

Considerations About Pollen Used for the Production of Allergen Extracts

Rosa Codina, PhD^{a,b}, Rodger C. Crenshaw, MS^c, and Richard F. Lockey, MD^b Lenoir, NC; and Tampa, Fla

Pollen is a biological product obtained to manufacture tree, weed, and grass allergen extracts, used to diagnose and treat allergies. Genetic and environmental factors affect the composition of pollen, e.g., the plant varieties from which pollen are obtained, weather, and levels of air pollution during plant growth. Therefore, appropriate guidelines and training of personnel to perform the activities associated with pollen are essential to produce appropriate allergen extracts. Various regulatory institutions, which vary in different countries, including the Food and Drug Administration (FDA) in the USA, control how such products should be produced. For example, the FDA regulates the manufacturing of pollen extracts but not the quality of the pollen used to prepare them, relying on each manufacturer to set its own standards to do so. To the contrary, European regulatory agencies, including the European Medicines Agency, control both the quality of the pollen and the manufacturing process to produce pollen extracts. Regulatory agencies, allergen manufacturers, scientific institutions, and pollen collection entities should collaborate to develop and implement guidelines appropriate for worldwide use for both the collection and processing of pollen raw materials. This article provides an overview of the subject of pollen for use in allergen extracts. © 2015 American Academy

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The factors responsible for the quality and composition of allergen extracts used to diagnose and treat allergic diseases include the raw materials used to produce these extracts.¹ Raw materials are diverse in origin and have different characteristics, including the particle size containing the allergenic components present in them.

For pollen, verifying their identity and purity and avoiding cross-contamination from other biological products are essential. In addition, phylogenetically and nonphylogenetically related pollen taxons often have a high level of cross-reactivity, which should receive appropriate attention to assure that the materials contain clinically relevant, nonredundant allergens, and that pollen extracts are prepared from the appropriate genera and/or species.

A major challenge is associated with the fact that both genetic and environmental factors are responsible for the allergenic composition of pollen. These variables, difficult to control, include the characteristics of particular plant species and varieties²⁻⁷ as well as the climatic and geographical locations where individual plants grow.⁸⁻¹¹ Likewise, pollen collection, purification, and storage conditions¹²⁻¹⁵ can affect the purity and allergenic composition of pollen.^{3,9,10,14} Although it is possible to address and monitor some of these variables, it is not possible to control all of them, in particular, the weather and soil composition where individual plants grow and the pollen is collected.

Molecular engineering techniques, including cloning-specific genes, can be used to increase plant nutrition and resistance to pesticides.¹⁶⁻¹⁸ Such techniques can also be used to produce plants with reduced allergenicity.¹⁹ Theoretically, a similar approach could be used to obtain pollen with ideal allergenic characteristics for the production of allergen extracts. However, there are safety concerns regarding potential human exposure to such modified pollen because molecular engineering techniques involve the use of microbial genomes, which could perhaps expose humans to them and potentially impact health.²⁰

Recombinant allergens to diagnose and treat allergic diseases are also practical alternatives to circumvent the caveats of using natural pollen allergens.²¹⁻²³ Although this science continues to evolve, natural products most likely will continue to be used for many years to come because of the varying limitations regarding the production and use of recombinant allergens. For example, it is currently not possible to obtain recombinant forms of all clinically relevant allergens. In addition, the safety and efficacy of

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^aAllergen Science and Consulting, Lenoir, NC

^bDivision of Allergy and Immunology, Department of Internal Medicine, University of South Florida Morsani College of Medicine, Tampa, Fla

^cRetired mycologist

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Corresponding author: Rosa Codina, PhD, Allergen Science and Consulting, 923 Barrington Dr., Lenoir, NC 28646. E-mail: rosacodina@allergenscienceandconsulting.com.

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Abbreviations used

CBER - Center for Biologics Evaluations and Research
FDA - Food and Drug Administration

such allergens need to be properly documented through appropriate clinical trials, which takes time and is extremely expensive.

