

Pollen production by *Pinus taeda* growing in elevated atmospheric CO₂

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Summary

1. Rising concentrations of atmospheric CO₂ may have important consequences for reproductive allocation in forest trees. Changes in pollen production could influence population dynamics and is likely to have important consequences for human health. This is the first study to evaluate pollen production by forest trees in response to rising atmospheric CO₂.

2. Our research objective was to quantify pollen production by Loblolly Pine (*Pinus taeda* L.) trees growing in elevated CO₂ (ambient + 200 11⁻¹) since 1996.

3. Trees grown in high-CO₂ plots first began producing pollen while younger and at smaller sizes relative to ambient-grown trees. Pollen cone and airborne pollen grain abundances were significantly greater in the fumigated stands. We conclude that the greater number of mature trees in high-CO₂ plots resulted in greater pollen production at the stand level.

4. Precocious pollen production has important implications for fertilization and pollen dispersal from young, dense stands. Increasing levels of airborne pollen raise concerns for escalating rates of human respiratory disease.

Key-words: carbon dioxide, climate change, FACE, fecundity, *Pinus taeda*, pollen, reproductive allocation, respiratory health, strobili

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Introduction

Rising concentrations of atmospheric CO₂ may have important consequences for plant reproductive biology (LaDeau & Clark 2001; Jablonski, Wang & Curtis 2002). Because of its dependence on carbon balance, pollen production is expected to respond to rising atmospheric CO₂ concentrations. Increased airborne pollen could have potentially devastating effects for many people who suffer from asthma and respiratory disease. But few studies have explicitly quantified pollen production in an elevated-CO₂ atmosphere; these few provide ambiguous results and none applies to trees. Studies using herbaceous species have sometimes found increased flower production and increased stand-level pollen production in response to CO₂ fumigation (Ziska & Caulfield 2000; Jablonski, Wang & Curtis 2002; Prasad *et al.* 2002; Wayne *et al.* 2002). The current study provides a first perspective on pollen production by a forest tree at future CO₂ concentrations in a fully controlled, multiple-year experiment.

Pollen production is a key attribute of reproductive success and fitness in forest communities. Most temperate tree species are wind-pollinated (Burns & Honkala 1990; Smith, Hamrick & Kramer 1990), and successful fertilization demands copious amounts of airborne pollen (Bramlett 1981; Bridgewater & Trew 1981; Holsinger 1991). Inter-annual variation in pollen production drives variable seed production in many temperate tree species (Grano 1973; Shibata *et al.* 2002; Mosseler *et al.* 2000; Knapp, Goedde & Rice 2001; Satake & Iwasa 2002), including coniferous species (Bramlett 1972; Knowles *et al.* 1987; Fatzinger *et al.* 1988; Sork *et al.* 2002). Differential responses in male and female reproductive effort to high CO₂ could therefore affect plant reproduction and fitness. Changes in the amount of pollen produced, or in the developmental timing of pollen release under higher atmospheric CO₂ concentrations, could have a direct impact on the growth and genetic diversity of tree populations. Shifting the onset of pollen production to smaller or younger trees may also have important implications for population dynamics. Short and dense canopies have reduced access to wind currents that disseminate pollen over long distances (Katul, Williams, & Siqueira 2005). Thus pollen produced by dense stands of relatively short trees is more

likely to be dispersed locally and could lead to higher rates of self-fertilization (Bramlett 1981; Cheliak *et al.* 1985). Pollen is the dominant vector of gene flow among tree populations. Heterozygosity (a key measure of genetic diversity) can depend on pollen exchange over a large geographical range (Greenwood 1986; Dyer & Sork 2001). High rates of pollen production from tall trees may be required to maintain the cohesion of gene pools in migrating or patchy populations.

