Production of allergenic pollen by ragweed (*Ambrosia artemisiifolia* L.) is increased in CO_2-enriched atmospheres

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Background: The potential effects of global climate change on allergenic pollen production are still poorly understood.

Objective: To study the direct impact of rising atmospheric CO_2 concentrations on ragweed (*Ambrosia artemisiifolia* L.) pollen production and growth.

Methods: In environmentally controlled greenhouses, stands of ragweed plants were grown from seed through flowering stages at both ambient and twice-ambient CO_2 levels (350 vs 700 $\mu L\ L^{-1}$). Outcome measures included stand-level total pollen production and end-of-season measures of plant mass, height, and seed production.

Results: A doubling of the atmospheric CO_2 concentration stimulated ragweed-pollen production by 61% ($P = 0.005$).

Conclusions: These results suggest that there may be significant increases in exposure to allergenic pollen under the present scenarios of global warming. Further studies may enable public health groups to more accurately evaluate the future risks of hay fever and respiratory diseases (eg, asthma) exacerbated by allergenic pollen, and to develop strategies to mitigate them.

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INTRODUCTION

Global environmental change has received significant attention in the fields of conservation biology, agriculture, and economics. Only recently, however, has research begun to address how a changing global environment may affect public health. [1] Outbreaks and expansion of diseases transmitted by vectors sensitive to climatic shifts (eg, malaria, dengue fever, and equine encephalitis) have been linked to environmental change. [2,3] Thinning of the ozone layer is expected to increase the incidence of melanomas. [5] Recent studies have also shown a link between warming trends within the past 50 years and the phenology and abundance of allergenic pollen released by a number of European tree species.[6,7] However, only limited data are currently available to evaluate the direct effects of rising atmospheric CO_2 concentrations on pollen production by allergenic plants and its potential impact on public health.[8]

Human allergic responses to the pollen of certain plant species (hay fever, allergenic rhinitis, pollinosis) is a serious environmental health issue.[9] Aeroallergens, including pollen, also play a role in the exacerbation of asthma.[10] The prevalence of both hay fever and asthma has increased significantly in recent decades.[11,12] Little research has been devoted to understanding how various components of global environmental change influence allergenic pollen production and, thus, the potential for pollen-related disease.

An increase in the concentration of atmospheric CO_2 is one of the most certain predictions of climate change models. CO_2 concentration has increased by 29% since preindustrial times, and is expected to double again sometime between 2050 and 2100.[13] Increased CO_2 concentrations stimulate plant net photosynthetic rate,[14] increase water use efficiency,[15] decrease carbon loss to dark respiration,[16] and alter phenology[17] and allocation patterns.[18] The net result of these responses is that plants grown in CO_2-enriched atmospheres generally grow faster and are larger at maturity, although the magnitudes of growth and physiologic enhancements vary considerably with environmental conditions and species identity.[19,20] Whereas significant CO_2-induced changes to reproduction have been documented,[21,22] nearly all studies to date have focused on the responsiveness of female reproductive structures (ie, flowers, fruits, and seed). In one recent study, Ziska and Caulfield[8] found that exposing ragweed plants to the higher CO_2 concentrations predicted in the year 2100 doubled the quantity of pollen produced.

Ragweed (*Ambrosia artemisiifolia* L.) is a plant common to roadsides and disturbed habitats throughout most of the United States and Canada.[23] It is dioecious, with male and female flowers born on distinct axillary branches, allowing for independent control of allocation to sexes.[24] Throughout its distribution, ragweed pollen is one of the most abundant aeroallergens in late summer and fall, and it is one of the primary causes of seasonal pollen allergy in North America.[25] Consequently, ragweed pollen and specific allergens extracted from it have been used in many clinical studies, and the biochemistry and genetics of ragweed allergens and their impacts on the human immune systems are well understood.[26,27]

This study investigates the direct impact of rising CO_2 concentrations on pollen production in experimental populations of ragweed. The results will be used to more accurately evaluate the future risks of hay fever and respiratory disease exacerbated by allergenic pollen, and to develop strategies to mitigate them.

MATERIALS AND METHODS

To study pollen production by ragweed populations in elevated CO_2 atmospheres, seeds of *Ambrosia artemisiifolia* L. were grown to reproductive maturity in controlled-environment glasshouses. Seeds were initially collected from wild populations in Woodstock, Illinois. Seed were sown into 12 total 30-L growth containers (50 x 40 x 15 cm). Soil in each container was composed of a 4:3 mix of Pro-Mix compost (Red Hill, PA) and washed sand (Quickrete Co, Atlanta, GA). Containers were fertilized weekly with 500 mL of 20:20:20 NPK Peter's Solution

(Allentown, PA), and watered daily. Day/night temperatures were maintained at 26/21° C and ambient glasshouse light levels were approximately 70% of full sun.