The purpose of this article is to provide health care professionals and other interested individuals with insight into the topic of natural pollen utilized to prepare allergen extracts. This article also integrates some information about the regulations associated with natural pollen used to manufacture allergen extracts in both the USA and Europe and provides possible recommendations as to how to address some of the caveats associated with the use of these materials.

GENETIC AND ENVIRONMENTAL FACTORS RESPONSIBLE FOR THE ALLERGENIC COMPOSITION OF POLLEN

Genetic factors

A number of genetic varieties of botanical species exist because of agricultural practices and natural hybridization. Different plant varieties of the same species often produce pollen with distinctive allergenic characteristics. For example, a study that investigated the allergenicity of pollen derived from 6 different varieties of olive (*Olea europaea*) identified large differences in allergenicity, as determined by various chemical and immunochemical tests.¹⁰

The application of molecular engineering techniques since 1996 has steadily increased to obtain crops with certain desirable characteristics.¹⁶⁻¹⁸ Today, more than 50% of crops in the USA are transgenic.¹⁸ In some cases, allergen extracts are prepared from pollen derived from transgenic plants because of the difficulty of obtaining sufficient quantities of pollen derived from wild-type plants. These plants include many grass and some tree species, for example, corn (*Zea mays*) and olive (*Olea europaea*), respectively.

Because of a general fear about genetically modified crops plants, particularly in Europe, European regulatory agencies restrict the use of pollen derived from transgenic plants, and, if utilized, require that their use be appropriately justified.²⁴ However, scientific evidence, obtained from risk-analysis evaluations, is lacking to preclude the use of such pollen for pharmaceutical use due to potential health concerns.²⁵⁻²⁷

Environmental factors

Geographical location, local weather, and soil composition are responsible for plant growth and nutrition, one of the main factors that affect the qualitative and quantitative allergenic composition of pollen. For example, a study that investigated the Amb a 1 content of short ragweed (*Ambrosia artemisiifolia*) pollen collected from the same location during different years indicates that the concentration of this allergen can vary by 10-fold, as assessed by quantitative immunochemical tests.¹

Environmental contaminants can potentially affect pollen before and during collection, and include those naturally present in the environment and those generated by humans. Naturally occurring biologic contaminants are fungal spores and associated structures, bacteria, pollen grains derived from other species, plant debris, algae, and insect debris.

Human activities, such as the use of fossil fuels and fertilizers, cause the release of chemical products into the environment. The

potential effects of these activities and agents on pollen are largely unknown. There is some evidence that increasing levels of carbon dioxide in the atmosphere could augment the allergenicity of ragweed and other pollen species.²⁸ In addition, several observations suggest that pollen collected from areas with high levels of air pollution have greater pro-inflammatory properties than pollen obtained from nonpolluted locations.²⁹

POLLEN SPECIES COLLECTED FOR THE PRODUCTION OF ALLERGEN EXTRACTS

In 2004, the Food and Drug Administration (FDA) Center for Biologics Evaluations and Research (CBER), which regulates allergenic products, created a committee to review scientific data about the safety and efficacy of nonstandardized allergen extracts. Such extracts were classified into various categories according to the level of scientific information available and safety considerations to justify their use.³⁰

CBER proposed to remove all products with potential safety concerns or lack of therapeutic value (Docket #FDA-2011-N-599). Although this activity is taking place slowly, the number of pollen extracts currently available on the market will likely be reduced from 708 to 451 species in the near future. Allergen cross-reactivity should be considered to help determine which pollen genera and/or species are clinically relevant.

A reduction in the number of pollen extracts available on the market should be the first step to select additional allergens for standardization. These decisions should be based on their clinical importance and feasibility of obtaining the necessary reagents for standardization. The high level of effort and cost associated with allergen standardization requires a thorough evaluation of the potential benefits of this process.

Because of the increasing regulatory requirements regarding allergenic raw materials used in Europe, Lorenz et al³¹ in 2009 proposed the concept of "homologous groups" to classify allergen sources. This concept is based on similar biochemical composition and homology/cross-reactivity of allergens or allergen sources. European regulatory agencies adopted this concept,²⁴ and now require that allergen manufacturing companies obtain quality data for representative allergen sources classified in each homologous group, 7 of which represent pollen species.

