Estimates of Common Ragweed Pollen Production for Urban Ragweed Plants

Victoria T. Bankowski and Daniel W. Katz, PhD[1]
School of Environmental Health Sciences, University of Michigan, Ann Arbor, MI 48109
[1]To whom correspondence should be addressed: dwkatz@umich.edu

In this study we develop an allometric equation for wild grown urban ragweed plants. Previous studies estimate relationships for ragweed pollen production using greenhouse gown plants and not wild ragweed. The development of a different equation is important due to differences between urban grown ragweed (e.g., due to competition with other plants), and the protective environment within the greenhouse. An unbiased allometric equation is important because it will be used for estimates of ragweed pollen production in urban areas. To create an allometric equation we measured pollen per anther, anther per inflorescence, inflorescence per cm and length of the inflorescence per plant. Ragweed flower processing was completed by measuring total inflorescence, height, diameter, and total inflorescence length per plant. We also tested whether pollen per plant could be predicted by plant height and diameter using the linear regressions: Pollen per plant ~ height, pollen per plant ~ diameter, and pollen per plant ~ height and diameter. The ragweed plants produced 3.1×10^6 to 1.3×10^8 pollen grains and the average pollen production per plant was 4.7×10^7. There was a significant relationship between height and pollen production per plant; the R^2 was 0.45, $p < 0.0001$, but the relationship between diameter and pollen production per plant was weak; the R^2 was 0.10, $p = 0.12$. When both variables were included in a linear model, the R^2 was 0.63 and $p < 0.0001$, but there was an even stronger relationship between inflorescence length and pollen production; R^2 was 0.91 and $p < 0.0001$. These results are similar to those found in a greenhouse experiment that included inter-plant competition and mowing treatments and show that either our or their allometric equation can be used for estimating urban grown ragweed pollen production as a function of total inflorescence length per plant.

Introduction

Airborne ragweed pollen (*Ambrosia artemisiifolia* L.), has a negative impact on human health and the quality of life; 10 to 30% of adults and 40% of children suffer from allergic rhinitis commonly caused by an allergic reaction to pollen.[1] Concentrations of 6-10 pollen grains m^{-3} is enough to trigger an allergic response[2].

Ragweed pollen allergies are a serious public health concern, affecting millions of Americans every year[3]. Not everyone exposed to ragweed will develop an allergy; however, those who do may experience an immune system response (wheezing, chest tightness, shortness of breath, and/or chronic cough), and may develop a worsening of asthma symptoms[4], and exposure can even sometimes trigger a fatal asthma attack[5].

Ragweed populations are a problem in impoverished neighborhoods where they are abundant in large numbers[6]. It tends to grow along roadways, urban landscapes, and in freshly cleared grounds[7,8]. There is a need for determining how much ragweed pollen is produced in urban areas as 10% of Americans are sensitized to ragweed[9]. We may be able to reduce exposure by changing the way local municipalities; preside over vacant parcels of land which are scattered throughout urban areas; doing so could improve public health conditions of the surrounding neighborhoods[6]. We know there is more ragweed in these areas, but we are unaware of how much ragweed pollen is being produced which

has prevented reliable estimates of airborne ragweed pollen concentrations from being developed. Now, as part of a larger study, to estimate the effects of ragweed pollen exposure on health outcomes, we require estimates of the amount of ragweed pollen produced per plant.

Previous studies created allometric relationships for ragweed pollen production using greenhouse grown ragweed plants[9], instead of wild ragweed plants, and this may result in inaccuracies when these equations are applied to wild plants. Green house grown conditions give ragweed protection from things that could damage it (e.g., mowing, competition with other plants, herbivores, pathogens, and abiotic stressors), which could cause substantial variation in pollen production in nature. Urban grown ragweed plants are subjected to sporadic mowing (random, or never mowed); competition with other species; as well as other gardening processes (herbicides) used to get rid of the nuisance[10]. Due to these conditions ragweed pollen plants may have a very different allometric equation.

In this study, we calculated pollen production and developed allometric equations for wild grown urban ragweed plants. To do so, we collected samples of ragweed pollen plants from urban areas and compared our allometric equation with allometric equations from other studies based on ragweed grown in greenhouses[9,11]. To do so, we collected ragweed plants, measured their size, the number of flowers per plant, and the amount of pollen per flower.

