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LONG-DISTANCE PINE POLLEN STILL GERMINATES AFTER MESO-SCALE DISPERSAL[1]

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Viability of long-distance pollen links ecological models to the genetic structure of forest tree populations, determining how forests will adapt to climate change and how far genes flow from genetically modified (GM) pine plantations. Addressing this landscape-scale inquiry is feasible when the pollen source, the delivery system, and the receiver field can be made explicit. To this end, I measured long-distance pollen germination along a 160-km transect along the North Carolina coastline, including 45 000 ha of mature *Pinus taeda* plantations and barrier islands. Using this system, I tested three hypotheses: (1) pine pollen germinates after dispersal on meso-scale distances, (2) sodium chloride exposure reduces germination of pollen captured over open saltwater, and (3) viable pine pollen is present at high altitudes before local peak pollen shed. The experimental findings are as follows: pine pollen had germination rates of 2 to 57% after dispersal at distances from 3 to 41 km, sodium chloride solutions mildly reduced *P. taeda* pollen germination, and viable pine pollen grains were captured at an altitude of 610 m. GM pine plantings thus have a potential to disperse viable pollen at least 41 km from the source. Wind and rainfall, as integral parts of regional atmospheric systems, together exert a powerful influence on the genetic structure of forest tree populations.

Key words: aerosols; climate change; conifer reproductive biology; genetically modified (GM) forest trees; heterospory; long-distance dispersal; male gametophyte; North Carolina; Pinaceae; *Pinus taeda*; U.S. Forest Health Initiative.

The viability of long-distance pollen is the missing link between ecological models and understanding the genetic structure of forest populations. Long-distance pollen, if viable, influences how resilient forests will be to climate change (Davis and Shaw, 2001; Austerlitz and Garnier-Géré, 2003; Hamrick, 2004) and how much unwanted pollen escapes from genetically modified (GM) pine plantations (Williams, 2005; Bonfils, 2006; Kuparinen and Schurr, 2007). Dispersal of viable pollen is also part of the scientific framework behind the U. S. regulatory decision-making for GM forest trees (van Frankenhuyzen and Beardmore, 2004; Kuparinen, 2006; Smouse et al., 2007). As shown here, measuring long-distance dispersal (LDD) pollen is feasible for *Pinus taeda* because a landscape system composed of pollen sources, delivery system, and receiver field (or sink) can be explicitly defined for this well-studied species.

Pollination biology—*Pinus taeda* begins producing pollen between ages 10 to 15 yr. A *P. taeda* tree at 16 m releases roughly 81 g of pollen per day for 2–4 weeks (Parker and Blush, 1996; Williams, 2008). Pollen is mostly shed during daytime hours, peaking at 1000–1300 hours and again at 1500–1800 hours if there is no rain (Fig. 1) (Blush, 1986). Viable pollen is also shed at night (Williams, 2008). This pattern is a good approximation for this species in other parts of the range (Greenwood, 1986; Williams, 2008), but it does not hold for other conifers or aerial biota as a rule (Pulkkinen and Rantio-Lahtimaki, 1995; Rogers and Levetin, 1998; Westbrook and Isard, 1999).

Pinus taeda pollen registers a terminal or settling velocity or V_t of 2.3 cm·s^{-1} (Niklas, 1984; Williams, 2008), so it is unusually buoyant for its size (Cain, 1940; Jackson and Lyford, 1999). Its aerodynamic properties come from its spheroidal shape, but it is not clear whether the two sacci on either side of the germination aperture aid flight or simply flatten during transit. Either way, that pine pollen travels long distances is well-established. Pine pollen moves up to 3000 km from its source (e.g., Campbell et al., 1999), but these phenomenal distances are immaterial if pollen moves outside of the range of receptive female strobili or it cannot germinate.

Pollen sources—*Pinus taeda* covers the southeastern United States, a total land area of 368 038 km^2 (Al-Rabab'ah and Williams, 2002). More than 228 023 km^2 occurs east of the Mississippi River Valley, and of this area, 41.5% is concentrated in South Carolina, Georgia, and Florida (Al-Rabab'ah and Williams, 2002). Only a narrow latitudinal band within the species range reproduces at any one time. In Florida and Texas, *Pinus taeda* sheds pollen in February, but farther north in South Carolina, pollen shed occurs by mid-March. In North Carolina, Virginia, and Maryland, which constitute its northern range limits, pollen shed is complete by mid-April (Baker and Langdon, 1990).

The extensive range of *P. taeda* overlaps with close relatives within its New World hard pine subsection *Australes*; these include *P. palustris*, *P. serotina*, *P. echinata*, and *P. elliottii*. All produce pollen grains that are too similar to be distinguished (Cain, 1940). Hybridization does occur occasionally if pollen shed overlaps. Of these, *P. palustris* pollen shed is more closely

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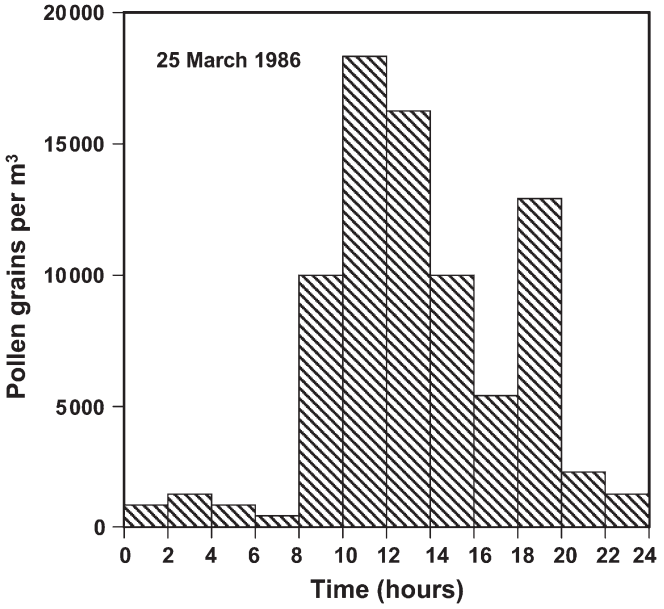

Fig. 1. The diurnal pattern for *Pinus taeda* pollen release shows two peaks: the primary peak occurs in the morning and a secondary peak occurs in late afternoon. (Reprinted by permission from Blush, 1986.)

synchronized with *P. taeda* than with either *P. echinata* or *P. serotina*, which shed later although exceptions do occur (Saylor and Kang, 1973; Edwards and Hamrick, 1995). *Pinus elliottii*, a southerly species sheds earlier than the rest in January and February even if planted north of its range (Baker and Langdon, 1990). Any F1 hybrids from such matings can mature into fertile adults capable of backcross and intercross matings. *Pinus taeda* has varying degrees of open-ended hybridization with close relatives throughout its range.

