancy is that it has two air-filled sacs or sacci attached to its otherwise round grain. Another school of thought suggests that the sacci function more as flotation devices in water rather than as aids to wind transport (Doyle and O'Leary 1935; Tomlin-

son 1994; Runions and Owens 1999). Support for this idea is shown in Table 2. Consider two closely related species, one of which has sacci and the other does not; however, terminal velocity values are similar. As an example, note in Table 2 that *Picea abies* (L.) Karst. (Norway spruce), which has saccate pollen, has a V_t of 5.6 cm·s^{-1} and *Picea orientalis* (L.) Link (Caucasian spruce) pollen, which has no sacci, has a V_t of 6.1 cm·s^{-1}. Only the saccate *Picea abies* pollen can float upwards in an aqueous solution. Hence, the conclusion that conifer pollen depend on its air-filled sacs for floating in aqueous fluids rather than floating in air (Runions and Owens 1999). Another explanation for the unusual buoyancy of *Pinus* spp. pollen might be that it retains its spheroid-like shape even when desiccated.

This property of shape retention also leads to the hypothesis that viability has no effect on terminal velocity measurements. This hypothesis runs contrary to the well-studied *Zea mays* L. (maize) pollen, which shrivels into a nonspheroidal shape upon drying (Aylor 2002). Pine pollen is shape constant whether dead and alive, so its terminal velocity should remain unchanged upon death.

Such small changes in terminal velocity are important because they can translate into large shifts in predicted dispersal distances. For example, dispersal distance for a pollen grain with a V_t value of 3.0 cm·s^{-1} is 49.6 km from source, whereas a V_t value of 7.0 cm·s^{-1} predicts a shorter distance of 26.8 km from source using turbulence conditions in a Durham, North Carolina, pine plantation (Katul et al. 2006). This has been shown for dispersal distances in other locations using simpler ballistics models (Koski 1970; DiGiovanni and Kevan 1991).

Pollen concentration: testing for the well-mixed condition

Uniformity of the pollen concentration within a reference space is another critical pollen cloud property for predicting trajectories of individual particles in turbulent flows. Many computational fluid mechanics and turbulence models derived using the so-called well-mixed condition (*wmc*), which states that, if a concentration of a passive scalar material is initially uniform at some time t_o, it will remain so at any future time t in the absence of sources and sinks (Thomson 1987).

Testing pollen concentration determines whether the well-mixed condition assumption is valid. However, because pollen grains have finite terminal velocity V_t and inertia, Thomson's (1987) assumption must be modified. The computational modifications proposed by Wilson (2000) are usually adopted for spatially explicit pollen dispersal models (Katul et al. 2005). For experiments in the actual forest canopy, there is some question as to whether pollen concentration will be uniform. Pollen is released in puffs or plumes around clusters of male strobili at the tips of the lower branches. How pollen concentration actually diffuses throughout the measurement space has yet to be tested experimentally.

Pollen uplifted above the canopy: is it viable?

Pollen grains are vertically uplifted above the plant canopy by updrafts or turbulent ejection events before they are moved horizontally far from source (Nathan et al. 2002). Turbulence here refers to a continuous succession of gusts, swirling eddies, and lulls accompanied by swift changes in wind direction or advection. Turbulence includes thermally generated vertical eddies inherent to uplifting and the LDD mode of dispersal (Lanner 1966; Koski 1970; DiGiovanni et al. 1996; Horn 2005). By adding a more complete modeling of turbulence in all directions, dispersal distance estimates now exceed the simple ballistics example (Katul et al. 2006). By including turbulence measurements in addition to horizontal wind speeds, small changes in V_t values now contribute to even larger shifts predicted LDD distances for pollen.

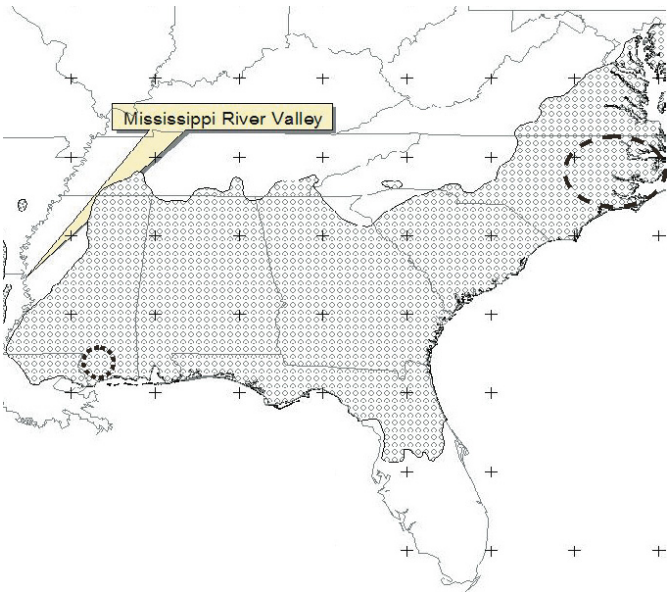
Measuring viability of pollen vertically uplifted above the canopy is a step toward resolving the larger question of LDD pollen viability. Viability, as measured by germination, is expected to be high. No vertical gradient is expected within or above the height of the forest canopy because pine, unlike *Z. mays* or *Agrostis stolonifera* L. (creeping bentgrass) pollen (Aylor 2002; Pfender et al. 2007), remains viable only for a few hours, pine pollen has persistent viability for weeks or longer.