European regulatory agencies also have proposed norms to reduce the number of allergenic preparations available on the market. These approximations are analogous in concept to that proposed by CBER in the USA, but are generally more rigorous, and the number of allergen extracts permitted are fewer than those currently available in the USA.³²

GENERAL REGULATIONS REGARDING POLLEN IN THE USA AND EUROPE

Allergen manufacturing companies in the USA must comply with CBER regulations for pollen allergen extracts, particularly with mandated limits for relative potency or major allergen concentrations of standardized pollen allergen extracts.³³ However, regulations or guidelines for nonstandardized extracts are lacking. In addition, the organization has not proposed regulations for pollen raw materials because it is assumed that the manufacturing process will assure that the final extracts are appropriate for human use.

In Europe, regulatory agencies control the quality of both the pollen used to manufacture allergen extracts and the derived extracts. Therefore, European allergen manufacturing companies

TABLE I. Comparison of general concepts, associated with regulations regarding pollen raw materials currently in place in the USA and Europe

Concept	USA	Europe
Regulatory focus	Final allergen extracts	Both raw materials and final allergen extracts
Number of pollen species allowed	Extensive	Reduced
Limits for biologic contaminants	Internally proposed by allergen manufacturing companies	Proposed by regulatory agencies
Limits for man-made contaminants	Not proposed	Testing requested
Recommendations to avoid contaminants	Avoid exposure	Avoid exposure and test for contaminants

have to comply with general regulatory guidelines for pollen raw materials.^{24,34} These regulations sometimes are specific to each country.

All guidance documents are subjected to interpretations that can differ among allergen manufacturing companies. Companies are responsible for justifying internal interpretations and documenting compliance. As expected, these guidance documents are continually being revised.

MEASURES TO ASSURE POLLEN QUALITY

Various considerations are necessary to obtain quality pollen for use in the manufacture of allergen extracts. Allergen manufacturing companies should have in place general quality measures appropriate for pollen raw materials,³⁵ as described below.

General measures to consider from pollen collection to extraction

The first recommendation to obtain pollen suitable for the production of allergen extracts is to assure its proper collection.^{36,37} Once pollen is received at the allergen manufacturing companies, batch records are initiated to provide a method to trace the pollen to its original source and collector. Subsequently, pollen identity and purity are assessed, and moisture content is determined to assure that it is sufficiently dry to prevent microbial growth. If pollen contains excessive moisture and/or foreign biological materials, it should be dried and/or cleaned, as appropriate, to meet manufacturing specifications.

European regulatory agencies have arbitrarily proposed maximum allowed concentration limits for some pollen biologic contaminants.^{24,34} The FDA does not regulate these components. Allergen manufacturing companies in the USA internally propose and follow their own guidelines, which are generally based on the feasibility of achieving the best levels of purity after the pollen has been cleansed.

European regulatory agencies have also proposed maximum limits of exposure to some man-made pollutants, such as heavy metals and pesticides. Such limits are based on toxicological data on gastrointestinal exposure using animal models. These same agencies require that the content of several heavy metals and a large number of pesticides in pollen be appropriately monitored^{24,34,38} and that the detected concentrations be below the limits proposed. However, many of the pesticides required for testing are no longer in use.

The FDA has not proposed threshold limits for chemicals in pollen mostly because proactive actions can minimize pollen

exposure to such agents, at least to some extent. For example, collectors are required to obtain pollen from nonurban, rural areas distant from roads with heavy traffic to minimize pollen exposure to man-made chemical agents.

European allergen manufacturing companies also have to perform extensive testing of the raw materials used to manufacture allergen extracts to assure that the qualitative and quantitative allergen composition of the products meets particular specifications. This testing is not required in the USA. Table I compares the general concepts and regulations associated with pollen currently in place in the USA and Europe.