Pollen delivery—In simplest terms, passive wind transport is the delivery system for pine pollen, but its movement is also affected by turbulence, a continuous succession of gusts, swirling eddies, and lulls accompanied by swift changes in wind direction or advection (Lanner, 1966; Koski, 1970; DiGiovanni et al., 1996; Horn, 2005). No one atmospheric motion (or synoptic) system is responsible for biotic movement (Westbrook and Isard, 1999). Four major synoptic systems wax and wane with the seasons, influencing daily weather as shown for Hatteras Island, North Carolina (Pielke et al., 1987). Low- and high-pressure systems, turbulent large-scale eddies and land–sea circulations are just some of the forces causing pollen grains to move vertically and horizontally through the atmosphere.

Pine pollen floats at altitudes up to 300 m above the earth's surface (Koski, 1970). If it floats even higher, then it may become part of the rich aerial biota composed of other pollen (17–58 mm), spores (1–30 mm), bacteria (0.25–8 mm), viruses (<0.3 mm) as well as wingless insects, spiders, and larvae (Gislén, 1948; Gregory, 1978; Aylor et al., 1982; Westbrook and Isard, 1999; Jones and Harrison, 2004; Rousseau et al., 2006). Biological particles become prevalent at these higher altitudes, accounting for 25–33% of the ice-crystal residues in clouds and perhaps influencing cloud formation as well as precipitation (Jaenicke, 2005). No direct evidence yet shows that pine pollen is present at altitudes higher than 300 m. This is an interesting issue because, as a rule, all types of pollen are thought to be distributed between 800 to 2600 m during the day then compressed into more shallow atmospheric layers at night in the southern latitudes (Westbrook and Isard, 1999).

Delivery speed—Simple ballistics models predict that a pine pollen grain will move horizontal distances of 47 to 60 km within 3 h if wind speeds reach 5 $m \cdot s^{-1}$ (Koski, 1970; DiGiovanni and Kevan, 1991). This estimate triples if turbulence is taken into consideration (Katul et al., 2005; Nathan, 2006). For instance, *P. taeda* pollen is predicted to move from 21 to 49 km within 48 min above the forest canopy (Katul et al., 2006). This delivery speed for pine pollen is so rapid that its capacity for germination should be retained especially because freshly released *P. taeda* pollen retains its state of robust germination for the first 24 h after release (Goddard and Matthews, 1981). The working hypothesis here is that pine pollen can move mesoscale distances and still retain its capacity for germination.

Experimental support for this hypothesis comes from *P. sylvestris* pollen sampled in Sweden and Finland where pine pollen captured before local shed had germination rates as high as 75% (Lindgren et al., 1995; Pulkkinen, 1994). The cool, dry Arctic climate at these latitudes favors persistent pollen viability so this finding may not hold for warm-temperate conifer species. Another line of reasoning is that abiotic stress mitigates against long-distance pollen germination, and one source of abiotic stress in maritime settings is saltwater spray. Sodium buffers inhibit germination, causing osmotic shock in *P. sylvestris* pollen (Bohne et al., 2005), suggesting that sodium chloride exposure reduces germination rates. The working hypothesis here is that sodium chloride exposure reduces germination of pollen captured over open saltwater.

Transport scales—Spatial movement of biological particles on atmospheric circulation can be classified as follows (Gage et al., 1999): (1) microscale, which covers <1 km^2 distances, a parallel to thermal shells and other forms of turbulence; (2) mesoscale, which covers from 1 to 10^3 km^2 distances, a parallel to thunderstorms, weather fronts, and hurricanes; and (3) macroscale, which covers >10^3 km^2, a parallel to large-scale synoptic events such as tradewinds, cyclones, and monsoons. Pine pollen moves on all three distance scales (Williams, 2009).

Pollination window—Asynchrony between female and male strobili development at the same location is a common condition and creates a pollination window (Greenwood, 1986; Pulkkinen, 1994). Some female strobili in a population reach receptivity several days before peak pollen shed occurs locally. This has been well described for *P. taeda* in Beaufort County, North Carolina (35.56°N, 77.07°W), which is within the 160-km transect used in this study. Female strobili were receptive by March yet local pollen did not peak until April (Fig. 2). Similarly, the pollination window lasts 2–3 d for *P. sylvestris* (Pulkkinen, 1994). The pollination window offers a critical time point for sampling pollen at high altitudes, leading to the working hypothesis here that viable pine pollen is present at high altitudes prior to the local peak for pollen shed.

Receiver field: Capture and germination—Either the pollination drop or raindrops wash the pollen grain into the micropylar chamber (Doyle and O'Leary, 1935; Bramlett and O'Gwynn, 1980; Greenwood, 1986; Brown and Bridgwater, 1987). Upon hydration, the pollen grain's germination tube emerges then penetrates the ovule's nucellar tissue, and completes pollination,

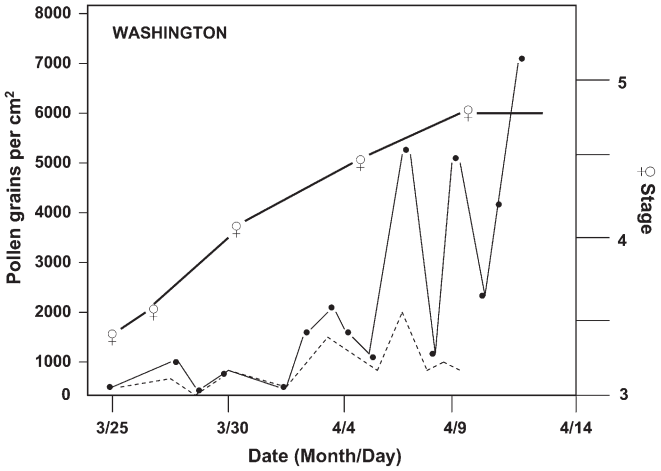

Fig. 2. Experimental data supporting the concept of a pollination window where female strobili are receptive just prior to local pollen shed. Note that female strobilus (symbol) receptivity occurs near stage 4.5, so many are approaching this stage on April 3–4 before the local spike in pollen count (solid line, round dots) occurs. Background pollen is shown as dotted line. (Reprinted by permission from Greenwood, 1986.)

even though fertilization does not occur for another year. The entire cycle from pollen release to seed maturity for *P. taeda* takes nearly 18 mo, so one experimental shortcut uses a standard agar-based germination assay highly correlated with seed set (Goddard and Matthews, 1981), thus approximating fertilization.