Pollen sampled at night: concentration and viability

Peak pollen release occurs midday for *P. taeda* (Blush 1986). A secondary peak of pollen can be released in the late afternoon, and nighttime pollen release may occur as a low steady concentration of *P. taeda* pollen if wind speeds within the canopy exceed 0.5 m·s^{-1} (Blush 1986). In contrast, *P. sylvestris* pollen concentrations in Finland are similar near peak pollen shed whether sampled during night or day (Pulkkinen and Rantio-Lahtimaki 1995) perhaps because nights are short and humidity is low in the cold arctic air. Night pollen is likely to be a combination of near-field or LND pollen and far-field or LDD pollen because pollen released during the day travels far from source during the night before deposition (Blush 1986). This phenomenon of nighttime pollen holds special interest because it occurs at the same time as ovular pollen capture within each female strobilus (Greenwood 1986; Brown and Bridgwater 1987). Pollen grains deposited on a receptive female strobilus sift down through the open cone scales until they stick on micropylar arms dangling from each inverted ovule. A pollination drop is exuded by each ovule between 2300 to 0700, and it retracts within the ovule's micropylar chamber, pulling the floating saccate pollen grains inside (Doyle and O'Leary 1935; Brown and Bridgwater 1987). Once inside the micropylar chamber, pine pollen grain hydrates. With hydration, its leptoma, located between the two sacci, opens for the germination tube and allows the tube to elongate. The germination tube continues to elongate into the ovule's spongy nucellar tissue, completing pollination.

The pollen drop is only exuded by each ovule as peak female receptivity approaches. Female strobilus receptivity often occurs before local pollen is shed (Greenwood 1986), so far-field pollen is first available then near-field (local pollen) itself becomes the dominant pollen source as peak pollen shed approaches (Greenwood 1986). Some authors show that early arriving *P. taeda* pollen shows no apparent advantage over late-arriving pollen (Greenwood 1986) in which case nighttime pollen would have the same chances as pollen released earlier in the day, but this conclusion is controversial (Brown and Bridgwater 1987).

Fig. 2. Eastern range of *Pinus taeda* extends from the Mississippi River to central Florida to coastal Maryland and Delaware. The small dotted circle shows the Harrison Experimental Forest within the US National Forest System (30°64′N, 89°14 ′W), where pollen was sampled for the terminal velocity measurements. The broken line encloses the area where pollen concentration and viability studies were conducted: (*i*) 18-year-old *Pinus taeda* plantation at the Blackwood Mountain division (30°64′N, 89°14′W) within the Duke Forest near Research Triangle Park, North Carolina, as well as locations for heat sum calculations for the same Duke Forest site and (*ii*) the Croatan National Forest near Havelock, North Carolina.



The purpose here was to experimentally measure four properties of pine pollen clouds all of which bear on the accuracy of spatially explicit pollen dispersal models: (*i*) terminal velocity, (*ii*) uniformity of pollen concentration, (*iii*) viability of pollen uplifted above the canopy during peak daytime release, and (*iv*) pollen concentration and viability of nighttime pollen. The experiments described here sampled pollen from *Pinus taeda*, a major timber species indigenous to the southeastern United States.

Methods

Pinus taeda (Pinaceae) is a wind-pollinated, outcrossing, and monoecious conifer with an extensive natural range throughout the southeastern United States. The eastern part of the range studied here extends from the Atlantic Seaboard and Gulf Coast extending inland from Maryland, Texas, and Florida (Fig. 2; Baker and Langdon 1990).

Pollen development and handling

Heat sum calculations

Pollen shed and female strobilus receptivity are latitude-dependent phenomenona that can be predicted using heat sum (Boyer 1978). Date of peak pollen shed for *P. taeda* in its indigenous range starts in mid-February at the southerly latitudes of the species' range then moves up towards the northerly latitudes of the species' range where peak pollen shed occurs by mid-April (Baker and Langdon 1990).

Classifying male strobilus development

The *P. taeda* male strobilus classification system (Bramlett and Bridgwater 1989) was used to described male strobilus and pollen development through peak pollen shed. For the terminal velocity study, pollen was harvested at peak shed from five *P. taeda* accessions located at the USDA Forest Service's Harrison Experimental Forest in Saucier, Mississippi (30°64′N, 89°14′W), in mid-March 2006.

Germination assay

Germination was tested using the following protocol because it reliably predicts pollen fertilization as measured by total seed per cone (Jett et al. 1993): (*i*) 0.625 g of Difco Bacto agar is added to 125 mL distilled water, (*ii*) sterilized in an autoclave for 20 min, and (*iii*) poured as a thin layer into each 90 mm Petri dish and cooled to room temperature. Pollen was then dusted lightly on the agar surface of each plate using a dry camel hair brush then the plate was placed in a 28 °C oven for 48 h before counting pollen grains with germination tubes. Only grains with a pollen tube length exceeding the pollen grain width were counted.