Pollen collection and cleaning practices

Pollen collection is tedious and requires a high level of specialization. The pollination seasons associated with particular botanical species typically vary depending on climate and geographical location.³⁹ In addition, many pollen species often need to be collected over short time intervals and at specific times of the day due to rapid fluctuations of the environmental parameters, for example, ambient humidity, that affect pollen release.

A few large pollen collection producers utilize their own land to cultivate the desired plants for pollen collection, under conditions that minimize exposure to man-made pollutants (Figure 1). However, this is an emerging strategy and often does not result in sufficient quantities of pollen necessary for manufacturers that produce pollen extracts.

A large number of small family-owned pollen collection entities also obtain pollen for pharmaceutical use. A close collaboration between pollen collection entities and allergen manufacturing companies is essential to maximize the efficacy of the collection activities and assure that sufficient quantities of the necessary pollen species are obtained.

There are 3 main methods used to collect pollen from wind-pollinated plants: the water-set; the vacuum; and the cut/dry/sieve method. Although many pollen species can be collected using any of these 3 methods, the decision as to which collection practice is utilized depends on the expertise of particular collectors and the resources they have available. Each of the 3 collection methods has advantages and disadvantages.

The water-set method consists of placing plant parts that contain mature anthers on trays of water. As the anthers open, pollen falls onto a clean surface from which it is collected. The main advantage of this method is that it results in very clean pollen. On the contrary, it is very labor intensive, requires large facilities, and the resulting pollen can be affected by moisture and subsequent microbial growth.



FIGURE 1. Field planted with lamb's quarter (*Chenopodium album*) for the purpose of pollen collection.

The vacuum method is recommended to collect pollen from plants that grow in pure stands, that is, most grasses and some weeds. As soon as the plants begin to bloom, the pollen is collected. Before beginning this process, the field is carefully inspected to remove any infected or different plants that bloom simultaneously. The main advantage of this method is that it is relatively simple to perform. The main problem with this method is that the resulting pollen often contains high levels of biological contaminants, which can be difficult to remove.

The cut/dry/sieve method is recommended to collect pollen from plants that produce male catkins such as alder (*Alnus* spp.), oak (*Quercus* spp.), and birch (*Betula* spp.). The advantage of this method is that it allows for the collection of large quantities of pollen. The catkins are removed from the trees as they begin to open and are placed on clean surfaces in a heated room. However, the pollen collected by this method needs extensive cleaning, comparable to pollen obtained by vacuuming.

Regardless of the collection method, the pollen is then dried to reduce moisture content and prevent microbial growth and potential allergen biodeterioration. This activity is usually performed in moisture-controlled environments.

When the pollen is sufficiently dry, plant parts, fungal spores, insect fragments, and foreign pollen grains are removed. The 2 primary methods to clean pollen are mechanical gradation sieving and air classification, both of which are described below.

Mechanical gradation sieving is the simplest and most widely used method to clean pollen because sieving with various micron-sized meshes can remove biologic contaminants that differ in size. The air classification method can separate particles that differ in both shape and size, which results in very clean pollen (Figure 2). However, air classification equipment is expensive and the cleaning process often disrupts pollen, which makes its allergenic quality difficult to assess. Some cytoplasmic materials that contain allergens can be lost during the cleaning process.

The procedures utilized to clean pollen depend on the resources available at pollen collection sites. The utilization of particular collection and cleaning methods is not associated with one particular pollen species versus another or with standardized or nonstandardized allergen extracts. Allergen manufacturing

companies use the pollen available at a given time regardless of the methods used to collect and purify it.

Established programs to qualify pollen collection entities and/or individual collectors are lacking in both the USA and Europe. Therefore, allergen manufacturing companies usually propose the criteria for approval of pollen collection entities and individual collectors.

Pollen identification and evaluation of biological contaminants

The identity of the pollen species collected to manufacture allergen extracts must be verified. Pollen collectors are requested to provide portions of the plants to verify plant identity from which pollen is obtained. Allergen manufacturing companies ideally should employ expert botanists to perform this task.

