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# Bridging allergologic and botanical knowledge in seasonal allergy: a role for phenology

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**Background:** Grass pollen is a worldwide cause of respiratory allergy. Identifying the causative species is essential, for example for choosing the appropriate immunotherapy, because not all grass allergens are totally cross-reacting, and the pollen calendars provide only a gross estimate. Phenologic analyses allow identification of the pollen release for each individual grass.

**Objectives:** To assess, using phenologic analyses, the true flowering periods of grasses and to compare the data with the standard pollen calendar.

**Methods:** Phenologic analyses were performed of the following grasses: black grass, sweet vernal grass, common wild oat, barren brome, cocksfoot, tall fescue, Yorkshire fog, ryegrass, Timothy grass, bulbous meadow-grass, Kentucky bluegrass, and Bermuda grass. Sampling was performed every 10 days, starting in April 2009, at 50 stations distributed across Italy. The flowering phase was assessed using a stereomicroscopy-based method for the detection of spreading stamens. The official pollen calendar was used for comparison.

**Results:** Relevant differences were found between grass pollen count and effective flowering of the grass species. Only some species contributed to the pollen peak, and a relevant pollen load for other species was also present out of the peak. Important Pooideae, such as Timothy grass, were not present during the pollen peak in northern and central Italy, and the same occurred with Bermuda grass.

**Conclusions:** The various species of grasses release their pollen grains at different times during the pollen season, and this information is missing with pollen calendars. This may have a relevant effect on the choice of an appropriate immunotherapy.

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## INTRODUCTION

Grass pollen is a major worldwide cause of respiratory allergy.<sup>1</sup> In clinical practice, the diagnosis of grass-induced asthma/rhinitis usually does not represent a problem because it is based on the presence of typical symptoms and on the demonstration of specific IgE using either skin testing or serum IgE assay.<sup>2</sup> In addition, the seasonal pattern of symptoms, which correlates with the dispersion of pollens in the environment (the pollen season), strongly supports the causal role of the plant. Certainly, symptoms depend also on the type of pollen, which is affected by meteorologic and environmental conditions.<sup>3</sup> Correct identification of the causative grass spe-

cies is marginally important when allergy is treated with symptomatic drugs but becomes relevant when allergen specific immunotherapy (SIT) is prescribed. In fact, SIT is an antigen-specific therapy, and, therefore, the vaccine should contain all the responsible allergenic molecules. In the case of grasses, there is extensive cross-reactivity among the allergens of some species,<sup>4</sup> but this is not always true for all species and for all families (eg, Chloridoideae and Pooideae).<sup>5</sup> Despite this, the detection of allergenic pollens in the environment is usually achieved by means of air sampling with pollen traps to obtain pollen counts and pollen calendars. This method, which uses optical microscopic examination, does not distinguish the pollen grains of subfamilies or tribes that belong to wild grasses because they are morphologically similar.<sup>6</sup> Thus, the pollen counts and calendars refer generically to “grasses,” although several hundred different grasses exist. For example, in Italy, there are more than 450 known species,<sup>7</sup> and their distribution largely varies according to microclimatic environment, geographic area, and altitude.

A more detailed assessment of the distribution of grass pollens can be obtained by using the so-called phenologic evaluation. Phenology,<sup>8</sup> which is based on visual and microscopic observations, is the study of periodic plant and animal life cycle events and how these events are affected by seasonal and interannual climate changes. Grasses are wind-pollinated plants; therefore, the pollen develops in the anther, the terminal part of the stamen, which opens when ripe and

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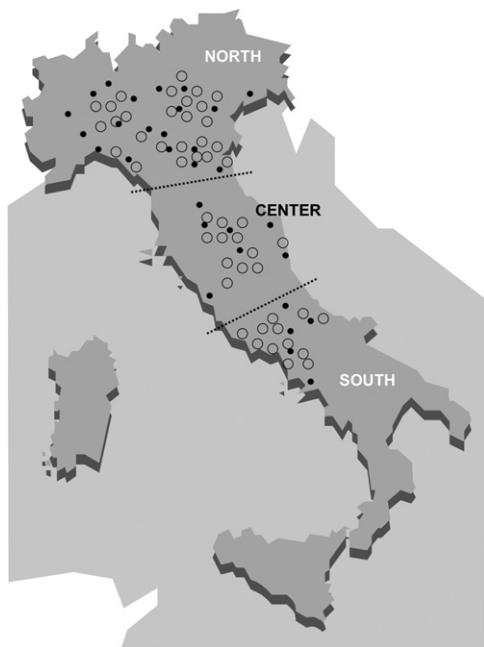


Figure 1. Map of Italy showing the subdivision into the northern, central, and southern macro-areas. The empty circles indicate the phenologic sampling locations, and the full circles indicate the pollen count stations.

releases a large amount of grain into the atmosphere to be transferred, by wind, to female flowers. We performed a phenologic study to assess the effective flowering period and pollen release of different grass species and compared the data obtained with the standard pollen count and calendar.