Terminal velocity testing using dyed and undyed pollen

Dyed pollen is detected in the falling tower with greater ease, so two dyes were tested here along with undyed viable pollen. The effect of dye on viability was not known at the start of the experiment, and this question was addressed here because dyed pollen, if viable, could be used in later experiments to follow a rare dispersal event through to pollination and fertilization.

Pollen was sieved, dried below 10% moisture content, and divided into 1 g lots. The first pollen lot, once tested for viability, was then stored without further processing (control or treatment 1). The second pollen lot was rinsed for 30 min in 50 mL of a dye solution composed of 0.50 g aniline blue dissolved in a 7% sucrose solution then redried before storage at –20 °C (treatment 2). The third pollen lot was rinsed for 30 min in a 50 mL fluorescent dye solution composed of 0.02 g rhodamine 123 dissolved in a 7% sucrose solution then redried and stored at –20 °C (treatment 3). All three pollen lots were rehydrated at room temperature for 24 h prior to terminal velocity measurements on 19 April 2006.

Terminal velocity measurement using a fall tower

Terminal velocity measurements were made using a clear glass tube in a darkened room as described in Aylor (2002). The experiment was conducted in the Rare Book Room in the Duke University Library because it had high ceilings and climate-controlled conditions: temperature held at a constant 22 °C and humidity was held at 39%. The 2.4 m glass settling tube, which had an inner diameter of 0.02 m, was illuminated using a cool fiber-optic light source at a distance of 0.3 m from the base of the glass tube. The top of the tube was covered with a thin diaphragm of aluminum foil which had a central pinhole of 0.2 mm diameter; here, the sample of pollen grains was placed before being tapped into the tube for timing the pollen as it fell 2 m below the start mark of 0.4 m.

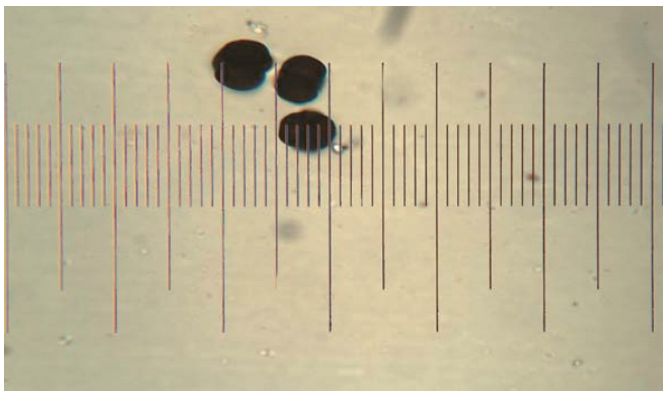
Falling pollen grains were also captured for particle verification, viability, and sizing. Before recording terminal velocity measurements, a series of four microscope slides,

Table 3. Terminal velocity values (means ± SD) and other measurements of *Pinus taeda* pollen from this study.

Treatment (10 runs)	Terminal velocity ($cm \cdot s^{-1}$)	Total pollen per plate	Pollen viability (%)	Pollen moisture content (%)
1	2.05±0.033	213	93.9	10.0
2	2.16±0.026	24	29.2	9.1
3	2.14±0.031	40	0	10.4
Mean	2.12±0.295			

Note: Treatments 1, 2, and 3 refer undyed pollen, blue-dyed pollen, and rhodamine-dyed pollen, respectively.

Fig. 3. *Pinus taeda* pollen grains measured here were captured at the base of the terminal velocity tube. Each gridline marks 10 μm.



each with double-sided cellophane tape, were successively placed beneath the base of the tube during a single pollen fall event to test whether sample particles were pine pollen grains or contaminant particles. Each microscope slide was examined on site using a portable 10× light microscope before the terminal velocity experiment. Only the third and fourth slides, which trapped slower particles, showed any pine pollen grains.

Falling pollen was also collected at base of the glass tube using Petri plates filled with 0.5% agar. The plates were then incubated for 48 h at 28 °C before scoring germination tubes. Size, or diameter, of measured pollen grains was checked as part of Aylor's (2002) protocol using a Zeiss Axioplan microscope set at 20× power. Measurements were made using a scale micrometer where each line marked 10 μm. The remainder of each pollen lot was then tested for pollen moisture content by taking an initial mass of the sample before drying it in a laboratory oven set at 50 °C then reweighing hourly until the sample mass no longer changed.

Pollen concentration and viability sampled along a vertical transect

During daylight hours, pollen was sampled at four heights along a meteorological tower within an 18-year-old *P. taeda* plantation located in the Blackwood Mountain division of the Duke Forest (see schematic diagram in Fig. 1). The plantation tower was located in Ring 1 of the free-air CO_2 enrichment (FACE) pine plantation Ameriflux site in the Duke Forest (35°97′N, 79°09′W). Planting density is 1730 $stems \cdot ha^{-1}$, and the genetic composition is local woodsrun seed. Its reproductive onset was roughly age 16 years (LaDeau and Clark 2006; Williams et al. 2006).