Pollen microscopic analysis is performed to further verify pollen identity and assess its purity. Pollen purity assessments include counting and identifying biological components that contaminate the pollen. However, this method of evaluating pollen purity may not permit assessment of the potential clinical importance or amounts of specific contaminants compared with the pollen in the final sample. Although the potential importance of many pollen contaminants is unknown, volumetric counting instead of particle counting could provide a better estimation of the amount of foreign biological materials contained in the collected pollen. However, standardized and approved methods to perform this evaluation are lacking.

For the purpose of microscopic pollen identity and purity evaluations, samples are stained and examined under various magnifications, even though the staining cannot selectively differentiate viable from nonviable pollen grains. Staining solutions able to differentiate viable from nonviable pollen are available, mainly for agricultural purposes.⁴⁰ Currently, these solutions are not used to evaluate the quality of the pollen collected for pharmaceutical use because it is assumed that both viable and nonviable pollen are equally allergenic. Nevertheless, this assumption may not be true because pollen allergenicity may be compromised under conditions that render pollen nonviable, for example, exposure to high temperatures during transportation and processing.

Microscopic pollen analysis can also provide valuable information regarding pollen quality. For example, the presence of a large number of plant parts and a variety of miscellaneous fungal spores generally indicates that the pollen is not sufficiently clean and that additional activities to remove biological contaminants are necessary. On the contrary, the presence of large amounts of one single spore type, hyphae, and/or sporulating structure indicates that fungi have infested the pollen for a period, most likely because of excessive moisture.

Qualified individuals should ideally perform the pollen identification and purity assessments in appropriate in-house laboratories at allergen manufacturing companies. However, programs to train individuals and certify such laboratories are currently lacking. A precedent to support the implementation of such programs exists regarding the analysis of airborne fungal spores for indoor air quality purposes.⁴¹

It is essential to perform all activities associated with pollen, including identification and purity assessments, in dedicated areas of allergen manufacturing companies where other types of raw materials are not present. Maximizing proper ventilation, cleanliness, and avoiding passive transport of allergens is

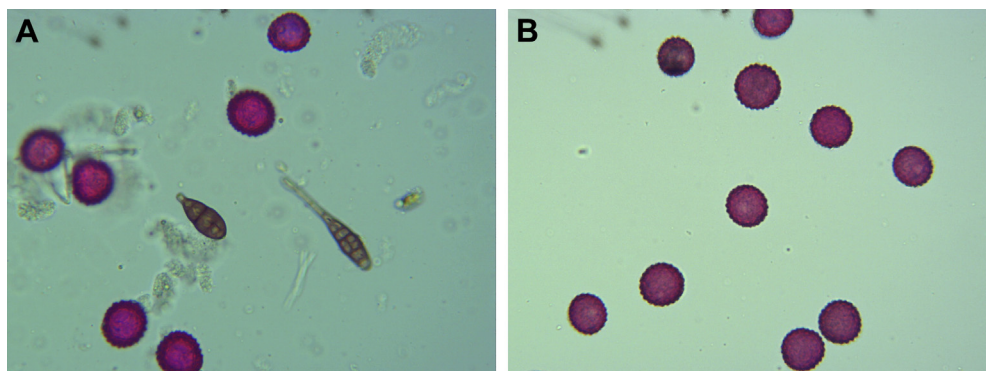


FIGURE 2. Micro-photograph of short ragweed (*Ambrosia artemisiifolia*) pollen before (A) and after cleaning (B).

necessary to prevent potential cross-contamination among different pollen species and other allergenic materials. Measures to prevent this potential passive cross-contamination are necessary.

Pollen storage

Pollen should be properly stored to assure its integrity until extraction. Although the FDA has not proposed expiration dates for pollen raw materials, European regulatory agencies generally indicate that pollen should be used within 5 years following collection. However, scientific evidence to substantiate this 5-year period is lacking.

A study that investigated the stability of 2 grass pollen species, stored under different temperature and moisture conditions over a 10-year period, indicates that no allergen degradation occurred as long as the pollen was properly dried and stored frozen.⁴² Real-time stability studies are necessary to perform, generally following proposed guidelines.⁴³ The information derived from the completion of these studies will likely identify selective expiration dates for particular pollen species, as expected, due to the diverse nature of different pollen allergens.