Pinus taeda L. (Pinaceae) is one of the most important timber species in the south-eastern USA, with a continuous range from Maryland south to Florida, and west to Texas. The importance of long-distance pollen dispersal is evidenced by considerable gene flow throughout this extensive range (Al-Rabab'ah & Williams 2002). Our study examines CO₂ effects on pollen production by *P. taeda* trees, which generally do not initiate production of male strobili (pollen) for several years after first producing female strobili (Greenwood 1980; Schmidting 1983). Open-grown trees in seed orchards produce significant amounts of pollen only after 15–20 years (Bramlett 1981). *Pinus taeda* trees growing at elevated atmospheric CO₂ (ambient + 200 mol mol⁻¹ CO₂) produce more seeds than trees growing in ambient conditions, and they begin producing seeds earlier (LaDeau & Clark 2001). If these changes indicate a fundamental shift in ontogeny towards more rapid development and early reproductive maturity, then trees growing in elevated CO₂ would also be expected to initiate pollen production earlier, and at smaller sizes. However, seed development is a stronger carbon sink than pollen (Wardlaw 1990; Avila-Sakar *et al.* 2001), and seed production may benefit disproportionately from increased photosynthate under high-CO₂ concentrations. In this study we use data from multiple years to determine whether CO₂-fumigated trees produce more pollen than ambient-grown trees. We explicitly evaluate the size at which trees begin producing pollen to test the hypothesis that trees grown in high CO₂ initiate pollen production earlier than ambient-grown trees.

Materials and methods

This experiment was conducted at the Duke Forest free-air CO₂ enrichment (FACE) site, located in an unmanaged Loblolly Pine plantation in the Piedmont region of North Carolina (Orange County, 35 97' N, 79 09' W). In August 1996, FACE technology was installed in a 13-year-old Loblolly Pine plantation (Hendrey *et al.* 1999). The fully replicated experiment consists of six 30-m-diameter circular plots of forest surrounded by 32 vertical pipes that deliver ambient or elevated CO₂ (ambient + 200 L⁻¹ CO₂) atmosphere at multiple canopy heights during daytime hours (prior to 2003 CO₂ fumigation was 24 h, for details see <http://face.env.duke.edu>). Three plots receive elevated CO₂. Central towers (22 m tall) in each plot provide canopy access. *Pinus taeda* at this site are even-aged,

maternal half-sibs and in 1996 accounted for 98% of the woody basal area. In 2004, pine basal area per plot ranged from 2.1 to 3.2 m² among ambient plots and from 2.2 to 3.6 m² among fumigated plots. Tree diameters at breast height (d.b.h.) were censused in 1998, 2002 and 2004.

Pinus taeda trees are monoecious, bearing female strobili on branches near the top of the canopy, and male strobili on slow-growing branches lower in the canopy (Greenwood 1980; Greenwood & Schmidting 1981). All male cones on a tree develop and release pollen synchronously over a 2–3-week period (Grano 1973). Although female strobili are pollinated in the spring following bud initiation, pollen-tube growth is arrested before reaching the ovule and fertilization does not occur for another 12 months. Seeds are dispersed more than 2 years after reproductive buds are initiated.

Because pollen cones were difficult to quantify on trees, we used a diverse set of experiments to evaluate individual tree- and stand-level pollen production in ambient and elevated atmospheric CO₂.

INDIVIDUAL TREE POLLEN PRODUCTION

Annual censuses were conducted to identify trees with pollen cones in each plot from 2002–05. Censuses were conducted from the central towers each April prior to pollen dissemination. In 2005, pollen-bearing trees were categorized according to the estimated proportion of branches with pollen cones (low, trees with cones on <25% of branches; moderate, 25–75%; high, ≥75%).

STAND-LEVEL POLLEN CONE PRODUCTION

Beginning in 2001, dehisced pollen cones were collected monthly from 12 seed traps (0.16 m²) located randomly in each plot (1.92 m² total ground area sampled per plot). Traps were constructed from laundry baskets lined with screen and covered with a 2.5-cm wire mesh to deter rodent predation. Analyses presented here assume that pollen cones sampled from litter traps derive only from trees within the experimental plots. This is a safe assumption given the dense tree canopy and relatively short stature of trees within plots. Because slight differences in woody basal area among plots could affect results, estimates of male strobili production are expressed as numbers per woody basal area of pine present in each plot. Seed cones were also censused from central towers in the autumn of each year of this study (LaDeau & Clark 2006).

