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ESTIMATION OF POLLEN AND SEED PRODUCTION OF COMMON RAGWEED IN FRANCE

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Abstract: Common ragweed (*Ambrosia artemisiifolia* L.) is an invasive weed of field crops and human-disturbed habitats in Europe. As well as in its natural range (North America), common ragweed is a threat to human health due to its abundant allergenic pollen release. Most studies have been focused on airborne pollen monitoring, but to date, no data have been available on precise individual plant pollen and seed production related to plant traits growing in natural environment and on their corresponding source of variation. The aim of this study was to evaluate pollen and seed production of common ragweed plants in several populations in France. Seasonal pollen production per plant ranged from 100 million to 3 billion and seed production from 346 to 6,114, depending on plant size and habitat. Common ragweed plants developing in field crops produced more pollens and seeds than those growing in other habitats. Pollen and seed production was closely related to plant volume and biomass, thus providing a means of estimating potential pollen and seed production in given target areas. Such biological data could be integrated into population management strategies or into airborne pollen modelling.

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INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.) is an annual wind-pollinated plant belonging to the Asteraceae family. This plant species, which is common in North America, was introduced into Europe in the second half of the nineteenth century [2] and has since invaded many countries where it is now considered a serious threat [11, 12, 15]. It is a pioneer and opportunist species, invading field crops and open disturbed habitats or roadsides [1]. Common ragweed is potentially a threat to human health due to its abundant allergenic pollen [15], which are regarded as very potent aeroallergens [6, 8]. Common ragweed is the most important cause of hay fever in North America and may generate a dermatitis among people who are not necessarily sufferers from hay fever [1]. The Carpathian basin, Northern Italy, and some parts of France are the main areas in Europe affected by ragweed pollen pollution [8, 15].

During the last few years, studies of airborne pollen survey [6, 8, 10, 12, 14], of common ragweed have been increasing. However, even though some studies looking for the influence of atmospheric CO_2 on ragweed presented approximated plant pollen production in a controlled environment [13, 17], little is still known about the pollen production of individual plants growing in natural environments, or about the relationship of pollen production with plant traits, such as size or weight for example. Bouillène and Bouillène [3] reported that *Ambrosia* sp. plants could produce about 2.5 billion grains of pollen per day, but they did not relate that production to any other factors Surprisingly, these numbers, which are occasionally cited in literature [e.g., 4, 8], were not based on experimental data or using identified *Ambrosia* species. Seed release also appears as a key point parameter in understanding the invasive capacity of common ragweed, but little is known about per plant seed production in Europe. The aim of this work was to

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evaluate the individual pollen and seed production of different common ragweed populations introduced in France, in relation to plant size and habitat types.

MATERIALS AND METHODS

Initially, the pollen and seed production of 30 individuals from 5 populations of common ragweed were studied in 2004 in France. However, due to some mortality that occurred between pollen sampling and final seed and plants harvesting, the pollen and seed production were finally studied on a total of 121 individuals from the 5 populations sampled (Tab. 1). Populations were sampled in both disturbed (wasteland) and field crop habitats across their distribution area in the eastern part of France (Tab. 1). All populations were located at about 200 m above sea-level. The mean daily temperatures for winter and summer seasons ranged from 3.3-20.9°C for central populations (3 and 5) to 2.5-18.6°C for northern ones (1, 2 and 4). The annual total rainfall ranged from 748 mm for the central populations to 803 mm for the northern ones.

Before pollen release (less than 5% of open flowers per plants), 5 male flower heads per plant were collected from the tips of 5 separate branches. The sampled plants were marked for collection later in the growing season, after seed production. For each plant, each of the 5 male flower heads was dissected under a binocular microscope (×30) to extract one closed flower per male flower head. Pollen grains from the 5-flower set per plant were extracted and prepared according to the technique described by Loublier *et al.* [9] and developed for plants belonging to the same family. Extracts of pollen were sonicated (Bransonic 12 FTZ, 50 kHz) to separate pollen grains which were then counted using an automatic particle counting machine (Coulter® Multisizer™). Each sample was counted 3 times and mean pollen numbers and diameter were computed. Pollen counts obtained from 20 random samples were also estimated using microscopes (×100) with Malassez cells to validate the automatic counting method. Mature labelled plants from each site were harvested one month later and several life history traits were measured: plant height, width (maximal plant diameter), dry biomass, number of primary and secondary stems, total number of racemes, number of male flower heads on 5 different racemes and number of flowers on 5 male flower heads. The volume of plants (V) was calculated using the formula for the volume of a cylinder ($V = \pi \times R^2 \times H$, where R^2 is plant width and H is plant height). Using the total mean number of pollen grains per flower automatically counted (Pf), the racemes number (R), the mean male floral heads number per raceme (Fh) and the mean flower number per male floral head (F), we computed the number of pollen grains produced per plant ($Po = R \times Fh \times F \times Pf$). Finally, the seeds were collected from the same plants, cleaned and counted using another automatic counting machine (Contador E®).