COST/BENEFIT CONSIDERATIONS OF ASSESSING THE QUALITY OF POLLEN RAW MATERIALS

Many pollen allergen extracts have been safely used for years to diagnose and treat allergic subjects, regardless of the absolute quality of the pollen raw materials utilized to make them. Therefore, the necessity and cost-effectiveness of the extensive testing that European regulators require to assure the qualitative and quantitative purity of pollen raw materials should be reevaluated.

It is assumed in the USA that the manufacturing process associated with the production of allergen extracts assures the consistency and quality of such products. Although this is partially true for the available standardized pollen extracts, nonstandardized extracts are not subjected to similar quality measures. As a result, large lot-to-lot variation in allergenic content may exist, partially attributed to the selective allergenic content of the pollen raw materials used to produce them. Extracts from different manufacturers and even from the same manufacturer, obtained at different times, often differ in diagnostic and therapeutic value.

Some level of control regarding the pollen raw materials used to manufacture both standardized and nonstandardized allergen extracts is necessary to assure optimal qualitative and quantitative allergenic composition of allergen extracts. Therefore, appropriate

testing is necessary to verify that pollen raw materials are appropriate for their intended use.

Preventive measures to minimize the potential impact of air pollution on pollen can be implemented at a relatively low cost by selecting appropriate collection areas and by visually inspecting the plants used for pollen collection. The utility of measuring the types and levels of a large number of pesticides and several heavy metals, as requested in Europe, is controversial and expensive, and should be investigated.

Biological contaminants of pollen are impossible to avoid, and the procedures used to clean pollen do not remove all of them or assure that they have not affected the quality of pollen. Practical guidelines should be developed to properly interpret the results derived from pollen purity evaluations. As previously discussed, an expert assessment and interpretation of the biological contaminants in pollen should properly address this issue. However, and because expertise to perform these assessments is scarce, European regulatory agencies request very specific testing to determine the potential impact of microbial growth in pollen.

KEY POINTS AND HIGHLIGHTS

- Proper understanding of the processes associated with the collection and processing of pollen and the regulations in place in the USA and Europe to do so are essential to improve the quality of pollen allergen extracts.
- Multiple factors before, during, and after pollen collection can potentially impact the quality of pollen raw materials.
- The proper selection of the genera, species, and/or varieties of plants used to obtain pollen and the collection and cleaning practices employed are critical for the manufacture of pollen extracts.
- The strict European regulations regarding the quality of pollen used to manufacture allergen extracts are generally based on the “worst case scenario” and not on adequate scientific principles. This may limit the availability of clinically relevant pollen species in the future.
- The accurate identification of the pollen collected for pharmaceutical use and the proper evaluation and interpretation of its purity are essential to assure the quality of allergen extracts.

RECOMMENDATIONS AND FUTURE DIRECTIONS

- The potential selective allergenicity of pollen derived from different plant varieties that belong to a particular species

needs investigation to identify the most clinically relevant pollen.

- The potential combined impact of biologic and man-made contaminants in pollen should be investigated by both the scientific and regulatory communities. The question is “how much purity is needed and why?”
- A combination of scientific data, feasibility, cost-effectiveness, and practicality of obtaining pollen needs to be considered, according to current regulations and guidelines. Risk-analysis models should be used to properly address this matter.
- Allergen manufacturing companies should have the proper infrastructure and dedicated spaces to store, process, and assure optimal quality of pollen used to manufacture allergen extracts.
- Scientific organizations should collaborate with industry and the regulatory communities to reevaluate the entire process to obtain, purify, and store pollen for allergen extracts.
- Manufacturers should develop programs to train and qualify individuals to oversee the process of producing pollen for allergen extracts.

CONCLUSION

A close collaboration among regulatory agencies, allergen manufacturing companies, scientific organizations, and pollen collection entities is necessary to properly address the current limitations regarding pollen used to manufacture allergen extracts and propose universal guidelines for worldwide use.

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