POLLEN GRAINS PER CONE

Pollen cones sampled from trees near the central canopy towers were bagged and removed from trees prior to ripening in March 2004. Samples were allowed to ripen in open plastic bags in the laboratory (Beers *et al.* 1981; Sprague & Snyder 1981). Seventeen cones were

collected from nine trees: nine cones from five ambient-grown trees, and eight from four trees in the high-CO₂ plots. Once cones ripened and disseminated pollen, 100 ml water was added to each bag to create a pollen slurry. The number of pollen grains per aliquot of slurry was quantified using light microscopy. Results were averaged over 10 aliquots per cone sampled. Counts of pollen grains per cone were divided by cone length to standardize for differences in the length of male cones. The effect of elevated CO₂ on the length of pollen cones was also evaluated.

POLLEN GRAINS FROM AIR SAMPLES

Airborne pollen was quantified during peak production in April 2004 and 2005. Sampling dates fell between 16 April and 28 April 2004, and between 6 April and 22 April 2005. In both years, samples of airborne pollen were collected on microscope slides coated with petroleum jelly. Two slides per plot were exposed to air on the central towers, one just below and one above the main canopy (at 18 and 14.5 m). Slides were secured horizontal to the ground and exposed for 60 min on six dates over the 2-week period. The total number of pollen grains per sample (4.8 cm² area) was quantified using light microscopy.

STATISTICAL METHODS

To quantify the complex relationship between individual tree diameter and maturation (here 'maturation' refers to the onset of pollen production) we implemented a model that estimates the effect of elevated [CO₂] on tree size (d.b.h.) when pollen production is initiated. The probability that tree *i* is mature in year *t* (θ_{it}) increases with diameter d_{it} as a gamma cumulative-density (CGAM) function with shape (a_θ) and rate (b_θ) parameters

$$\theta_{it} = CGam(d_{it}; a_\theta, b_\theta) \quad \text{eqn 1}$$

tree $i = (1, \dots, n)$; year $t = (t_1, \dots, T_i)$

with distribution mean a_θ/b_θ . Our goal was to compare the mean diameter at which trees are likely to reach maturity. We explicitly test the hypothesis that trees in the high-CO₂ plots initiate pollen production at smaller diameters than trees growing in ambient plots ($a_{\theta e}/b_\theta < a_{\theta a}/b_\theta$) by comparing posterior parameter estimates where the shape parameter is fit separately to ambient ($a_{\theta a}$) and elevated CO₂ ($a_{\theta e}$) treatments. Note that the second parameter for the gamma cumulative-density function is the same for both treatments, thus the treatment effect is taken up in the first parameter. For the gamma distribution, the mean and variance are not represented by independent parameters, making this approach reasonable. Estimation is accomplished with a Gibbs sampler, which involves simulating the posterior density of parameters by sampling alternately

from conditional posteriors and updating values. Non-informative priors were used for parameters in the maturation schedule θ . Significance levels were assessed by 95% credible intervals from model output. The sampler was run for 100 000 iterations with a 25 000 iteration burn-in period. Gibbs samples were resampled systematically to remove correlation. Further details of this modelling approach are given by Clark *et al.* (2004).

Estimating CO₂ effects on pollen production relied on cone and pollen data from seed traps and air samples, as described above. Standard statistical techniques were used to evaluate differences in mean values between ambient and elevated CO₂ samples. Linear mixed-effects models were used to compare longitudinal data sets of pollen-cone abundance in litter traps. These models estimate year effects and random plot effects in addition to the CO₂ treatment response (Venables & Ripley 2002, pp. 271–300). Significance levels were assessed at $\alpha < 0.05$. All analyses were implemented using the R package ver. 2.0 (2004, © R Foundation, from <http://www.r-project.org>).