Freshly released pine pollen tends to have moderate germination rates between 20–80% before processing (Williams, 2008). Freshly released pollen of *P. sylvestris* germinates at only 45–65% after peak shed (Pulkkinen, 1994). Oddly, processed pine pollen often has higher viability after it has been sieved and dried under laboratory conditions (Williams, 2008).

Next I describe a series of experiments where three hypotheses were tested in relation to the landscape-scale system: (1) that pine pollen can germinate after moving meso-scale distances across open water, (2) that sodium chloride exposure reduces pollen germination sampled over openwater and (3) that viable pine pollen is present before local pollen shed.

MATERIALS AND METHODS

Experimental approach—Most of coastal North Carolina is planted with a single hard pine species, *Pinus taeda* L. (Pinaceae) although scattered stands of *P. serotina*, *P. palustris*, and *P. echinata* and a few plantings of *P. elliottii* are also present. Characterizing the pollen source required the following variables: (1) the source population's age, height, and release patterns; (2) atmospheric conditions during pollen dispersal including wind speed and direction; and (3) pollen capture location. All assumptions were independently verified using ownership records, weather stations, meteorological studies, a vegetation survey, and waterway features for the North Carolina coast (Fig. 3).

Pollen sources—The chief source was mature *P. taeda* plantations within a 160-km transect running south to north through nine North Carolina counties: Carteret, Pamlico, Craven, Beaufort, Hyde, Martin, Washington, Tyrrell, and Dare. This area has over 45 000 ha of *P. taeda* plantations at 15 yr or older in a flat topography (<0.1% slope) at less than 100 m above sea level. Over 35 200 ha of these non-GM *Pinus taeda* plantations are held by Weyerhaeuser Company, and the other source was the plantings in the Croatan National Forest adjoining Cherry Point weather station, the Neuse River ferry, and Pamlico County. There are 64 750 ha of pine plantations, 23% of which is either *P. taeda* or *P. palustris* (Oswalt and Johnson, 2002). This combined estimate of 45 000 ha is conservative because naturally occurring *Pinus* spp. stands and pine plantations owned by individuals or partnerships were omitted from the estimate. Along this 160-km transect, pollen shed starts south and moves northward between late March and early April (Greenwood, 1986; Williams, 2008).

Pollen delivery—Wind speed and direction and temperature were recorded at the Cherry Point, North Carolina (WBAN ID# 13754) weather station (34.89°N, 76.88°W) located near the Croatan National Forest and at Billy Mitchell Airport (WBAN ID# 93279) on Hatteras Island (35.23°N, 75.62°W). These local climatological data were accessed on 11 May 2009 (NNDC Climate Data Online, website http://www7.ncdc.noaa.gov/CDO/cdo). Weather and global positioning system (GPS) data were collected in the helicopter. Synoptic classification gives March as the first day of spring on Hatteras Island (Pielke et al., 1987) because this is when the prevailing wind switches direction from north to south-southwest.

Pollen capture—The Burkhard spore sampler model (Spiral Biotech, Norwood, Massachusetts, USA) includes a 90-mm Petri plate filled with 15 ml of 0.5% agar. Each plate sampled pollen concentrations at timed intervals using an air intake rate of 20 $L \cdot min^{-1}$. Each Petri plate sample was incubated at 28°C for 48 h before counting pollen with germination tubes.

Experiment 1.1. Hatteras Island—Three pollen plates were sampled at each of three locations along Hatteras Island's Pamlico Sound site on 3 April 2009 (Table 1; Fig. 3). More plates were taken on the Hatteras ferry en route to Ocracoke Island. The northernmost part of this 90-km-long island is part of a national seashore and wildlife reserve, Pea Island. Here, terrain is mostly dunes and shrubby vegetation from Pea Island Wildlife Sanctuary southward to Rodanthe. The closest indigenous pine stands are located at Nags Head Island due north of Hatteras (Bratten and Davison, 1987). A survey also showed a mixed *Pinus* spp. planting at Bodie Lighthouse, which shed 2 wk before the North Carolina mainland in 2006 and again in 2009. Residential landscaping at Frisco southward includes young or stunted horticultural pine plantings. At its southern end, Hatteras is separated from Ocracoke Island by Hatteras Inlet, a 0.8-km-wide strait. The smaller Ocracoke Island borders the Pamlico Sound, a saltwater body of water roughly 45 km wide (Kearney, 1901), but faces the Atlantic Ocean on its other side (Fig. 3).

Experiment 1.2. Ocracoke-Swan Quarter Ferry over the Pamlico Sound—Pollen was sampled during its trajectory across open saltwater (Table 1) along the ferry route between Ocracoke Island and the Swan Quarter ferry landing in Hyde County North Carolina (Fig. 3). A pollen sample was taken along with its GPS coordinates at the start and finish of each of three ferry runs on the Ocracoke-Swan Quarter ferry.

Meso-scale pollen dispersal was defined as the distance between Swan Quarter and the Petri plate sample taken closest to Ocracoke Island. At best, this was a minimum distance because inland *Pinus* spp. sheds pollen at distances of 300 km or more in the westerly direction (Williams, 2008). No pine pollen came from the barrier islands due to the strong prevailing westerly wind direction and an absence of pines on Ocracoke Island.