Containers were randomly assigned to two blocks, each containing two modules maintained at either ambient 350 μL L^{-1}) or double ambient (700 μL L^{-1}) CO_2 concentrations (ie, three containers per growth module). Thirty plants were established in each growth container and arranged in a regular grid. This resulted in a density of 150 plants m^{-2}, a density commonly observed in natural field populations. [28] To minimize any edge effect, only the central 12 plants per container were measured and used in analyses.

Pollen was collected and pooled from the 12 central plants in each stand after 84 days of growth, during the peak of the flowering season. All pollen bearing shoots on each plant were vigorously shaken within a large Teflon-coated (DuPont, Wilmington, DE) funnel that opened at its narrow end into a collection vial filled with 50 mL of ethanol. To estimate pollen concentration, a 5-mL sample of well mixed pollen-ethanol solution was transferred to a glass vial and dried for 24 hours in an oven. The dried pollen was then mixed with 4 mL of concentrated salt water (Instant Ocean, Mentor, OH) and subsamples of this solution were analyzed for pollen particle number and size using a Coulter Z-series Particle Count and Size Analyzer (Hialeah, FL). Calibration for particle size was conducted by using 50-μm beads using the protocol suggested by the manufacturer. Particle number was calibrated against solutions of pure salt water. After pollen removal from plants, all mature seeds were removed from each shoot, and along with total shoot biomass, oven-dried and weighed to the nearest g^{-4}. Shoot height was measured immediately before pollen removal. Persons responsible for pollen collection and measurements of particle counts and plant size, were not blinded as to which CO_2 environments plants were grown in.

For all traits, including total pollen production, mean pollen size, shoot biomass, height, and seed weight, differences between treatments were evaluated with analysis of variance that compared the effects of CO_2 to the larger of Block x CO_2 and container-to-container variation.

RESULTS AND DISCUSSION

We found that stand-level pollen production was 61% higher in elevated versus ambient CO_2 environments (F = 15.16, P = 0.005); however CO_2 did not significantly influence the average size of pollen grains (Fig 1). CO_2-induced growth stimulation of stand shoot biomass was similar to that of total pollen production (63%, F = 9.08, P = 0.017; Fig 1). Both shoot height and total seed mass were also greater in elevated CO_2 environments (9% and 31%, respectively); however, these effects were not statistically significant (P = 0.057 and P = 0.3781; Fig 1). Our observation of a CO_2-induced increase in pollen production parallels the results reported by Ziska and Caulfield, [8] who reported an even greater magnitude of pollen increase. Similarly, our observed CO_2-induced enhancement to shoot biomass is similar to average species enhancements values (54%) observed in surveys of fast-growing wild plants. [18]

Figure 1. The effects of a doubling of the atmospheric CO_2 concentration (350 vs 700 μL L^{-1}) on components of reproduction and growth of ragweed (*Ambrosia artemisiifolia* L.) plants grown in stands in climate-controlled glasshouses. Significant differences are indicated with an asterisk and a P value. Error bars represent ± 1 standard error of the mean.

Detailed data on individual plant pollen production and reproductive development were not investigated in this study. Nevertheless, it is possible that in addition to increasing stand-level pollen production through increased plant size, high CO_2 may have resulted in plants allocating proportionally more resources to pollen relative to seed or total shoot mass. Previous studies with ragweed have shown that adding essential resources to stands (eg, nitrogen) results in plants investing in proportionally more male versus female reproductive structures. [29] More generally, studies with ragweed and other wind-pollinated species suggest that larger and taller plants within populations tend to be male more often. [30,31]

It will be challenging to accurately predict the future threat to public health caused by CO_2-stimulated pollen production. As with most environmental health issues, many factors are involved, and in the specific case of climate change, the future state of many of the factors themselves is uncertain. Based on previous climate change studies evaluating the responses of plant growth and yield, it is likely that plant pollen production will also be influenced by factors expected to change in concert with CO_2, including temperature, precipitation, and atmospheric pollutants. [15,32,33] Over longer periods of time, these factors are likely to impact the relative abundance and geographic distribution of plant species, [34] possibly altering the demographics of populations currently exposed to allergenic species. In fact, recent models suggest that climate change scenarios will favor the spread of ragweed throughout Europe. [35]

CONCLUSION

Despite these uncertainties, our observation that a doubling of the atmospheric CO_2 concentration markedly stimulates ragweed pollen production suggests that the incidence of hay fever and related respiratory diseases may increase in the future. Additional research is warranted to more accurately evaluate the future impact of allergenic pollen on public health and to help develop effective ecologic, public health, and policy strategies for mitigating these threats.

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