A novel method to suppress the dispersal of Japanese cedar pollen by inducing morphologic changes with weak alkaline solutions

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ABSTRACT: Inhalation of airborne pollen causes irritative symptoms in humans, known as pollinosis. The changing global climate and increased pollution contribute to enhance the release of pollen, thereby increasing the number of people suffering from allergies. We examined the effect of spraying weak alkaline solutions onto cedar trees, the main allergenic culprit in Japan, on pollen release. Weak alkaline solutions were sprayed onto Japanese cedar blossoms to disrupt the external walls of the pollen, and to induce swelling of the cytosolic components containing the nucleus. This morphologic change of the pollen grains depended on the pH of the suspending solution, with a threshold pH of near 7.5. As the breakdown of the external walls and swelling of the cytosolic components are inhibited by high osmolarity, the influx of water triggered the morphologic changes. Weak alkaline solutions sprayed onto cedar blossoms decreased the amount of pollen released from the anthers in a pH-dependent manner. The addition of detergent to the sodium bicarbonate solution facilitated this effect on cedar pollen release. We suggest that spraying cedar and cypress forests with a weak alkaline solution might prevent the scattering of pollen that causes allergies in humans.

Key Words: Pollinosis, *Cryptomeria japonica*, alkaline solutions, pollen release

Introduction

Various environmental substances cause allergic diseases (*1*). The inhalation of airborne pollen leads to irritative symptoms such as runny nose, sneezing, and eye hyperemia, an allergic reaction known as pollinosis. The allergens in airborne pollen have been purified, and their molecular structures have been analyzed (*2*). Environmental factors such as microparticles generated from diesel engines (*3,4*) and pathogenic bacteria (*5*) worsen the allergic symptoms of pollinosis. Various types of host cells are involved in pollinosis (*6*), such as acquired immune cells like helper T cells and immunoglobulin-producing B cells (*7*), innate immune cells like dendritic cells and macrophages (*8*), mast cells and eosinophils (*9*), mucosal epidermal cells, *etc*. These cells communicate with each other through the cytokine network (*10,11*), and inflammatory mediators such as histamine cause the allergic pathology (*12,13*). These aspects of the molecular mechanisms of pollinosis are extremely complicated. As our understanding of the system is currently insufficient, it is difficult to develop effective drugs to target pollinosis at the molecular level.

The number of patients suffering from pollinosis is increasing, along with the number of patients suffering from other allergic disorders such as atopy and asthma. One reason for the worldwide increase in pollinosis is that the increased concentration of atmospheric carbon dioxide and the accompanying global warming promotes pollen release from trees (*14,15*). When plants are raised in high concentrations of carbon dioxide or high temperatures, metabolic processes such as photosynthesis and water uptake are facilitated, and the production of pollen is promoted (*16-19*). Both the concentration of carbon dioxide in the air and the climate temperature are predicted to increase over time. Therefore, it is anticipated that the number of patients suffering from pollinosis will further increase due to a global overproduction of pollen. To cope with the situation, methods targeting pollen release from trees might be effective. The development of tree races that produce less total or less allergenic pollen has been attempted (*20*). Planting these new races in place of widely distributed anemophilous plants, however, is time-consuming and costly. Thus, simpler and more large-scale methods to suppress pollen dispersal are necessary.

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In Japan, pollinosis caused by Japanese cedar pollen (*Cryptomeria japonica*) is considered to be the most problematic (*21*). As a result of historical large-scale cedar planting, cedar trees are distributed throughout the country. For geographic reasons due to monsoons, pollen grains are likely to scatter in the air and reach the cities. Moreover, once pollen grains fall onto the paved roads in cities, they tend to remain there and are easily swept up into the air, thereby coming into contact with people. Therefore, a method that efficiently inhibits pollen release from cedar trees might be useful for suppressing pollinosis. In this report, we suggest a novel way to reduce the amount of pollen released from trees by focusing on the characteristics of cedar pollen, which undergoes morphologic changes in weak alkaline solutions.

Materials and Methods

Pollen

Pollen from cedar and cypress was purchased from the Japan Forest Tree Breeding Association. Cedar branches and blossoms were collected at the Gunma Prefectural Forestry Experiment Station (Gunma, Japan) in March, 2007.

Observation of morphologic changes of pollen grains

Pollen grains in a plastic tube were suspended in distilled water (pH 6), 25 mM sodium- or potassium-phosphate buffer solution (pH 5-9), or 1 M sodium bicarbonate solution (pH 9). After incubation at 20˚C for 10 min, an aliquot was observed under a microscope.