Ragweed Morphology Overview

Common ragweed may grow just a few centimeters tall, to less than two meters in height. The stems are upright or straight, lay along the ground or surface with the extremity curving upward. The leaves may be arranged alternately or oppositely, and they are irregularly lobed. The leaf blades vary, sometimes they are pinnate or palmate into lobes. The edges are smooth or can be toothed. Many are hairy, and most are glandular[12].

Common ragweed is monoecious, and most produce inflorescences that contain staminate and pistillate flowers. Inflorescences are often arranged in the form of a raceme (spikes) made up of mostly staminate (male) flowers with some pistillate (female) flowers clustered at the base of the inflorescence. The male flower heads (inflorescence) have stamens surrounded by whitish to yellowish florets. Along the mid-stem leaf and sub-leaf, the female heads can be found. Pistillate flower heads have fruit-yielding ovules surrounded by many phyllaries (one of the bracts forming the involucre or the head (green cap) or inflorescence of a composite plant) and fewer and smaller florets. The pistillate flowers are wind pollinated, and then the fruit develop. Each has burs, or wings with knobs, and spines. Each male flower is referred to as a floret and has 5 stamens and anthers[12].

Ragweed flowering occurs late in the summer, when temperatures drop lower that 60° F and the length of nights increases[4,13]. In the Midwestern United States, the typical pollen count during ragweed season is estimated at 200-400 grains/m^3 and it has been estimated that symptoms after exposure to ragweed pollen can begin sensitivity as few as 5-20 pollen grains/m^3 [10]. Ragweed pollen grows in very large numbers; one plant can release 1 billion pollen grains in a season[4]. Individual plants can produce between 10^8 and 10^9 grains per m^2 [9].

Material and Methods

Study Area and its Characteristics

The ragweed used for the development of our allometric equation was collected from Ann Arbor, Michigan, U.S.A. (Longitude: -83.7108, Latitude: 42.2947). Ann Arbor is approximately 35 miles west of Detroit. The elevation ranges from 750 feet (230 n) to 1014 feet (309 m) and has an annual temperature of 49.2* F and an average annual precipitation of 30.67 inches. The continental climate is characterized by four distinct seasons[14].

When collecting the ragweed plants used for the study, the location for each plant (latitude and longitude) was obtained from a GPS and we pulled out each sample by their roots along with the dates the samples were collected.

Ragweed male flower processing was completed by measuring the total spike length in cm per plant, height of the plant (cm), and total length of all male inflorescence for each plant. To count the number of male inflorescence heads we randomly selected ten inflorescences per plant, and ten random spots per inflorescence to cut 1 cm sections from. Analyses of the 1cm section was completed by counting the total number of involucre hoods (cap), and randomly selecting one cap from analyzed sections of inflorescence. The total number of flowerets per cap was recorded and random unopened florets were collected (anther) to be preserved for later analysis (ten total).

The final step uses the hemocytometer to count the pollen. Using a glass stir rod to crush the anthers and homogenize the pollen concentration of each centrifuge tube containing the ten anthers. Using a pipette, transfer 0.1 mL, of the con-

Figure 1: Left Photo urban ragweed (Ambrosia artimisiifolia L.) growing alongside of the road, Middle Photo-Individual urban ragweed inflorescence, Right Photo-Male inflorescence growing off the side of the road. Photographer-Victoria Bankowski

tents of the centrifuge tube solution on to the hemocytometer's counting surface. The counting was completed, and data entered onto sheets.

Analysis

To calculate the amount of ragweed pollen produced by each plant, we multiplied the pollen per anther (P) by the anther per inflorescence (A) by the inflorescences per cm (C) by the total length of the inflorescences per plant (I).

Pollen Production per Plant = (P)*(A)* (C) * (I)

We also tested whether pollen per plant could be predicted by plant height and diameter. To do so, we ran three linear regressions: Pollen per plant ~ height, pollen per plant ~ diameter, and pollen per plant ~ height and diameter. We also extracted information from greenhouse-based studies to compare our results to theirs. Analyses were conducted in Microsoft Excel and in R.

Results

We measured 33 plants. The heights of the plants ranged from 32 to 80 cm and the average height was 49.03cm. The diameter of the plants ranged from 1.56 to 8.11 mm and the average diameter was 4.46 mm. The total length of inflorescence per plant ranged from 24 to 579 cm and the average of the spike length per plant was 128.73 cm. The inflorescences per plant ranged from 262 to 4866 with an average of 1243 inflorescence per cm, and inflorescences ranged from 81 to 6948 inflorescences, with an average of 1246 inflorescence per plant. The range of anthers per plant was 4333 to 59752 with an average of 50291 anthers per plant, the ragweed plants produced 3.12×10^6 to 1.3 $\times 10^8$ pollen grains and the average pollen production per plant was 4.7×10^7.