## METHODS

The personnel of the Laboratory of Palinology and Aerobiology of the University of Perugia sampled the following grasses of the Pooideae subfamily: *Alopecurus myosuroides* (black grass), *Anthoxanthum odoratum* (sweet vernal grass), *Avena fatua* (common wild oat), *Bromus sterilis* (barren brome), *Dactylis glomerata* (orchard), *Festuca arundinacea* (tall fescue), *Holcus lanatus* (Yorkshire fog), *Lolium perenne* (ryegrass), *Phleum pratense* (Timothy grass), *Poa bulbosa* (bulbous meadowgrass), and *Poa pratensis* (Kentucky bluegrass). *Cynodon dactylon* (Bermuda grass), belonging to the Chloridoideae subfamily, was also sampled. Sampling was conducted in 50 different locations across Italy and surrounding the stations where official pollen counts are performed (Fig 1). The data collected were subsequently pooled according to the 3 macro-regions (northern, central, and southern Italy), as is done for the official pollen calendar. The stations covered a broad spectrum of altitudes, population densities, and climates, and each station was chosen to cover an area as homogenous as possible regarding topography and vegetation.<sup>9</sup>

Assessment of the phenologic phase of the flowering (corresponding to the main period of pollination) was performed using direct observation and stereomicroscopy, evaluating the presence of styles spreading and releasing pollen grains. This allowed identification of the start of flowering, full flowering with pollen release, and the end of flowering.<sup>4</sup>

The observations were conducted using the following phenologic key in 4 steps: (1) inflorescences fully emerged—stamens not extruded; (2) start of flowering—stamens partially extruded, release of pollen; (3) full flowering—all stamens extruded, full pollen emission; and (4) end of flow-



Figure 2. Examples of the phenologic identification phases of *Phleum pratense*: inflorescence emerged but with stamens not extruded (left) and full flowering with stamens extruded (right).

ering—all stamens withered, no pollen emission. According to this key, pollen emission occurs in steps 2 and 3. The assessment was performed every 10 days starting in April 2009, and at each station, at least 25 plants were considered for each species. The beginning of each step was defined as the day on which the corresponding phenologic phase was observed on at least 50% of plants in each station.

In each macro-area, the period of flowering with pollen emission is the mean of all observed flowering dates at the sampling stations. This allowed identification of the effective flowering period for each species.<sup>10</sup> Examples of the phenologic identification phases are shown in Figures 2 and 3. The Italian official pollen calendar<sup>11</sup> was used for comparison.

## RESULTS

According to the pollen calendar, grass pollens began to appear in the first and second week of April, with minimal differences among the 3 macro-regions. The pollen peak, defined as the week with the highest pollen concentration, corresponded in northern areas to May 18 to 24, in central areas to May 4 to 10, and in southern areas to May 25 to 31. Nevertheless, looking at the phenology readings, it was apparent that not all grasses were present during the period of maximal pollen count. In particular, important Pooideae, such



Figure 3. Example of the phenologic identification phases of *Cynodon dactylon* by means of stereomicroscopy ( $\times 10$ ): end of flowering with withered stamens and the female flower fertilized.

as *P pratense*, appeared in the northern and central areas only after the pollen peak, and the same occurred with *C dactylon* across the entire territory. In contrast, the flowering of *P bulbosa* and *B sterilis* invariably preceded the pollen peaks. As a consequence, the pollen peak is due to some and not all pollen species, and, in turn, a relevant pollen load for some species is present also out of the pollen peak. Figure 2 shows the flowering of Timothy grass, and Figure 3 shows the flowering of Bermuda grass using stereomicroscopy. Figure 4 summarizes the periods of effective pollen grain release (phases 2 and 3) of each grass species in relation to the pollen count and the pollen peak (the week with the highest pollen concentration) in the 3 areas.

## DISCUSSION

Grass pollen is one of the most important and represented allergen sources worldwide. Its presence is commonly detected and quantified by appropriate pollen traps, which provide a gross and cumulative estimate of the pollen load during the year. Nonetheless, there are hundreds of different species of grasses, which do not pollinate simultaneously in Europe<sup>10</sup> and the United States.<sup>12</sup> This may be relevant in interpreting the circa-annual pattern of symptoms and may explain why in some grass-sensitized patients the occurrence and severity of respiratory symptoms does not parallel the standard pollen counts or why symptoms worsen far from the pollen peak. In addition, not all the allergens of grasses are fully cross-reacting. This is well-known in the case of Bermuda grass, which is poorly cross-reacting with the Pooideae, because it lacks major group 2 and 5 allergens,<sup>13</sup> and the same happens with ryegrass and Bahia grass.<sup>14</sup> This is of central importance also for the correct choice of a vaccine for immunotherapy, which should, in principle, contain all the relevant allergens. It is reasonable to expect that an extract lacking some of the major allergens is incompletely effective in patients generically sensitized to grass but exposed to the allergens of different species, as previously observed for ragweed.<sup>15</sup> Actually, in the United States, immunotherapy for grass allergy is generally performed by using multiple grass extracts,<sup>16</sup> which are chosen according to the results of skin tests and taking into account the cross-reactivities. In this case, skin prick test or IgE assay results may be sufficient to correctly identify the sensitizing grasses to be used for vaccination, but it is also true that the information provided by phenologic analysis would be helpful in selecting the appropriate diagnostic tests to be used in a given geographic region. On the other hand, in Europe, “grass mix” extracts are generally used for in vivo diagnosis, and the use of single grass extracts is considered a valid option on the grounds that they contain all the relevant allergens. In this case, more detailed knowledge of the sensitizing grasses and their flowering periods is relevant. In fact, the data we obtained by means of phenologic evaluation suggest that the different grass species may have variable importance in the sensitization process because of a dissimilar behavior during the pollen season. In particular, it is notable that some grasses seem to be less important be-