Results

INDIVIDUAL-LEVEL POLLEN PRODUCTION

Trees initiated pollen production at smaller diameters when grown in high CO₂ (Table 1). Modelled transition to maturity occurred at diameters that were an average 3 cm smaller for trees grown in the high-CO₂ atmosphere relative to ambient-grown trees (95% credible intervals do not overlap; Fig. 1a). Estimated maturation size was well correlated (85%) with the size of observed pollen-producing trees, demonstrating that the model adequately fits observed data. The proportion of mature trees per plot increased over time (Fig. 1b), although there was considerable variability among years in the numbers of trees observed with pollen. During our study, an average 16.4% (± 4 SD) more fumigated trees contributed pollen compared with ambient trees. Because trees growing at this site are evenly aged, differences in annual transition rates reflect differences among trees for age at maturation. The results presented here are similar to findings for

Table 1. Parameter estimates from Gibbs sampler for gamma function (a_θ, b_θ) and estimated diameter at maturation for trees grown in ambient and elevated-CO₂ atmosphere

| Parameter | Estimate | SE | 95% CI |
|----------------------|----------|------|--------------|
| $a_{\theta A}$ | 4.73 | 0.48 | 3.84, 5.62 |
| $a_{\theta B}$ | 3.92 | 0.45 | 3.09, 4.73 |
| b_θ | 0.23 | 0.03 | 0.18, 0.27 |
| Ambient d.b.h. (cm) | 20.71 | 0.41 | 19.94, 21.56 |
| Elevated d.b.h. (cm) | 17.14 | 0.37 | 16.45, 17.86 |

Mean estimates shown with standard error and 95% credible interval.

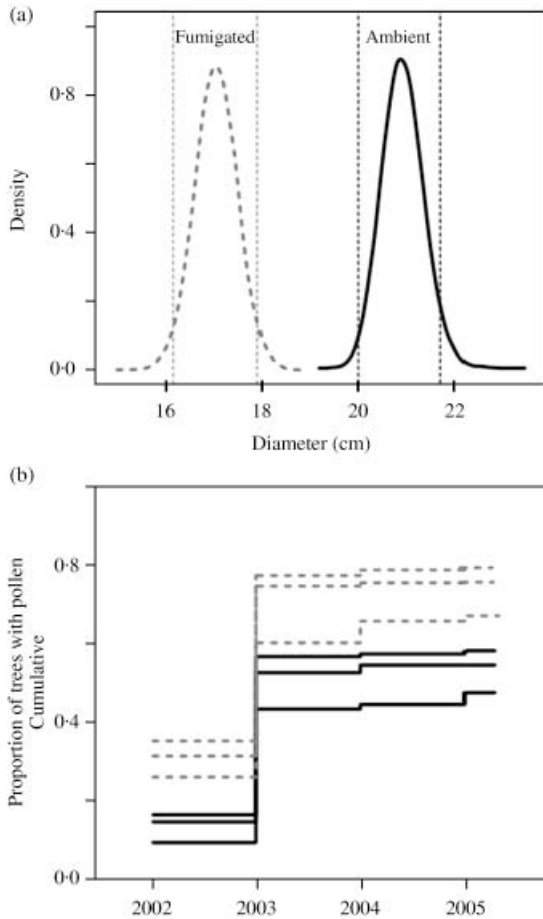


Fig. 1. (a) Mean diameter at which 50% of trees are reproductive in ambient and CO₂-fumigated plots. Dotted lines, 95% credible intervals. (b) Cumulative proportion of trees in ambient (solid black line) and high-CO₂ (dotted grey line) plots with pollen cones from 2002–04.

initiation of seed production at this site (LaDeau & Clark 2006). By spring 2005 63% of all trees growing in high CO₂ had produced both pollen and seeds, *vs* only 36% of trees in the ambient plots. Ambient-grown trees were more likely to allocate resources toward only seed cones (15 *vs* 6% of elevated trees) or only pollen cones (24 *vs* 18%).

In spring 2005 we assigned tree-abundance categories depending on visual inspection of pollen cone numbers. In the elevated-CO₂ plots, an average 32% (± 2 SD) of pollen producing trees were in the low category and 17% (± 4) in the high category. Among ambient trees that produced pollen in 2005, 39% (± 4) fell in the low category and 12% (± 3) in the high category. Proportions did not differ between ambient and fumigated plots in any category (Pearson's χ^2 test, $\chi^2 = 2.44$, *df* = 2, *P* = 0.29).