Experiment 1.3. Atlantic Ocean—Pollen was captured over the Atlantic Ocean then tested for germination (Table 1). Dispersal distance was calculated from the Duke Marine Laboratory to the last sampling location in the Atlantic Ocean, but this too was a minimum dispersal distance. Five agar-filled Petri plates were used to collect pollen over the Atlantic Ocean during the day (set 1 from 0900 to 1700 hours ET on 6 April 2006), and two more plates were used to sample pollen at night (set 2 from 1700 to 0300 hours ET on 6–7 April 2006). Each set also included a closed-plate control loaded with viable pollen; this served as an adjustment for any adverse pollen handling conditions during the voyage. The ship's GPS coordinates were 34.29.99°N, 76.37.65°W at the start of set 1 on 6 April 2006 and 33.58.85°N, 76.17.75°W at the start of set 2 at 1703 hours.

Experiment 2. Germination response to sodium chloride—The exposure study (Table 1) used stored *P. taeda* pollen which had been harvested at peak shed from five *P. taeda* accessions at the USDA Forest Service's Harrison Experimental Forest in Saucier, Mississippi (30°64N, 89°14W) in mid-March 2006. These were frozen until laboratory testing as described elsewhere (Williams, 2008).

Stored pollen was rehydrated at room temperature then immersed in one of three solutions for 10, 20, 30 or 40 min. The first treatment (0) was pure ddH_20

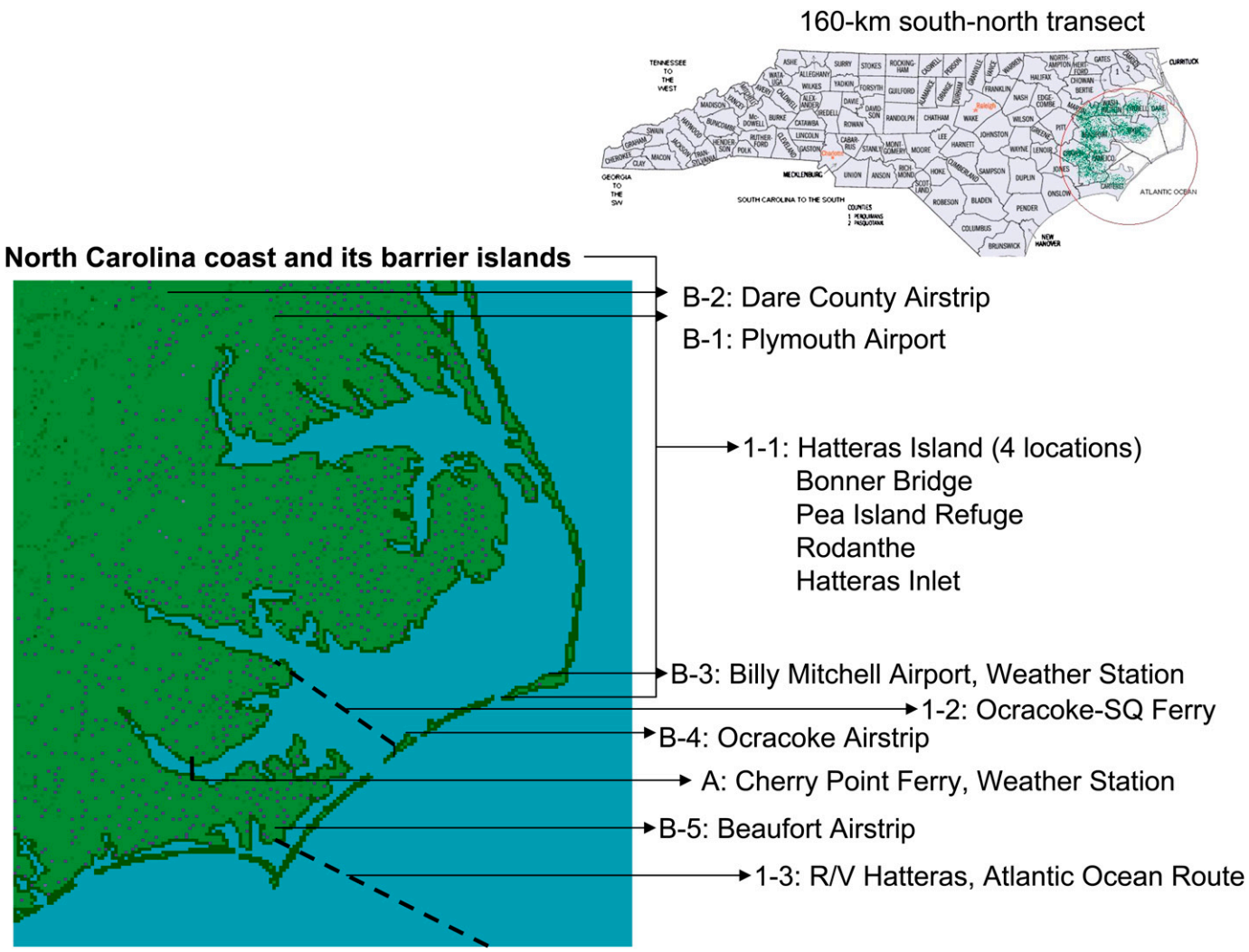

Fig. 3. The map of North Carolina's coast line shows the nine-county, 160-km transect from Beaufort County Airport (south) to Dare County Airport (north). Pollen sampling points are also shown for Hatteras, Ocracoke, Swan Quarter-Ocracoke ferry, Neuse River ferry and all airports. The Swan Quarter-Ocracoke ferry route's total distance of 41 km serves as the map's scale.

and the other two treatments had 0.45% and 0.90% sodium chloride (w/v). Although this salt content was within the range of the Pamlico Sound (0.5 to 35 parts per thousand or ppt), these treatment levels were lower than the Atlantic Ocean's levels which are around 3.49% sodium chloride or 34.9 parts per thousand. To ensure uniform submersion for floating pine pollen, each sample was applied to a 1-cm^2, clean, natural sponge, already saturated with its respective treatment solution, then turned upside down in the solution for the timed exposure before being transferred to a Petri plate of 0.5% agar.

Experiment 3. Helicopter sampling during the pollination window—The helicopter pilot and crew flew south–north transects while sampling pollen with the Burkhard spore sampler and its agar-filled Petri plates (Table 1). We collected

TABLE 1. Experimental plan for testing germination of long-distance pollen along the North Carolina coast (experiments 1.1, 1.2, 1.3), under laboratory conditions (experiment 2), and at high altitudes using helicopter sampling (experiment 3). Airport locations for sampling control plates were Plymouth, Dare County, Ocracoke and Beaufort.