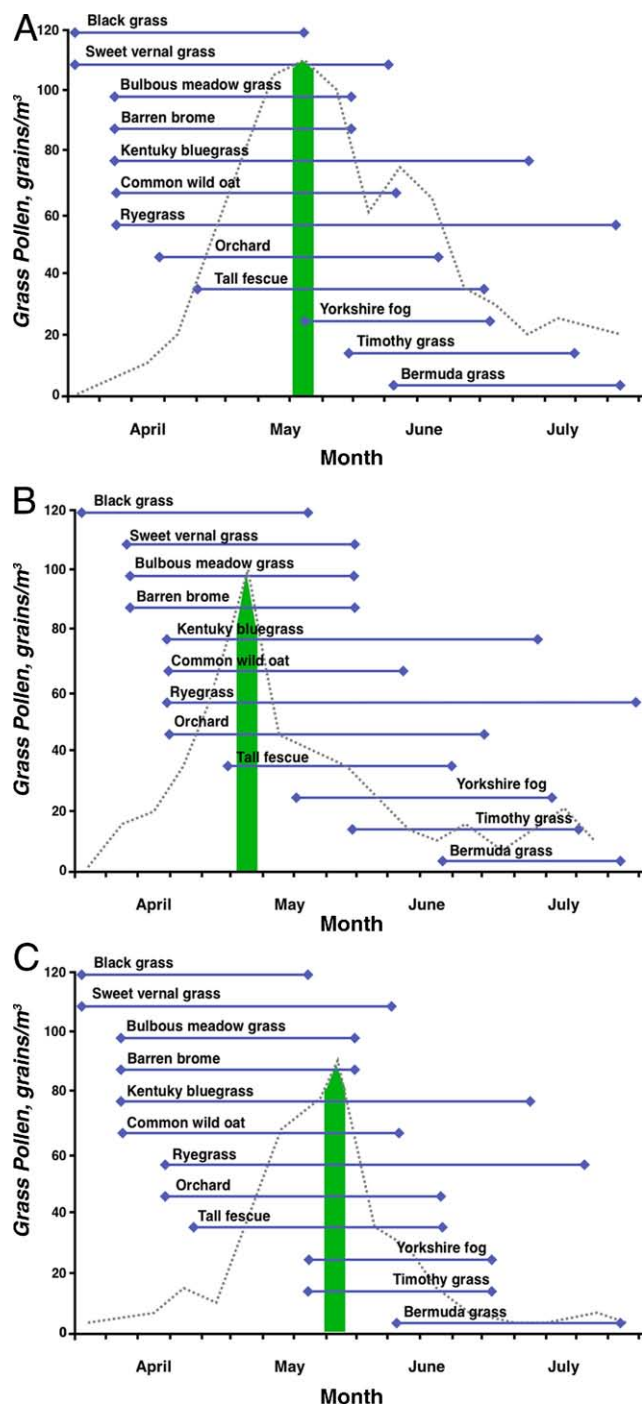


Figure 4. Pollen counts and phenologic analyses in the 3 Italian areas: northern (A), central (B), and southern (C). The blue lines indicate the duration of effective release of pollen grains (phases 2–3) as assessed by means of phenologic analysis for each grass species. The pollen count according to the official pollen calendar is shown as a dotted line. The pollen peak is highlighted in green.

cause they release their pollen in July, when usually grass-allergic individuals have few or no symptoms. Thus far, phenologic analysis has been mainly used to evaluate the effects of climate changes on ecosystems,<sup>17</sup> but it could represent a useful tool for evaluating the true exposure to allergenic grass pollens.

The present findings show that the various species of grasses release their pollen grains at different times during the pollen season, whereas the traditional pollen count, which does not distinguish the different species,<sup>18</sup> provides only partial and incomplete information. Concerning Italy in particular, the use for SIT of a mixture of different grasses seems to better reflect the natural occurrence and has the potential to provide a more complete interaction with the immunologic profile of grass-sensitized patients. Similar studies using the phenologic approach should provide useful data from other geographic areas, and studies comparing phenologic data with symptoms in a population of grass-allergic patients should strengthen such observations by providing a clinical evaluation. In addition, the phenologic evaluation could also be useful in planning clinical trials with medications for grass pollen allergy, and phenologic data could be combined with those obtained using component-resolved diagnostic approaches.

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