The Burkhard spore sampler had a 90 mm Petri plate filled with 0.5% agar, which collected pollen concentration data at 15 min intervals at four heights. Its air intake was 20 $L \cdot min^{-1}$. Each Petri plate was incubated for 48 h at 28 °C before scoring for germination. Aside from the first daytime sampling date, which had a single replicate at each tower height, all other sampling dates had at least two replicates per height. The six sampling dates in 2007 were as follows: (*i*) 24 March (day 83 of the year) from 1200 to 1344, (2) 26 March (day 85) from 1145 to 1223, (3) 28 March (day 87) from 1142 to 1217, (4) 30 March (day 89) from 1300 to 1505, (5) 2 April (day 92) from 1413 to 1604, (6) 4 April (day 94) from 1230 to 1303. Note that sunrise and sunset occurred at 0658 and 1940, respectively, on 4 April for Durham, North Carolina. Germination of pollen released inside the canopy was compared with germination of pollen uplifted above the forest canopy.

A linear model was used to test for differences among tower heights and among sampling dates using SAS statistical software (SAS Institute Inc. Cary N.C.). The model for the GLM procedure was as follows:

$$Y_{ijk} = \mu + T_i + D_j + \varepsilon_{ijk}$$

where μ is the mean, T_i is the tower height, D_j is the date, and ε_{ijk} is the random error. Each sampling date used in this analysis had two or three replicates at each tower height.

Night sampling using an elevated platform in an open field

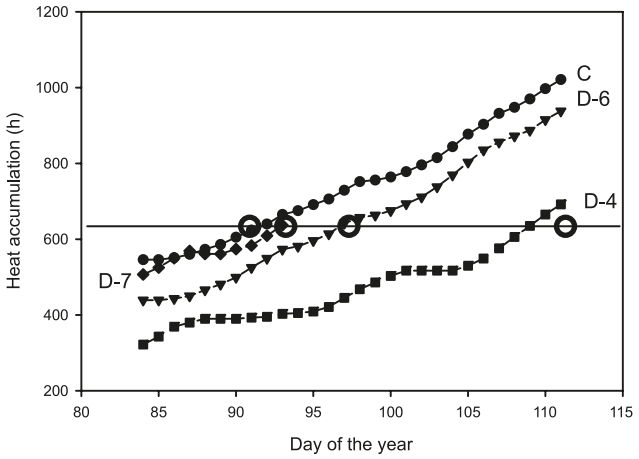
Pollen concentration and germination data was collected using the Burkhard spore sampler at a height of 5 m on a platform in an open field 600 m from to the Duke Forest tower site (see Fig. 1). Each night sample consisted of eight 15 min samples. The three sampling dates in 2007 were (1) 29 March (day 88) from 2339 until 0306 30 March (day 89), (2) 1 April (day 91) from 0100 to 0308, and (3) 2 April (day 92) from 1112 h to 0119 on 3 April (day 93).

The GLM procedure was used to test whether sampling dates were statistically different in either concentration or germination levels. Standard errors for binomial proportions of living (p) and dead ($1 - p$) pollen proportions were estimated using $\sqrt{p(1-p)}/n$, where n was defined as total pollen count.

Results

Terminal velocity measurements for *P. taeda* pollen had a mean value of 2.12 $cm \cdot s^{-1}$ using a fall-tower protocol (Table 3). Desiccated pollen grains retained the spheroid-like shape and could be sized at a width of 50 μm (Fig. 3). Terminal velocity values were statistically similar for all three treatments: undyed (2.05 $cm \cdot s^{-1}$), aniline-dyed pollen (2.16 $cm \cdot s^{-1}$), and rhodamine-dyed pollen (2.14 $cm \cdot s^{-1}$). Note that terminal velocity values were similar even though germination rates varied widely among treatments. Rhod-

Fig. 4. The heat sum predictive equation (Boyer 1978) closely predicted peak pollen release for indigenous *Pinus taeda* seed sources planted in North Carolina's Piedmont and coastal regions. The Boyer (1978) equation had a baseline temperature of 55°F (or 12.78 °C) and a heat sum accumulation start date of 1 January. The heat sum accumulation equation assumed peak pollen shed would occur at the 636 h threshold for peak pollen shed (horizontal line). Equation was tested at the Croatan National Forest (34°83′N, 76° 95′W; coded as C) in 2006, peak pollen shed was predicted (and observed) on days 91 and 92 of the year. For the Blackwood Mountain plantation within the Duke Forest (35°97′N, 79°09′W), peak pollen shed predictions also coincided with observed dates for 3 years: 2004 (coded as D-4), 2006 (coded D-6), and 2007 (not shown). Predicted days of the year 111 and 112, 96 and 97, and 91 and 92 (respectively) were a close fit to observed peak pollen shed on days 111 and 112, 96 and 97, and 93, respectively.



amine-dyed pollen (treatment 3) did not germinate, whereas the undyed pollen (treatment 1) had 93.9% viability after 4 weeks of low-moisture storage (Table 3). The blue aniline-dyed pollen had intermediate germination values (29.2%).