Table 1. Location and habitat of French common ragweed populations analyzed for pollen and seed numbers, with number of plants sampled (n).

Population	Location	Longitude (E)	Latitude (N)	Habitat	n
1	Lux	5° 12’ 56’’	47° 27’ 03’’	Wasteland	29
2	Labergement	5° 14’ 27’’	47° 14’ 34’’	Wasteland	26
3	La Boisse	5° 01’ 49’’	45° 49’ 41’’	Wasteland	29
4	Bey	4° 56’ 03’’	46° 47’ 34’’	Field-crop	12
5	Meyzieu	5° 04’ 43’’	45° 43’ 57’’	Field-crop	28

Statistical analyses were performed with SYSTAT 11® software for Windows ($p < 0.05$). Automatic and estimated counts were compared using paired t-test. Pollen and seed productions per population were analyzed using one-way ANOVA with population as fixed effect. Relationships between Log_{10}(plant volume), Log_{10}(plant dry biomass) and both Log_{10}(number of pollen grains) and Log_{10}(number of seeds) were analyzed using linear regressions. Slope differences were tested using analysis of covariance (ANCOVA) with population as the fixed effect and Log_{10}(plant dry biomass) as the covariate.

RESULTS AND DISCUSSION

No significant differences were found between automatic counting and estimation of pollen numbers ($p = 0.729$). Time saved by automatic counting allowed us to analyze a larger amount of samples than when using classical estimation based on Malassez cells. Common ragweed plants produced on average 1.19 ± 0.14 billion pollen grains. Mean pollen production per population (Fig. 1) ranged from 100.13 ± 14.21 million to 3.04 ± 0.33 billion (min: 4 million, max: 10 billion). The mean number of pollen grains was significantly different between populations ($F = 17.29$, $p < 0.001$). These values of total pollen production per plant by season were lower than the 2.5 billion grains of pollen per day estimated by Bouillène and Bouillène [3] on *Ambrosia* sp. plants. Pollen production per plant (Fig. 2a) was significantly and positively correlated to plant dry biomass ($F = 606.88$, $r^2 = 0.84$, $p < 0.001$) and plant volume

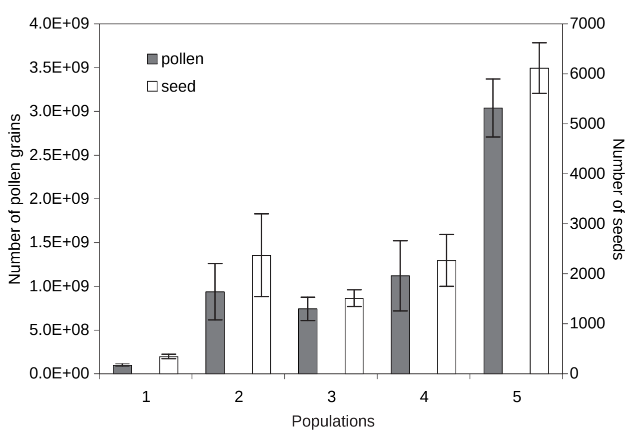

Figure 1. Mean (± standard error) number of pollen grains and seeds produced by common ragweed from different populations.