In Figure 2: Pollen production as a function of height; we found significant relationships between plant height and pollen production per plant; the $R^2 = 0.45$, ($p < 0.0001$); we also found there was no significant relationship between diameter and pollen production per plant; the R^2 was 0.10, ($p = 0.12$). In Figure 3: Pollen production as a function of plant diameter; when both variables were included in a linear model, the R^2 was 0.63 ($p < 0.0001$); in Figure 4: there was an even stronger relationship between total inflorescence length and pollen production; R^2 was 0.91 ($p < 0.0001$) (Fig. 5). In Figure 4: We found strong correlations in pollen per plant as a function of diameter and height (point color), and in Figure 5; we found a strong correlation in pollen per plant (mean+/- standard deviation) as a function of total inflorescence length in cm. Height and diameter explained 63 percent of variation in estimated pollen production. In Figure 5: Pollen per plant (mean +/- standard deviation) as a function of total inflorescence length in cm; found strong correlation in pollen per plant (mean +/- standard deviation) as a function of total inflorescence length in cm. In Figure 6: Data comparison for Simard and Benoit (2011) and our study (2017) found simi-

| Variables Measured	
Variable	*Symbol*
Pollen per Anther	*P*
Anthers per Inflorescences	*A*
Inflorescences per cm	*C*
Inflorescences(cm) per Plant	*I*
Height of Plant	*H*
Diameter of Plant Stem (mm)	*D*
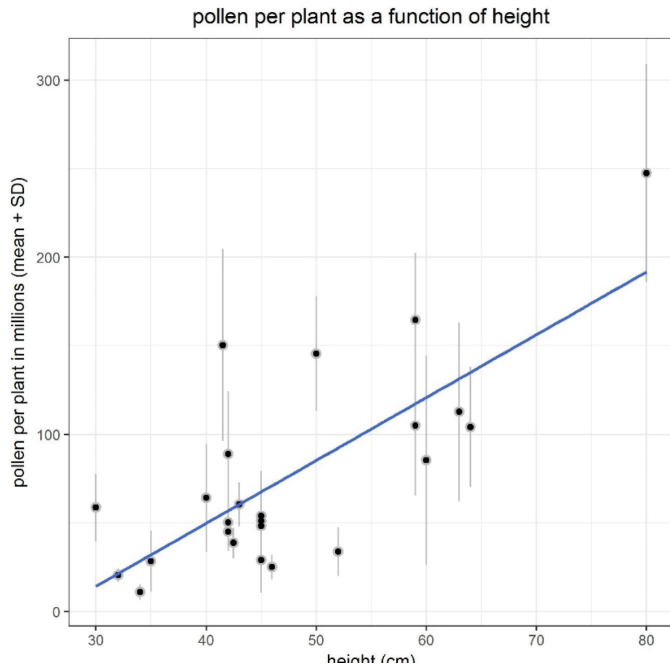
Figure 2: Pollen production as a function of height found a significant relationship between plant height and pollen production per plant; the R^2 was 0.45, (p < 0.0001); we also found there was no significant relationship between diameter and pollen production per plant; the R^2 was 0.10, (p = 0.12).
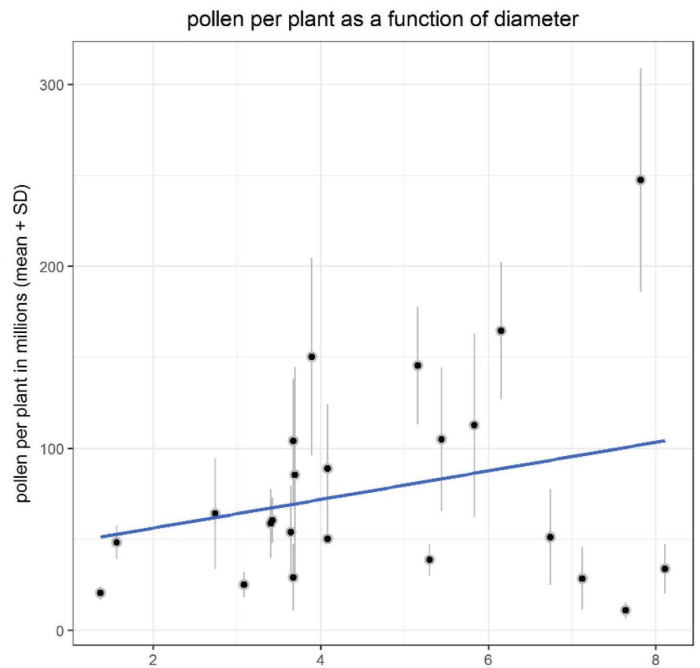
Figure 3: Pollen production as a function of plant diameter, when both variables were included in a linear model, the R2 was 0.63 (p < 0.0001); in Figure 4: there was an even stronger relationship between total inflorescence length and pollen production; R^2 was 0.91 (p <0.0001) (Fig. 5).
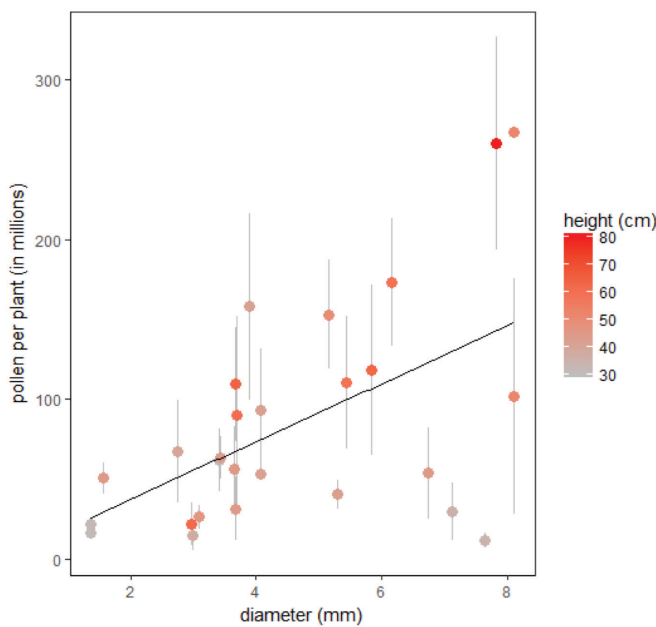