Measurements of pollen concentration and viability at the Duke Forest site were completed before, during, and after peak pollen shed. Pollen season lasted at the Duke Forest plantation for 10 days (Fig. 4). Pollen was first detected on site on 24 March; only two pollen grains were sampled at 11 m along the entire vertical gradient. Similarly, the next sample taken on 26 March rose slightly to a total of five grains (5.8 m), nine grains (11 m), ninen grains (17 m), and nine grains (22 m). Peak pollen shed occurred on day 93 of the year (2 April) as indicated by male strobilus development stages (Table 4), and it showed a close fit between the heat sum predictive equation at several locations (Fig. 4). Peak pollen shed was also detected by the spike in mean pollen count (Fig. 5A) on 2 April: daytime maximum pollen count was 1495 pollen grains.

By comparison, the previous sample on 30 March had only a total of 494 grains, and the next sample on 4 April had a total of 894 grains. But even so, the maximum pollen count from a single sample had only 444 pollen grains. This plate count was collected over a 15 min interval using the Burkhard spore sampler, which translated into a pollen concentration of 1480 grains·m^{-3}.

Pollen concentration was uniform along the tower's vertical gradient; no statistical differences could be detected using sparse pollen counts and four transects. Starting on 28 March, pollen concentration among tower heights were not significantly different at the 5% level (P = 0.1801). This was also the case on 30 March, 2 April, and 4 April, where the probability of a greater F value for tower height was not statistically significant at the 5% level or higher (P = 0.3252, 0.2117, and 0.5537, respectively). This was also the case for earlier sampling dates, which showed either no germinating grains (0 of 2) for 24 March or extremely low viability (3 of 32 grains) for 26 March. No gradient could be detected for pollen concentration sampled during daylight hours.

However, uplifted pollen sampled at tower height of 22 m had comparable germination with sample heights 5.8, 11, and 17 m within the canopy (Fig. 5B) for all dates except one. No gradient for daytime pollen germination was apparent until after peak pollen shed. Figure 5B shows that pollen germination was highest at a tower height of 11 m relative to other tower heights on 28 March (79.1%) and 30 March (59.1%). No germination peak or gradient coincided with peak pollen shed on 2 April or before peak shed.

However, after peak pollen shed, an interesting exception appeared. For 4 April, germination became statistically different across height (P = 0.0077) and a slight, inversely vertical gradient was detected for pollen germination although absolute germination values were declining across dates. Germination values for 4 April for tower heights 22, 17, 11, and 5.8 m were 33.1%, 13.4%, 7.1%, and 10.8%, respectively. Contrary to expected results, pollen germination was higher at the top of the tower than at the lower heights.

Night sampling began immediately after heavy rains on 29 March, and this meant that ambient pollen before the first sampling date was removed via precipitation washout. Despite the high humidity, nighttime pollen was viable on all sampling dates. On 29 March, the the pollen count was 54.75 ± 11.18 (mean ± SE), and its germination was 24.4%; however, on subsequent dates, these values declined. On 1 April, the count was 31.13 ± 4.51, and its germination was 28.51%. On 2 April, the mean pollen count decreased to 20.87 ± 4.25, and its germination dropped to only 1.20%. Notice that nighttime pollen concentration (Fig. 6A) and germination (Fig. 6B) were more variable compared with the daytime samples (Figs. 5A–5B).

Discussion

The experimental results showed the following: (*i*) terminal velocity was slightly lower for *P. taeda* relative to other pines, as previously reported (Niklas 1984); (*ii*) neither terminal velocity nor shape changed for dead, dyed, dessicated, or live *P. taeda* pollen; (*iii*) no gradient could be detected for *Pinus taeda* pollen concentration although its viability had a more erratic vertical pattern; (*iv*) pollen uplifted above the canopy germinated as well as pollen within or below the canopy; and (*v*) nighttime pollen concentrations were lower relative to daytime samples, but its uneven germination suggested a potential contribution to pollination.

Terminal velocity measurements were similar regardless of treatment, whether alive or dead. Introducing dyed pollen did not alter these V_t values either, and this result suggested that using dyed pollen to trace pollen trajectory should intro-

Table 4. Classification stages for development and dehiscence of *Pinus taeda* microsporangiate strobilus (adapted from Bramlett and Bridgwater 1989).

Stage	Microsporangiate strobilus development	Day of the year in 2007 for Duke Forest
1	Male strobili are encased in bud scales at tips of vegetative shoot	—
2	The individual male strobilus emerges from its bud scales	—
3	Male strobilus lengthens and exudes a clear liquid when pressed	83
3.3	Male strobilus exudes yellow fluid when pressed	85
3.6	Male strobilus exudes clear fluid when pressed; this occurs 3–5 days from dehiscence	86
3.9	Male strobilus exudes little, if any fluid when pressed; this occurs 1 or 2 days from dehiscence; at this stage, microsporophylls bend easily so that spaces are visible between them	87
4	Proximal end of male strobilus begins releasing pollen and dehiscence moves acropetally; <10% of pollen released at any given day over a period of 7–14 days	88
5	Maximum stage for pollen release; most strobili within a cluster are now releasing pollen	92–93
6	Pollen release is complete, and male strobilus is dried and brown in color	—

Note: The 2007 study observations began at stage 3 prior to reaching threshold heat sum accumulation (and peak pollen shed) on days 92 and 93 of the year.

duce no bias. It is interesting that the blue aniline-dyed pollen retained some of its viability (29.2%), which suggests it has potential value as a tracer pollen source when tracing rare LDD events from pollen release and transport to pollination.