Experiment	Site	Method	Design	Interval
1.1 Hatteras, Hatteras Inlet	Island	Burkhard	2 plates/site	9-min
1.2 Ocracoke	Open water	Burkhard	1 plate/section, 3 runs	9-min
1.3 Atlantic	Open water	Open plate	2 plates/section	8-h
2. Sodium chloride	Laboratory	Open plate	1 plate/treatment	0-10-20-30-40 min exposures
3. Helicopter	Altitude	Burkhard	1 plate/section	15-min
3.1 Ferry	Control	Open plate	2 plates/run	15-min
3.2 Airports	Control	Burkhard	1 plate/site	15-min
		Open plate	1 plate/site	15-min
3.3 Heat Sum	Verification	Temperature	—	—

15 samples over the entire 160-km transect from 1034 to 1834 hours ET at a single altitude of 610 m. Whether sampling occurred within the 2006 pollination window was tested using three methods: (1) ground sampling at five regional airports on the sampling day, March 30, 2006, (2) concurrent ferry sampling on the Neuse River near the Croatan National Forest, and (3) the heat sum equation (Boyer, 1978). Female strobilus receptivity was not measured.

Control 3.1. Airport sampling—This control was used to test whether pollen could be shedding at ground level and yet not present in the atmosphere. On March 30, 2006, ground-level samples were taken at four airports where the helicopter landed in North Carolina: B1 Plymouth (Washington County); B2 Manteo (Dare County); B3 Ocracoke Island (Dare County); and B4 Beaufort (Carteret County). Pollen was captured using both the Burkhard sampler and open-plate methods.

Control 3.2. Ferry over the Neuse River—This next control measured whether the helicopter crew sampled local peak shed and whether pollen could be shedding at ground level at the southern end of the transect yet not present at higher altitudes. Two open Petri plates filled with 0.5% agar were used per sample pine pollen for 15-min intervals while crossing the 2.4-km Neuse River ferry route between Cherry Point and Minnesott Beach, North Carolina, adjacent to the Croatan National Forest. Pollen samples were taken while crossing the Neuse River ferry using the paired open-plate method on March 30, 2006, and then again after helicopter sampling on April 1, 2006.

Control 3.3. Heat sum prediction—This control also tested whether the helicopter method sampled local shed. The heat sum method (Boyer, 1978) predicts the timing of peak pollen shed on the basis of three parameters: the start date of the growing season, the threshold temperature for accumulating hours, and a critical heat sum accumulation for the target event. For *P. taeda* populations in North Carolina, the heat sum equation is reliably predicted from a January start date, a threshold of day-heat units above 13°C (55° F), and an accumulated heat sum of 353°C (636° F) (Katul et al., 2006; Williams, 2008).

The heat sum was calculated from local climatological data for the Cherry Point weather station near the Croatan National Forest downloaded from the website http://cdo.ncdc.noaa.gov/CDO/cdo. These heat sum data and the observed peak pine pollen shed in spring 2006 have already been described previously (Williams, 2008). Male *P. taeda* strobilus development was verified daily during helicopter sampling at the Croatan National Forest using a pollen development classification system (Bramlett and Bridgwater, 1989).

RESULTS

The experimental findings were as follows: (1) meso-scale pine pollen had germination rates from 2 to 57% after dispersing over minimum distances of 3 to 41 km, (2) sodium chloride solutions had a mildly negative effect on pine pollen germination, and (3) viable pine pollen was sampled at an altitude of 610 m.

Sampling on Hatteras Island—Pine pollen was captured in late afternoon along the inland edge of Hatteras Island. These pollen samples had mean germination rates ranging from 13.6 to 49.1% after moving 11 to 35 km at minimum (Table 2). The sampling occurred during conditions favoring pollen capture: peak pollen shed had just started at the northern end of the transect, and the prevailing wind direction was W-SW (240 to 250°). The winds blew at steady speeds of 25 to 35 km·h^{-1} and occasional gusts reached 40 to 50 km·h^{-1}. At these wind speeds, pine pollen moved across the Pamlico Sound in minutes, not hours.

Germination was not tightly correlated with distance from the source (Table 2). The Pea Island Wildlife Sanctuary (samples 4–6) had a mean germination rate of 45.8%, and its estimated minimum travel distance was 21 km. Rodanthe (samples 7–9) had a lower germination rate of 13.6%, yet it was a similar distance from the nearest pine stand, 24 km (Table 2). Germination did not decline as distance increased.

TABLE 2. Germination of *Pinus taeda* after wind transport on meso-scale distances to Hatteras Island, North Carolina, USA during its peak pollen shed on April 3, 2009. Three Petri plate samples were taken at each of four locations using the Burkhard spore sampler. Burkhard spore sampler drew 20 L·min^{-1} for 9 min for each Petri plate. Daily weather data from the Billy Mitchell Airport on Hatteras showed wind direction of 240° and wind speed at 35.4 km, gusting to 51.5 km at 1651 hours ET (W-SW), a wind direction of 240° and a wind speed of 30.6 km, gusting to 46.7 km at 1751 hours ET (W-SW) and a wind direction of 250°and a wind speed of 25.7 km, gusting to 40.2 km at 1851 hours ET (W-SW).

Plate nos.	Sample location, latitude, longitude	Start time (hours ET)	Mean pollen count	Mean germination (%)	Distance to nearest pine pollen source (km)
1–3	Bonner Bridge, 35°45′58.54″, 75°31′33.23″	1815	19.0	49.1	11
4–6	Pea Island, 35°45′39.59″, 75°31′12.83″	1900	56.0	45.8	21
7–9	Rodanthe, 35°35′35.22″, 75°28′7 29″	1930	105.7	13.6	24
10–12	Hatteras Inlet Ferry, 35°12′ 31.33″, 75°45′19.53″	2100	120.7	34.0	35

Sampling over the Pamlico Sound—Pollen samples captured over open water between Ocracoke and Swan Quarter on 4 April 2009 germinated despite fluctuations in pollen count, direction, and minimum distance from the source. Run 1 from Ocracoke to Swan Quarter had pollen counts of 29 and 27 and germination rates of 13.8 and 7.4% (Table 3). Run 2 had higher pollen counts of 42 and 111, but germination rates were similar at 2.4 and 17.1%. Run 3 pollen counts rose to 40 and 273, and germination was 27.5 and 15.0%. Germinating pollen was moderate to low in all samples even though total pollen count rose gradually from morning to afternoon.