Figure 4: Pollen per plant (mean +/- standard deviation) as a function of diameter and height (point color). Found strong correlation in pollen per plant as a function of diameter and height (point color).
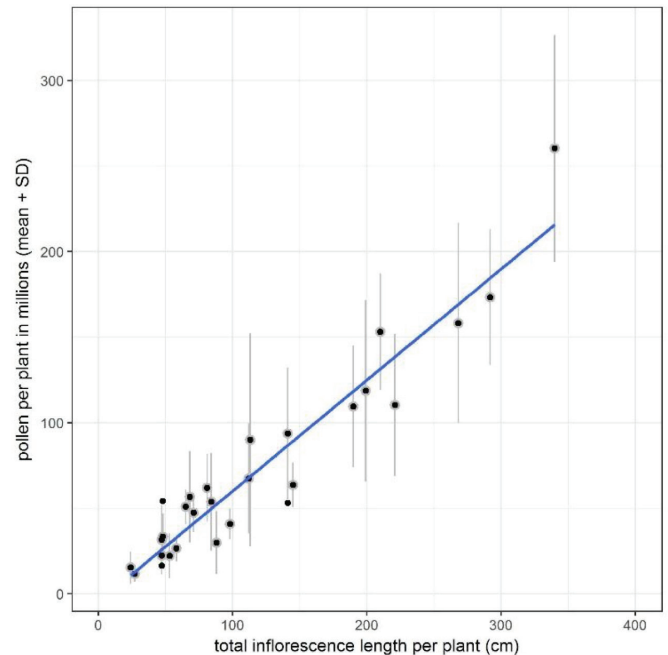
Figure 5: Pollen per plant (mean +/- standard deviation) as a function of total inflorescence length in cm; found strong correlation in pollen per plant (mean +/- standard deviation) as a function of total inflorescence length in cm.
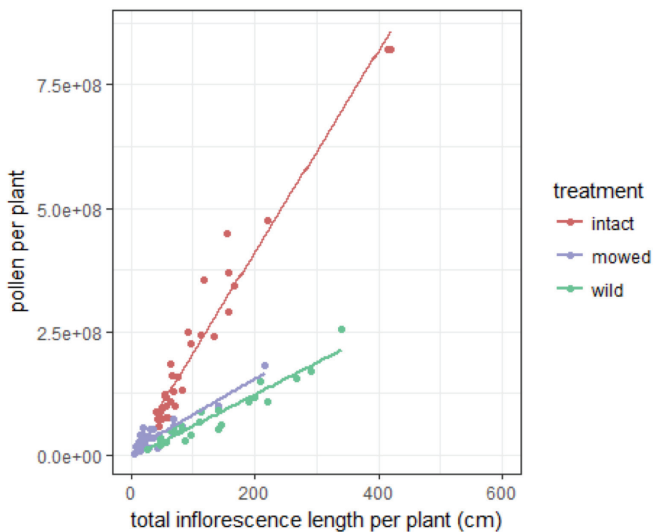
Figure 6: Total pollen per plant as a function of total inflorescence; data comparison for Simard and Benoit study (2011) and our study (2017) compared our results to other studies; found similarities in our wild grown ragweed data (2017) and their mowed greenhouse grown ragweed treatments data from the Simard and Benoit (2011).