The absolute value of V_t was lower than expected. As suggested by Jackson and Lyford (1999), protocol can bias V_t measurements, and these findings are no exception. It is possible here that truncating particle catch from the fall tower might have lowered V_t estimates. Conversely, if this estimate of 2.1–2.3 cm·s^{-1} is a true value for *P. taeda* pollen, then predicted pollen distances reported for this species are conservative because V_t values has been assumed to range from 3 to 7 cm·s^{-1} (Katul et al. 2006).

These findings for *P. taeda* pollen are distinctly different from *Z. mays* pollen (Aylor 2002). Water content for *Z. mays* pollen is higher (52% vs. 10% for *P. taeda*), particle diameter is larger (92 μm for *Z. mays* vs. 50 μm for *P. taeda*), and its terminal velocity (V_t) values differ by an order of magnitude (26.7 cm·s^{-1} for fresh *Z. mays* pollen vs. 2.1 cm·s^{-1} for *P. taeda*). *Zea mays* pollen is only viable for <24 h, so it has a far more limited range for gene flow than *P. taeda* pollen. Although *P. taeda* pollen has V_t values similar to *A. stolonifera* (1.9 cm·s^{-1}) here too, viability persistent is far greater for pines. *Agrostis stolonifera* pollen is viable for only 3 h (Pfender et al. 2007).

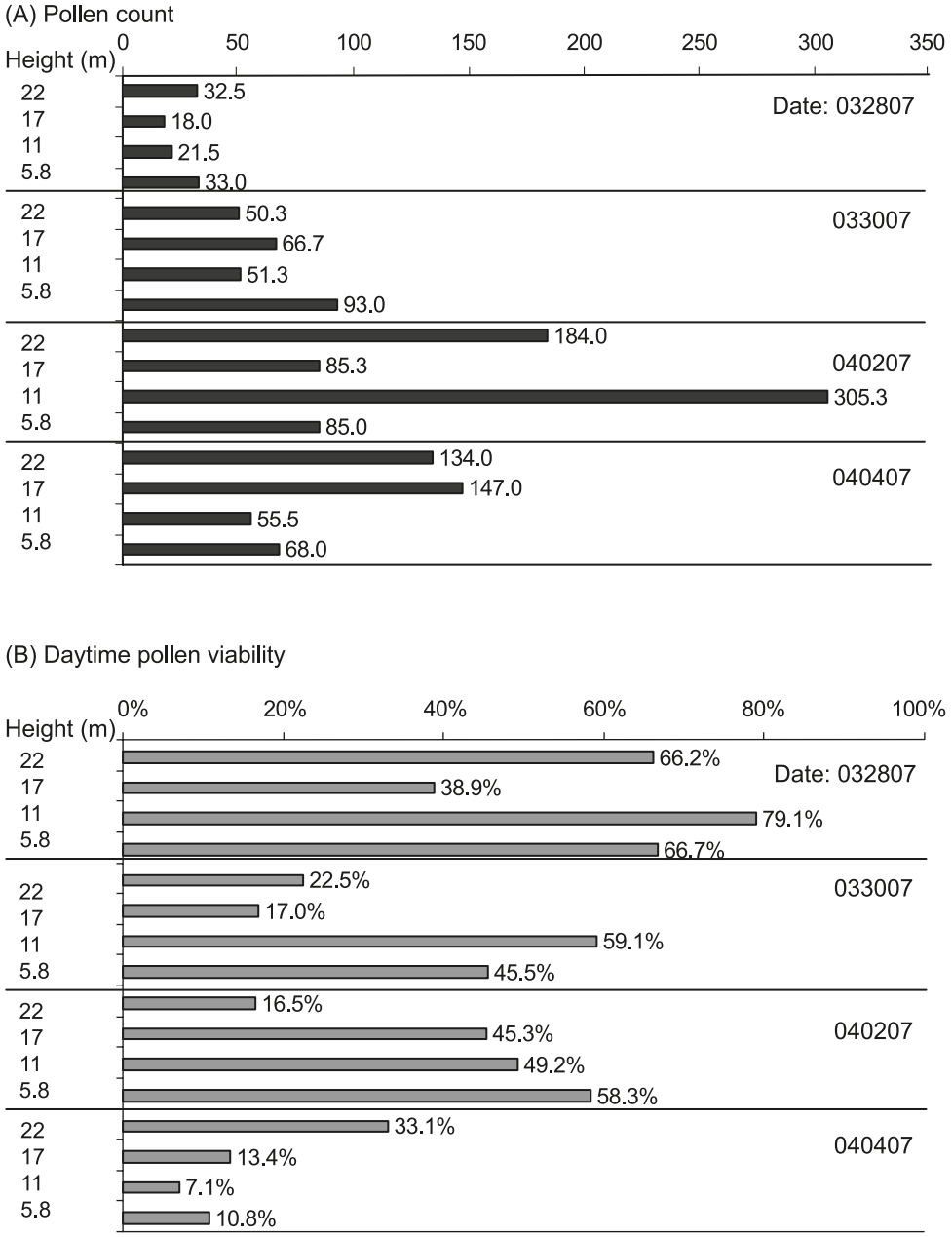
This study shows that *P. taeda* pollen has a higher nuisance value than other well-studied dispersal systems such as *Z. mays* and *A. stolonifera* not only because it has low terminal velocity, shape retention, and persistent viability but also because *P. taeda* pollen is perennial and its production increases with age and environment (LaDeau and Clark 2006).