Shifts in wind direction did not reduce capture of germinating pollen. These wind shifts were restricted to surface winds; the prevailing wind direction of W/NW was constant at all sampling times even at altitudes of 1000 to 4000 m. Even so, surface wind direction was less consistent for the Ocracoke sampling than for Hatteras (Table 3). Only run 1 had a prevailing W-SW surface wind direction. In all runs, wind speeds ranged from 18 to 25 km·h^{-1}.

Sampling over the Atlantic Ocean—Two sets of paired Petri plates were each exposed for 8 h while the R/V Cape Hatteras traveled over the Atlantic Ocean. Set 1 was opened at 30 km from port, and set 2 was opened 90 km away (Table 4; Williams, 2009). Pollen count was higher in set 1. The controls were sealed Petri plates sample of pollen at the start. These had germination rates of 82.2–96.5%, so handling alone could not account for reduced pine pollen germination. The higher sodium chloride concentration in the Atlantic Ocean reduced long-distance pollen germination.

Sodium chloride reduces germination—Exposing *P. taeda* pollen to sodium chloride reduced germination. Mean germination was 74.6%, 55.4%, and 29.6% after sodium chloride treatment

TABLE 3. Conditions for testing whether pollen meso-scale wind transport over the Pamlico Sound between Ocracoke Island (OC) and Swan Quarter (SQ), North Carolina, USA during peak pollen shed on April 4, 2009. The Burkhard spore sampler drew 20 L·min^{-1} for 9 min for each Petri plate. Daily weather data came from the Billy Mitchell Airport on Hatteras Island.

Run no., Direction	Starting plate	Ending plate
1, OC-SQ		
Description	SQ 1-22	OC 1-16
GPS coordinates	35°18.622′, 76°18.352′	35°11.736′,76°05.436′
Time (h)	0908	0800
Wind direction (°)	270 W	240 W-SW
Wind speed (km·h^{-1})	24	22.5
Distance (km)	9.82	31.05
Pollen count	29	27
Germination (%)	13.8	7.4
2, SQ-OC		
Description	SQ 2-24	OC 2-33
GPS coordinates	35°21.792′, 76°19.023′	35°10.866 76°03.548′
Time (h)	1017	1201
Wind direction (°)	310 W-NW	310 W-NW
Distance (km)	3.44	34.02
Wind speed (km·h^{-1})	17.7	25.7
Pollen count	42	111
Germination (%)	2.4	17.1
3, OC-SQ		
Description	SQ 3-43	OC 3-34
GPS coordinates	35°18.071′, 76°18.359′	35°07.735′, 76°00.124′
Time (h)	1509	1323
Wind direction (°)	290 W-NW	310 W-NW
Distance (km)	10.26	41.57
Wind speed (km·h^{-1})	14.5	17.7
Pollen count	40	273
Germination (%)	27.5	15.0

Notes: GPS and time were recorded at the start of each 9-min Petri plate sampling. Format for GPS coordinates is D.M.DD. Weather data are recorded hourly at Billy Mitchell Airport on Hatteras Island.

of 0, 0.45%, and 0.90%, respectively (Table 5). These sodium chloride concentrations were intermediate between the Pamlico Sound and Atlantic Ocean (Table 5). Even so, this *in vitro* experiment had no parallel to the sampling over the Atlantic Ocean where the pollen grains (and agar) were exposed to saltwater aerosols for 8 h.

Helicopter sampling at high altitudes—Results from helicopter sampling at an altitude of 610 m on March 2006 (Table 6) showed a combined total pollen count of 42 pollen grains for all 15 Petri plates and of these, only four grains or 9% germinated. Controls at ground level also showed sparse pollen count. The eight plates from airport sampling had a total count of 40 grains and of these, 17 germinated (Table 7). Sampling on the Neuse River ferry captured a total of 154 pollen grains in seven open Petri plates, but only 4.5% of the grains germinated (Table 8). Sparse pollen, indicative of the pollination window, was present at higher altitudes and at ground level. Long-distance pollen captured during the pollination window was sparse at higher altitudes and at ground level for all controls, but a few of these pollen grains did germinate.

Helicopter sampling occurred right before peak shed. As shown by the Neuse River ferry data, peak shed started 2 d later on April 1. Pollen count rose to 1818, and germination was 66% (Table 8). Observed peak pollen shed after helicopter sampling was consistent with heat sum predictions (Fig. 4). During sampling, the accumulated heat sum had only reached 586 on March, a value that fell short of the requisite 636 heat-sum hours. Heat sum values, predicted and observed, supported the helicopter sampling before peak pollen shed.

TABLE 4. *Pinus taeda* pollen was collected with the open plate method at meso-scale distances from source over the Atlantic Ocean and subsequent germination. Set 1 Petri plate samples were collected 30.5 km from port by the R/V Hatteras on April 6, 2006. Set 2 Petri plate samples were collected 91.2 km from port. Agar-filled Petri plate controls sealed with stored pollen were also present in each set throughout the voyage. Partial data were previously published in Williams (2009).

Set-Plate no.	Pollen count	Germination (%)
1-1	10	0
1-2	85	0
1-3	18	0
1-4	76	0
1-5	108	1
Subtotal	297	0.30
Control	84	96.4
2-1	2	0
2-2	0	0
2-3	5	0
2-4	2	0
2-5	3	0
Subtotal	12	0
Control	107	82.2

Using the Burkhard spore sampler for the helicopter sampling while using the open-plate method for the ferry control (Tables 7, 8) raised the question whether results can be directly compared. To test for any discrepancy, I ran the Burkhard spore sampler for 15 min while a processed pine pollen sample was introduced into the airflow vents using a camel's hair brush. This method collected 157 grains, of which 150 germinated (95.5%). The open-plate method had the same pollen sample introduced into the plate using the same brush. Of 103 pollen grains collected, 97 germinated (94.2%), so the method of pollen collection did not affect germination.

DISCUSSION

These findings extend existing knowledge about pollen dispersal and pine pollination biology. That pine pollen still germinates after meso-scale wind transport provides an explanation for the genetic diversity patterns observed for *Pinus* spp. populations while providing a measure of ecologically realistic conditions.