lar allometric equations; our wild grown ragweed allometric equation; $y = 624018 * x - 6025129$, $R^2 = 0.91$, $(p < 0.0001)$, and their mowed greenhouse grown allometric equation; $y = 727225 * x + 8537690$, $R^2 = 0.83$, $(p < 0.0001)$[15].

Conclusion

We found a strong relationship between height, diameter, total length of flowers, and the total amount of pollen produced. Thus, although there is inter-individual variation in variables such as the number of anthers per flower, that information is not required to be estimated for each plant. Knowing these relationships will be useful for estimating pollen production by ragweed plants in urban areas with a mix of land management regimes.

We found that ragweed plants produced an average of 4.7 x 10^{7} pollen grains; in contrast, Simard and Benoit (2011) found that ragweed plants produced an average of 2 x 10^{9} pollen grains, and pollen production had strongly correlations with plant diameter, plant height and inflorescence length. This difference is due to the size of the plants and does not mean there is a difference in the allometric equation. Sauliene et al. (2012) found that plants grown in a greenhouse in Poland produced an average of 7.4 x 10^{9} pollen grains per plant and found relationships in inflorescence

length and the number of male flower heads. Rogers et al. 2006 found that greenhouse grown plants produced an average of 0.7 x 10^{9} pollen grains per plant, but these plants were much larger than ours (average height = 100 cm). Pollen production per inflorescence was most strongly associated with inflorescence length, and number of inflorescences per plant, and obtained a negative correlation between inflorescence number and pollen production, which suggests some plants produce fewer pollen-rich inflorescences while others produce more inflorescences, and variance per plant producing less pollen per unit length.

This suggests that simply measuring ragweed height and diameter may be somewhat useful for rough calculations of pollen production. For more exact purposes, pollen production can be estimated by measuring the inflorescence length.

In Figure 6: Data comparison for Simard and Benoit (2011) and our study (2017) found similar allometric equations. Simard and Beniot' (2011) mowed greenhouse treatment data has a close relationship to our data; now we know that we can use this studies data for urban grown ragweed. This may be because the simulated the conditions that urban ragweed plants experiences: mowing and competition with other plants.

Now that we have an allometric equation for urban ragweed pollen production, it will be used in a larger study to predict ragweed pollen production across Detroit, MI, using models based on field surveys and remote sensing data. These estimates of ragweed abundance and pollen concentrations will be used to estimate airborne pollen concentrations and ultimately the health effects of ragweed pollen exposure. Thus, the allometric equation developed here will play a useful role in understanding both the health effects of ragweed and in the resulting management recommendations for reducing ragweed abundance.

Limitations of study

One of the reasons why our study results are different from the green house studies could be due to the date in which we began sampling. Ragweed season in the United States typically starts in mid-August and continues until the first frost, around mid-October,[13,16] our sampling began in mid-September and ended early in November. Because the plants were collected late into the ragweed pollen season, we were limited to the number of viable ragweed plants from which to sample from, limiting our sample size. In future studies it will be of great importance to begin sampling early in the ragweed pollen season to insure there is an abundance of ragweed pollen plants from which to test from

There were variances in the pollen counts when using the hemocytometer. We found it necessary to question the length of time needed after visualizing the capillary action. There were variances in the count when the hemocytometer was placed onto the microscope stage immediately after capillary action. When filling both chambers consecutively there was a lot of variation in those readings. The length of time needed to complete the process should have limited guidelines. In future studies, it will become increasingly important to investigate the issues we had while using the hemocytometer.

Acknowledgements

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