The assumption of the well-mixed condition for spatially explicit models appears to be valid for *P. taeda* pollen released in a plantation, although these data are limited. A vertical gradient for pollen concentration could not be detected using four sampling heights along the tower inside the plantation, even though the sparse pollen plume was released only at the lower canopy at heights between 5.8 and 11 m. This finding is preliminary and should be tested using (*i*) more sampling heights along the tower and (*ii*) multiple spore samplers at each height rather than one spore sampler moved along the vertical axis over time.

A gradient might be more apparent for open-ground *P. taeda* stands where pollen production is far greater. Blush (1986) reported pollen concentrations at 27 000 grains·m^{-3} in a mature *P. taeda* seed orchard using a Rotorod sampler; here, in this unthinned *P. taeda* plantation, the maximum pollen concentration at peak pollen shed was only 1480 grains·m^{-3}. In the same seed orchard, pollen shed lasted 4 weeks, yet pollen shed lasted 10 days in the Duke Forest plantation. Generalizing pollen concentration assumptions for the well-mixed condition would benefit from a wider sample of forest conditions.

In this study, pollen grains were equally viable above and within the plantation. It is apparent that pollen that escaped the canopy volume and now available for horizontal transport is no less viable than pollen that is deposited within the canopy itself. Further testing of LDD pollen germination is needed for two reasons. Firstly, uplifted pollen is predicted to move at far greater heights than the 22 m height tested here using the tower, so germination should be tested farther above the canopy. Secondly, once pollen is uplifted, it is then moved horizontally by winds above the canopy. To determine if LDD pollen is indeed capable of germination,

Fig. 5. (A) Daytime *Pinus taeda* pollen concentration was sampled along a vertical 23 m gradient inside and above Duke Forest plantation's canopy in 2007 (D-7). Dates are given as mmddyy. Daytime sampling was conducted before, during, and after peak pollen shed using a Burkhard spore sampler. Tower heights of 5.8–11.0 m correspond approximately to the canopy heights where pollen is released from the lower branches of the crown. These pollen concentration data validate the assumption of the well-mixed condition inherent to spatially explicit dispersal models. (B) Germination rates for uplifted pollen above the canopy was comparable with that for pollen collected within the canopy for all sample dates until after peak pollen shed (4 April 2007).