STAND-LEVEL POLLEN PRODUCTION

Plots exposed to elevated atmospheric CO₂ produced more pollen than ambient plots. The total number of pollen cones collected in litter traps varied considerably between 2001 and 2005, with the highest numbers

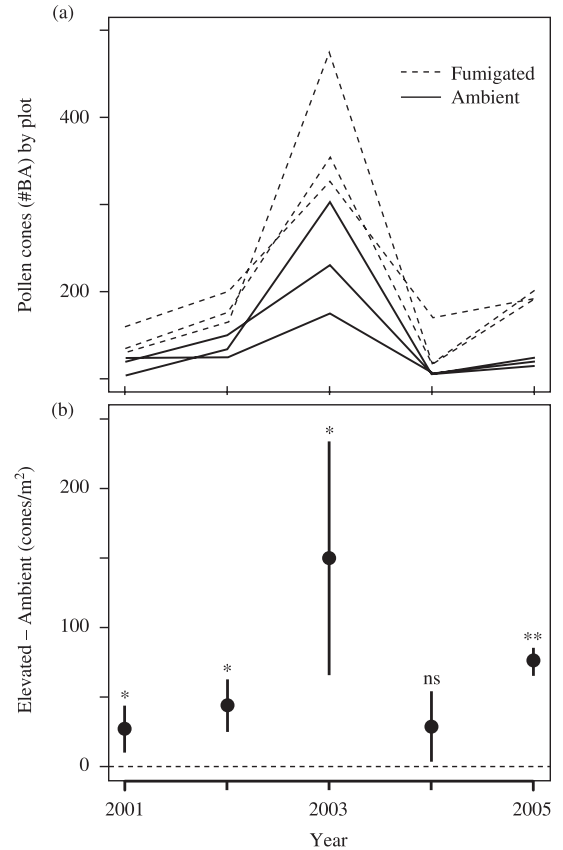


Fig. 2. (a) Total pollen-cone abundance from litter-trap collections from 2001–05, corrected for basal area of pine present in each plot. Dotted grey lines, three CO₂-fumigated plots; solid black lines, ambient plots. (b) Difference in mean pollen cone abundance between elevated-CO₂ and ambient plots. Error bars, standard errors. Asterisks identify significance levels for each year separately (one-sided *t*-tests: *, *P* < 0.05; **, *P* < 0.01).

(corrected for woody basal area) in each plot collected in 2003 (Fig. 2a). Prior to spring 2001, pollen production was uniformly low in all plots (S.L.L., personal observation). The total numbers of pollen cones collected in litter traps ranged from a maximum of 37 cones m⁻² woody basal area in 2001, to a maximum 473 cones m⁻² in 2003. The mean CO₂ enhancement of pollen cone production (sampled from litter traps) ranged from 15 more cones m⁻² basal area in 2004 to 148 more pollen cones collected in high-CO₂ plots in 2003 (Fig. 2b). We compared the longitudinal data for pollen-cone abundance over 5 years using a linear mixed-effects model with random plot effects. After accounting for differences among plots and years, CO₂ treatment was a significant source of variability ($F_{1/4} = 15.6$, *P* = 0.017). We did not find a difference in the number of pollen grains per cone, which ranged from 500 to 37 000 grains (two-sided *t*-test, *P* > 0.05). Pollen cone length, which averaged 2.4 and 2.9 cm in ambient and elevated treatment plots, respectively, was not enhanced by CO₂ fumigation (two-sided *t*-test, *P* > 0.05).