Meso-scale pine pollen is viable—Weather conditions favored capture of viable pine pollen in these experiments. Wind speeds gusted from 25 to 50 km·h^{-1} westerly from the mainland toward Hatteras during sampling (Table 2). Similarly, wind speeds were 17 to 25 km·h^{-1} from the westerly direction from mainland pine plantations toward Ocracoke (Table 3). Also, sampling coincided with pollen shed along the entire 160-km study transect.

Another condition favoring pollen viability is the sheer volume of pollen released from the pollen source, and this is not apparent from the experimental design. A rough approximation is that that a 16-yr-old pine tree releases up to 80 g of pollen per day (Parker and Blush, 1996), and if each *P. taeda* planting has

TABLE 5. Germination of *Pinus taeda* pollen after different exposures of NaCl concentrations.

NaCl concentration (%, w/v)	Exposure (min)	Germination (%)
0	10	73.8
0.45		66.4
0.90		53.7
0	20	75.4
0.45		61.9
0.90		21.8
0	30	73.9
0.45		46.9
0.90		29.1
0	40	75.2
0.45		46.6
0.90		13.7

1700 trees per ha, then daily pollen production per hectare can reach 34 L of pollen. This nine-county region includes 45 000 hectares of *P. taeda* plantations, so this is a maximum daily pollen release of 1.5 million liters of pine pollen per day over 2–4 weeks, and this estimate does not include the fraction of pine pollen that is deposited then resuspended (i.e., McDonald, 1962). This estimate is thought to be conservative because more than 45 000 ha of pine stands are releasing pollen especially if one considers inland pine pollen sources. This is difficult to reconcile with single-tree long-distance dispersal (LDD) models. At best, LDD is modeled as either very rare long-distance diaspore movement (~50 km) or many frequent events of more limited distances (~20 km) (e.g., LeCorre et al., 1997; Austerlitz and Garnier-Géré, 2003); the latter may be the more accurate. The sheer volume of long-distance pine pollen dispersed in this setting also suggests that LDD models for linking viable pine pollen dispersal to forest population structure may also be unduly conservative.

Sodium chloride exposure reduced germination rates—On the basis of the laboratory results, sodium chloride exposure mildly reduced germination of pollen moving across the Pamlico Sound. But these results do not explain the Atlantic Ocean samples; the 8-h aerosol exposure plus saltwater on the agar suggests that this experiment was not a suitable test of long-distance pollen viability, thus results for the Atlantic Ocean are not directly comparable to any other studies reported here. Germination is expected to be higher in future Atlantic Ocean studies if pollen sampling times are measured in minutes, not hours and captured pollen is placed on salt-free agar. Germination of meso-scale pollen is hypothesized to be viable whether captured over ocean or over land.

TABLE 6. Helicopter sampling of pine pollen was conducted at a constant altitude of 610 m over coastal North Carolina from 1034 until 1834 hours ET on March 30, 2006.

Plate no.	GPS coordinates (N, W)	Time (ET hours)	Pollen count	Germinating pollen grains
001	35°11.3, 77°00.5	1034	4	0
002	35°28.4, 76°53.5	1051	1	0
005	35°53.9, 76°37.6	1141	3	0
006	35°54.6, 76°11.7	1201	4	0
009	35°53.4, 75°38.4	1348	3	2
010	35°18.9, 75°31.0	1404	4	0
011	35°14.9, 75°36.3	1423	0	0
014	35°03.6, 76°02.1	1507	0	0
015	34°52.4, 76°17.5	1522	1	0
018	35°00.1, 77°22.8	1702	2	0
019	35°17.2, 78°02.1	1718	3	0
020	35°26.4, 78°24.2	1734	8	1
021	35°34.7, 78°45.5	1750	7	1
022	35°34.7, 78°45.5	1805	0	0
023	35°53.1, 79°27.6	1822	2	0

Vertical distribution of viable pine pollen—Finding viable pollen at an altitude of 610 m above the earth's surface raises the minimum altitude for pine pollen from 300 m, but it does not address the more interesting question of how pine pollen might be vertically distributed above the earth's surface. More sampling are needed before one can conclude that pine pollen ascends to higher altitudes of 800 to 2600 m as suggested by Westbrook and Isard (1999). Thus it is still not clear from these experiments whether pine pollen grains are an integral part of the aerial ecosystem composed of other types of pollen, spores, bacteria, and other biological particles. Another shortfall here is that viability of pollen captured using the helicopter may be a novelty, not a contributor to gene flow. Not only did this pine pollen have low viability, but it would only be a successful paternal parent under the "first come, first serve" hypothesis, which has been rejected for *Pinus* spp. (Greenwood, 1986; Varis et al., 2008).

Regional atmospheric systems shapes gene flow and the genetic structure of forests—Regional atmospheric systems are composed of more than strong winds; they also include other weather events such as rainstorms. Frequent late-afternoon rainstorms are common during *P. taeda* pollen shed, and these cleanse the atmosphere of ambient pine pollen (McDonald, 1962; Blush, 1986; Greenwood, 1986). Strong winds accelerate pollen movement but sudden rainstorms halt or punctuate such movement. More than halting pollen movement, raindrops serve as a proxy pollination mechanism, washing pollen down into the ovules (Greenwood, 1986; Brown and Bridgwater, 1987). Thus synoptic events have the capacity to expand and contract effective population sizes of forest tree populations from one year to the next, as suggested by Lanner (1966). As such, these findings are difficult to compare to other dispersal studies, so a couple of caveats are offered here.

Long-distance dispersal (LDD) requires many experimental methods—The study of LDD to date has been refined to single-tree-tracking experiments where a known quantity of pollen is released from a pollen donor then traced over measured distances. Single-tree tracking is not suited to testing meso-scale pollen profiles across a regional landscape scale for one tree or many trees, so no direct comparison is possible here. LDD pollen studies may require many methodological approaches, not only single-tree-tracking studies, especially if more than one long-distance dispersal process is operative.

Potential dispersal measured on a meso-scale is not directly comparable to DNA paternity analyses—Another caveat is that these long-distance pollen viability experiments are not directly comparable to gene flow distances estimated from DNA-based paternity analysis methods (e.g., Ennos, 1994). The experiments reported here measure potential dispersal, while DNA-based paternity analyses measure *effective* dispersal or successful fertilization events. DNA-based studies often report average

TABLE 7. Airport control for the helicopter sampling. Paired samples of open-plate (O) and Burkhard spore sampler (S) were taken for 15 min at ground level at four coastal North Carolina airports on March 30, 2006. This served as an altitudinal control for the helicopter sampling on the same day. Germination is reported as pollen count.