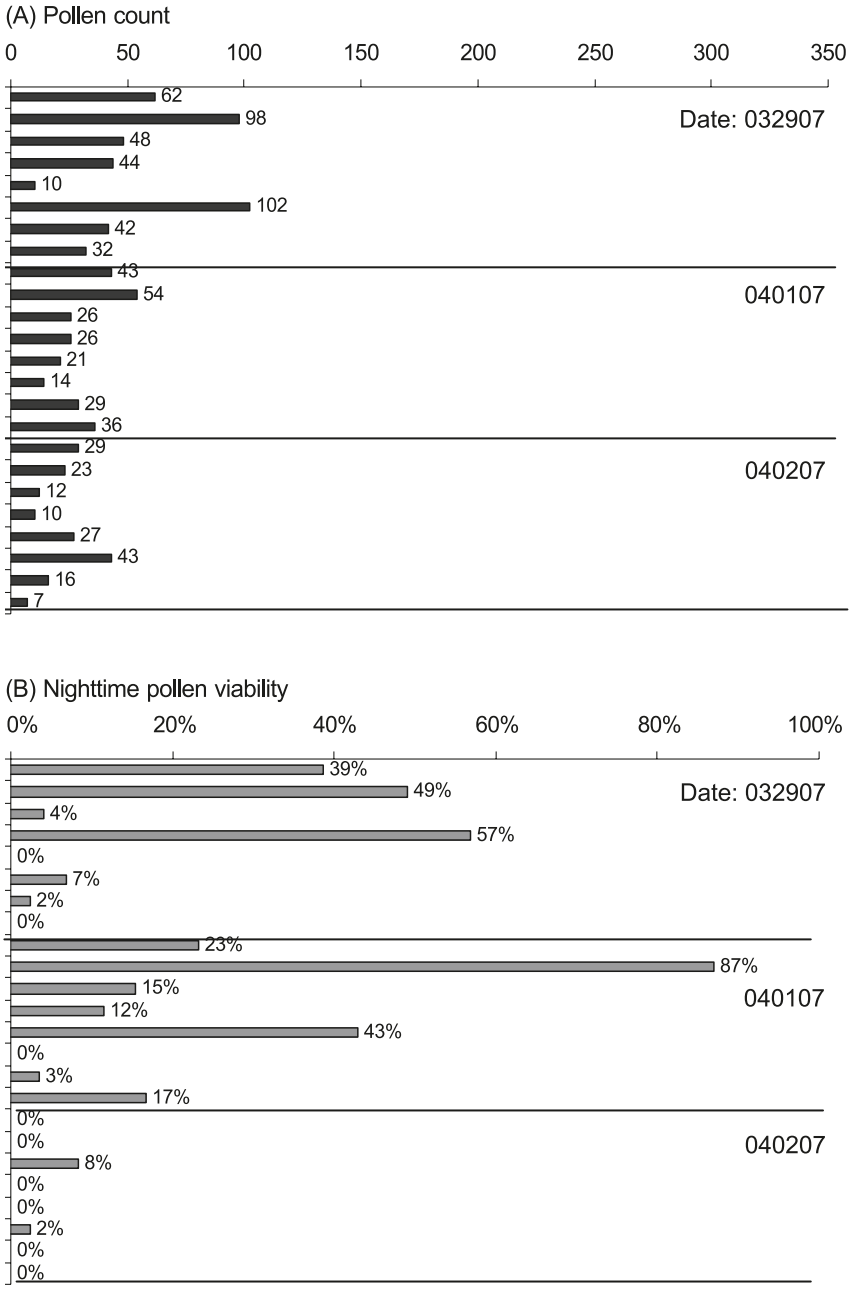


one would need to test at higher levels of uplifted pollen and then sample pollen transported in the horizontal directions.

Findings from night sampling had some parallel to the comprehensive study reported by Blush (1986), who showed low, steady pollen concentrations in a mature *P. taeda* seed orchard relative to daytime release. This contrasts with the *Pinus sylvestris* pollen shed in Finland, which had similar concentrations whether sampled at day or night (Pulkkinen and Rantio-Lahtimaki 1995).

This study adds further to this contrast: here, *P. taeda* pollen sampled at night had low, erratic viability patterns, yet the Finland study showed high pollen viability for both night and day. The Finnish results can be attributed to short nights characterized by cold, dry ambient conditions that favor sustained viability whereas the Durham, North Carolina, location had longer 12 h nights during which cool moist ambient conditions prevailed. The Durham study also used two different locations at the same site for sampling

Fig. 6. (A) Nighttime concentration of *Pinus taeda* pollen. Dates are given as mmddyy. Pollen was collected using a 5 m tall platform located in an open field, which was 600 m from the Duke Forest plantation right after rainfall. On 29 March 2007 (day 88 of the year), sparse pollen was collected just prior to peak pollen shed. Peak pollen shed occurred on 1 and 2 April. (B) Pollen germination values for nighttime samples. Viable pollen was aviable at the same time that ovules within receptive *Pinus taeda* female strobili capture windborne pollen on micropylar arms.



pollen at day and night, so these two data sets were not directly comparable as they are in previous studies (Blush 1986; Pulkkinen and Rantio-Lahtimaki 1995) In any case, the role of viable night pollen on gene flow deserves closer study.

Night pollen capture in this *P. taeda* study (Blush 1986) coincided with peak pollen shed and, presumably, female strobilus receptivity. If female strobili were indeed in the latter stages of receptivity so that the pollen drop is emerging each night to pull pollen into its micropylar chamber, then one can hypothesize that germinating night pollen has the potential to make a genetic contribution. Night pollen is likely to come from the nearby *P. taeda* plantation where daytime pollen was sampled (a near-field source), but it can also come from daytime pollen released at greater distances (far-field source). This question of viable night pollen source and its pollination success opens a new, largely untested dimension to studying pine reproductive biology because night pollen has not yet been quantified as a paternal donor.

Even with better estimates for aerodynamic properties of pine pollen, mechanistic dispersal models approximate transport distance on a landscape scale (Katul et al. 2006; Kupar-

inen 2006). Precise estimates of terminal velocity, pollen concentration, and pollen viability are only small parts of a complex gene flow process. Still, the findings in this study narrow the gap between donor and receptor approaches to studying the gene flow question for long-distance pollen transport.

Gene flow distances for various woody perennial species have been reconstructed using DNA-based methods, and these studies show distances <50 km (Schuster and Mitton 2000; Lian et al. 2001; Smouse et al. 2001; Robledo-Arnuncio and Gil 2005). These distances deviate from the pollen flow distances shown in Table 1. Two explanations are as follows: (*i*) LDD pollen may not be viable or (*ii*) LDD events are so rare that experiments must designed differently to detect them. The latter is more likely given that long-distance pollen in northern Sweden is reported to be viable (Lindgren et al. 1975). If so, the DNA-based experiments sampling existing populations should be designed around predictions from spatially explicit dispersal models (Katul et al. 2006) to increase the probability of sampling of rare long-distance events. Stepwise sampling at increasing distances from source would be effective because pine pollen which can travel 1000 km from source (see references in Table 1).

In summary, the results of this study add further to the growing assertion that *P. taeda* pollen has a higher nuisance value than either *Z. mays* or *A. stolonifera* pollen because of its perennial production, low terminal velocity, and persistent viability, and this bears on regulatory concerns for genetically modified *P. taeda* plantations within the species' natural range.

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