Airborne pollen grains sampled in 2004 and 2005 are compared in Fig. 3. We believe that airborne pollen sampling captured peak pollen dispersal in 2005, but

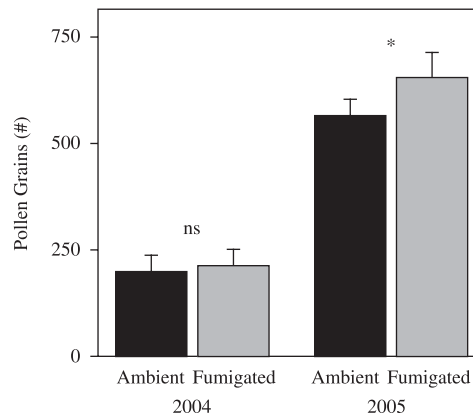


Fig. 3. Total number of pollen grains collected in 2004 and 2005 sampling efforts. Bars, average pollen grains collected on slides exposed for 60 min, over six dates, in ambient (dark bars) and fumigated (light bars) plots. Error bars, standard deviations; $n = 3$ for all bars. Asterisks identify significance levels for each year separately (one-sided t -tests: *, $P < 0.05$).

occurred after peak pollen release in 2004. We saw no difference in the number of pollen grains sampled in ambient vs fumigated plots in 2004 (one-sided t -test, $P > 0.05$). More pollen grains were collected from the three elevated CO₂ plots in spring 2005 than from ambient plots. The mean treatment effect in 2005 was 137 more pollen grains per 30-min exposure per unit area in the high-CO₂ plots (one-sided t -test, $t = 3.01$, $df = 3.0$, $P = 0.025$).

Discussion

There is little doubt that atmospheric CO₂ is increasing, with potentially profound implications for vegetative dynamics as well as for human health (IPCC 2001). Changes in pollen production may be a key determinant in evaluating potential impacts, but data are lacking. The 5-year study presented here shows the first analysis of pollen production by a forest tree growing in predicted future atmospheric [CO₂]. We find that *P. taeda* stands grown at high CO₂ produce more pollen per unit woody basal area than ambient-grown trees (Fig. 2). Trees fumigated with CO₂ began producing pollen at younger ages and smaller sizes (Fig. 1); thus evenly aged stands had larger proportions of trees contributing pollen under elevated CO₂. Our results did not show unequivocally that an individual tree grown in high CO₂ produced more pollen than an ambient-grown tree. However, earlier onset of reproductive maturation means increased numbers of pollen-producing trees in high-CO₂ plots and greater pollen production at the stand level. We believe this is supported by increased abundance of airborne pollen grains sampled in 2005 (Fig. 3), although we acknowledge that airborne analyses would be greatly improved with more intensive sampling.

Increased pollen production under future atmospheric CO₂ concentrations may have important consequences for human health. Pine pollen is not a

dangerous allergen for the public at large. However, even at current production levels, it does affect individuals with specific sensitivity (Harris & German 1985a, 1985b; Kalliel & Settupane 1988; Freeman 1993). Our study demonstrates the potential for future increases in pine pollen productivity, a result that may also apply to more allergenic species. For example, pollen production is enhanced 61–200% in ragweed (*Ambrosia artemisiifolia*) grown in high-CO₂ atmosphere (Ziska & Caulfield 2000; Wayne *et al.* 2002). Many other species have life-history traits similar to *P. taeda* and could experience significant reproductive enhancement under elevated CO₂.

Precocious pollen production has several potential implications for forest ecosystems. It could enhance the production of viable seeds by increasing the percentage of fertilized ovules (Barnett 1976; Greenwood 1980). More pollen disseminated from multiple-source trees may also increase rates of gene flow among stands, and could further reduce rates of self-pollination, indirectly enhancing the production of viable seeds. However, pollen dispersal from short, dense canopies may not have great ecological implications if the access of strobili to adequate wind currents is low (Farris & Mitton 1984; Feldman, Tomback, & Koehler 1999; Cain & Shelton 2001; Dyer & Sork 2001; Katul, Williams & Siqueira 2005). For example, Cheliak *et al.* (1985) found that outcrossing rates were significantly lower in young, dense Jack Pine stands relative to older stands. Restricted dispersal from relatively young canopies growing in elevated atmospheric CO₂ may negate the effects of high pollen production by leading to increased rates of self-pollination in dense stands.

Long-term population effects of changes in atmospheric CO₂ will be largely determined by reproductive effort. Understanding the consequences of increased atmospheric CO₂ for forest ecosystems demands longitudinal studies, like the one presented here, that explicitly evaluate fecundity and account for the multiple sources of variability inherent in nature.

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