30 March 2006	Location (airport)	County	Time (ET hours)	Pollen count	Germinating pollen grains
3-1-1-003-S	Plymouth	Washington	1116	6	2
004-O			1116	20	13
3-1-2-007-S	Manteo	Dare	1322	3	0
008-O			1322	5	2
3-1-3-012-S	Ocracoke	Dare	1444	0	0
013-O			1444	1	0
3-1-4-016-S	Beaufort	Carteret	1548	1	0
017-O			1548	4	0

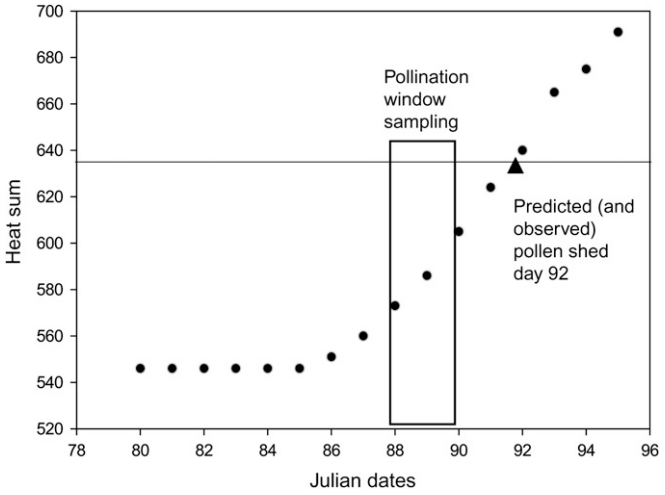

Fig. 4. Helicopter sampling occurred during the 2006 pollination window as indicated by the heat-sum equation based on data from the Cherry Point North Carolina (WBAN ID# 13754) weather station (34.89°N, 76.88°W). Symbols: triangle indicates peak pollen shed, round dots represent the observed heat-sum accumulation, and the line is the heat-sum threshold for 636 heat-sum hours.

distances across age, height, and occasionally generations. Distances are often reported in meters, although a few meso-scale exceptions have been reported (Schuster and Mitton, 2000; Robledo-Arnuncio and Gil, 2005). In part, DNA-based gene flow distances, if expressed as averages, are biased downward because average distances are weighted in favor of the more common local dispersal events (99%), not the rare long-distance dispersal events (1%).

Revising experimental design for effective dispersal is also suggested. The scale over which dispersal is measured in DNA analyses tends to be smaller than the scale of movement for viable pollen. However difficult, the experimental design should parallel prevailing wind direction so there is a need for designs that are not the radial or "wagon wheel" designs commonly used for DNA paternity analysis. Wagon-wheel designs are less desirable because they bias in favor of sampling short-range dispersal events and artificially truncate sampling along the full scale of movement. A better option is to sample along the prevailing wind direction to trace more ecologically realistic gene flow distances, as shown for creeping bentgrass (*Agrostis stolonifera* L.) (Watrud et al., 2004).

Relevance to forest adaptation to climate change—Regional atmospheric systems are directly affected by climate change just as forest tree populations are. Higher wind speeds are predicted to move pollen and seeds farther from the source (Kuparinen et al., 2009), and if this pollen is viable, then effective population sizes of forest tree populations will expand, not contract, under climate change. Similarly, enriched CO_2 concentrations causes *P. taeda* to triple its seed production (LaDeau and Clark, 2001), and this too could expand effective population sizes. These effects have yet to be fully figured into pollination biology and the rest of the diplohaplontic life cycle. More forest resilience in the temperate zones seems a likely outcome (Davis and Shaw, 2001; Hamrick, 2004; Aitken et al., 2008). More gene flow, pollen or seed, among populations contributes to higher within-population levels of genetic diversity relative to among-population levels (Hamrick, 2004). However, another outcome that must not be overlooked is the non-optimal adaptation commonly observed for many temperate-zone forest tree populations even now (Westfall and Millar, 2004). Even so, dispersal of viable long-distance pollen bears on population structure within an east–west latitudinal band, not the entire *P. taeda* range.

TABLE 8. Neuse River ferry control for the helicopter sampling. Paired open-plate (O) samples were taken for 10 min at the midway point on March 30, 2006 and again on April 1, 2006. This served as a control for helicopter sampling on March 30.

Date (2006)	Time (hours ET)	Total plates	Total pollen count	Germination %
30 March	1347–1716	7	154	4.5
1 April	1317–1905	12	1818	66.2

Relevance to U. S. regulation of genetically modified (GM) forests—The regulatory problems for GM forest trees are multiplying (Strauss et al., 2009) as agencies struggle with the long timelines and fecundity incumbent to temperate forest tree species. For years, gene flow information has been partial, incomplete, or missing. This information has been lacking although most of the reproductive biology protocols required to test these hypotheses were developed in the public domain by U. S. timber company researchers decades ago (e.g., Blush, 1986; Greenwood, 1986). In any event, these findings show that long-distance pine pollen remains viable even after meso-scale transport and that the escape of GM transgenes into less-managed forest ecosystems is a certainty that cannot be reversed (Bonfils, 2006). The unintended effects of these transgenes, unknown at this time, have the potential to persist on an ecological and evolutionary timeline exceeding any regulatory agency's purview (Williams and Davis, 2005).

Persistence of transgenes from GM pine plantations extends to close relatives of *P. taeda*. Natural hybridization is rare yet a certainty within the *Australes* subsection. Phenological conditions do not often overlap among species from year to year, but hybridization results in fertile adults capable of crossing back to other hybrids, parental species, or even a third related species. The viability of long-distance GM pollen has implications not only for *P. taeda*, but for its close *Australes* relatives.

In summary, pine pollen can still germinate after traveling on meso-scale distances from the source. These findings also pro-

vide an ecologically realistic explanation for the high degree of within-population genetic diversity typical of *P. taeda* and many other wind-pollinated conifers, temperate and tropical. Regional atmospheric systems are powerful forces shaping the dispersal of pine pollen and ultimately the genetic structure of conifer forests, whether GM or not, under human-induced climate change.

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