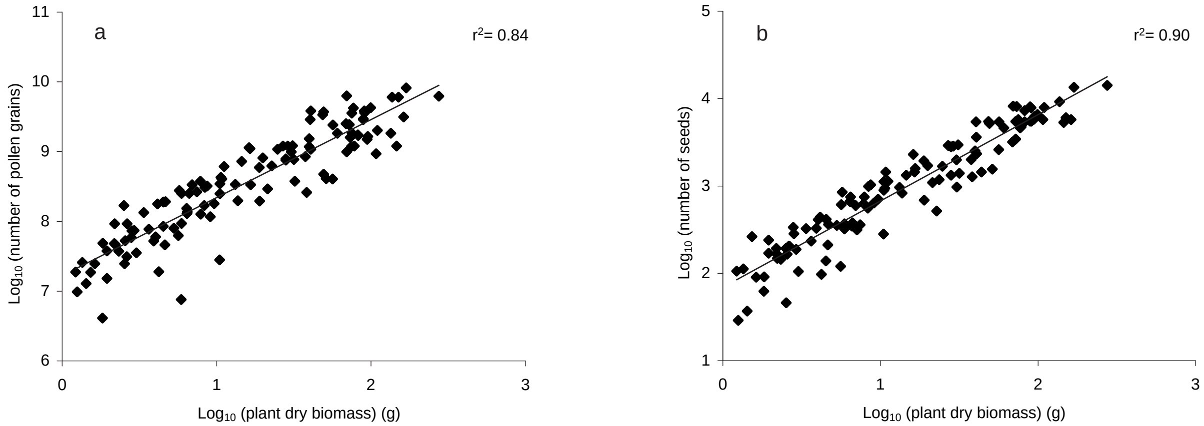

Figure 2. Relationships between Log_{10}(plant dry biomass) and Log_{10}(number of pollen grains) (a) or Log_{10}(number of seeds) (b) of 121 common ragweed plants from five French natural populations. Linear regression lines (—) and coefficient (r^2) are shown on the diagrams.

($F = 325.93$, $r^2 = 0.73$, $p < 0.001$). The common ragweed population that produced the highest amount of pollen grains per plant was found in the field crop habitat, whereas the least productive population was found in the wasteland habitat (Fig. 1). Such a pattern can be explained by the dependence of plant size on the growing conditions (temperature, humidity, soil nutrients, competition, etc.) and by the aforementioned relationship between plant size and pollen production. No significant differences were observed among ragweed populations when pollen production was regressed across either plant biomass ($F = 0.78$, $p = 0.540$) or plant volume ($F = 1.39$, $p = 0.241$). Consequently, the number of pollen grains (Ypb, Ypv) produced by common ragweed could be properly estimated using plant biomass (Xb): $Log_{10}(Ypb) = 7.22 + 1.12\ Log_{10}(Xb)$, or on plant volume (Xv): $Log_{10}(Ypv) = 4.19 + 0.92\ Log_{10}(Xv)$. Mean pollen size per population varied from 13.02 ± 0.12 µm (population 4) to 14.86 ± 0.16 µm (population 2), which is more than 25% lower than that previously described by Jones [7] on North American common ragweed.

Common ragweed plants produced on average $2{,}518 \pm 271$ mature seeds with values (Fig. 1) ranging from 346 ± 46 to $6{,}114 \pm 508$ (min: 22, max: 18,605). The mean number of seeds produced was significantly different between populations ($F = 12.69$, $p < 0.001$). These results were 10 times lower than published estimates for North American common ragweed [5]. As in the case of pollen, the number of seeds produced per plant was significantly and positively correlated to plant dry biomass ($F = 1{,}100.44$, $r^2 = 0.90$, $p < 0.001$) and plant volume ($F = 520.78$, $r^2 = 0.81$, $p < 0.001$) (Fig. 2b). Slopes of linear regressions between number of seeds and plant dry biomass were not significantly different among populations ($F = 1.66$, $p = 0.165$) although differences were found among populations with respect to regressions between number of seeds and plant volume ($F = 3.46$, $p = 0.010$). Consequently, the equation that could be used to estimate seed number produced (Ysb) per plant should be based on plant dry biomass (X): $Log_{10}(Ysb) = 1.84 + 0.99\ Log_{10}(X)$. On the other hand, as regression lines using plant volume were different between populations, seed number estimation (Ysv) from plant volume (Xv) based on a single linear equation should be used carefully and taken only as an approximate seed number estimation ($Log_{10}(Ysv) = -0.91 + 0.83\ Log_{10}(Xv)$). Therefore, seed estimation from field measures or greenhouse experiments should be more realistic using plant dry biomass than volume.

Large common ragweed plants produced more pollen grains and seeds than small ones. Common ragweed plants developing in field crops were bigger than those from wasteland habitats, but, conversely, their density per square meter was lower. According to the linear regressions obtained and the knowledge on plant density, pollen and seed productions of common ragweed could be estimated in various populations. Indeed, the spectra of plant variation according to geographical location, environmental conditions and plant sizes which have been analysed, was representative of the overall variation of plant populations.

The strong relationship detected between plant biomass or volume and pollen or seed production is a useful tool for common ragweed management. Such data could be used to provide risk estimation maps to localise problematic populations before pollen release. It can also be used for scientific purposes [16, 17], because direct counting of pollens and seeds is an arduous task, and such allergenic and invasive plant species can be difficult to study in greenhouse or common garden experiments. Furthermore, this kind of data could be used in modelling pollen flow and plant demography or in biological, chemical or mechanical experiments, which need to quantify the effects of treatments on pollen and seed plant productions.

CONCLUSION

Results obtained from common ragweed developing under natural conditions were different from those previously published. Pollen and seed production per individual were also lower than previously mentioned [3, 5] and are probably more representative of natural patterns of such invasive European plant populations. In the context of common ragweed spread and its control in Europe, the methods presented here to estimate pollen and seed production could be applied in scientific studies of this plant species.

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