Measurement of the number of pollen grains released from blossoms

Cedar branches with blossoms (0.4-0.7 g) were sprayed with 10 mL of distilled water, 0.1 M phosphate buffer solution (pH 5-8), or 1 M sodium bicarbonate solution. The branches were dried for 3 h on a paper towel and placed in a 50-mL plastic tube. The tubes were shaken vigorously for 10 sec with a vortex mixer. The blossoms were collected and their weights measured. The collected pollen was suspended in 1 mL of distilled water. An aliquot of the suspension was observed under a microscope and the number of pollen grains was counted using a cytometer. The average number of pollen grains collected from three branches was calculated.

Results

Morphologic changes of Japanese cedar pollen in weak alkaline solutions

In the nasal cavity of humans, the external walls of cedar pollen grains break down, which results in the release of the cytosolic components surrounded by a transparent membrane (*22*). The high pH (> 8) of the mucus is considered to contribute to this reaction. Morphologic changes of cedar pollen were induced in 50 mM Tris/HCl solution (pH 8) (Figure 1). The external walls of the pollen grains were fluorescent under a fluorescence microscope, but the released cytosolic particles surrounded by the transparent membrane were not (Figure 1). We suspended cedar pollen in buffered solutions with various pHs, and counted the number of pollen grains that underwent morphologic changes after 10 min. Weak alkaline solutions induced morphologic changes of the pollen, with a threshold of pH 7.5 (Figure 2). The transparent membranes of the cytosolic particles disappeared when the morphologically changed pollen was incubated with pectinase (data not shown), indicating that the membrane covering the particle is made of pectin. Because the transparent membrane was not degraded by organic solvents or detergents (data not shown), lipids are not responsible for maintaining the rigidity of the transparent membrane.

Japanese cypress (*Chamaecyparis obtusa*) is another gymnosperm that causes pollinosis (*23,24*). The structure and antigenicity of the cypress pollen components are similar to those of cedar pollen (*24,25*). Therefore, we assumed that cypress pollen might also undergo morphologic changes in weak alkaline solutions. We suspended cypress pollen in 1 M sodium bicarbonate solution, and observed the samples under a microscope after incubating them for 10 min at room temperature. The majority, 50% to 90%, of the cypress pollen grains released cytosolic particles from the external walls (Figure 3), whereas in distilled water most pollen did not change morphologically. Thus, as with cedar pollen, weak alkaline solutions induced morphologic changes in cypress pollen.

The external walls of cedar pollen grains are permeable to water, ions, and small hydrophilic molecules (*26,27*). We hypothesized that water absorption through the external walls is the initial step of the morphologic changes in cedar pollen. If so, the process might be influenced by osmotic pressure. Thus, we tested the effect of high osmolality on the morphologic changes in cedar pollen in weak alkaline solutions. The addition of 1 M sucrose to 1 M sodium bicarbonate solution decreased the number of swollen cytosolic particles (Figure 4). This finding indicates that a pH shift increases the permeability of the external surfaces of cedar pollen, leading to a rapid uptake of water, which results in the breakdown of the outer walls and the release of transparent particles. Further swelling of the transparent membrane was inhibited by 5 M sucrose. Therefore, the swelling is also triggered by the osmotic inflow of water.

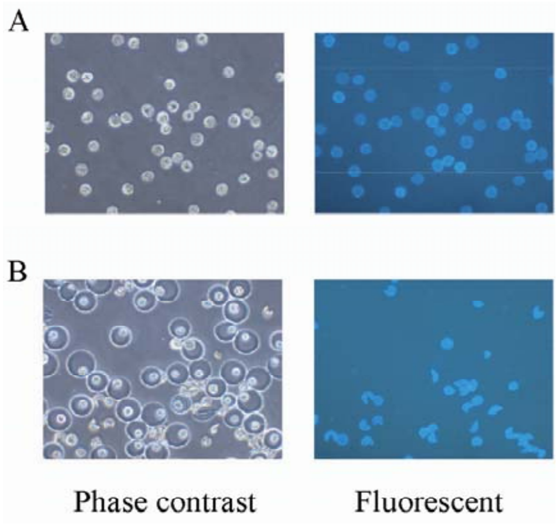

Figure 1. Morphologic changes of Japanese cedar pollen in weak alkaline solutions. Japanese cedar pollen was suspended in distilled water (pH 5-6) (A) or 50 mM Tris/HCl (pH 8) (B), incubated at 25˚C for 10 min, and samples were observed under a microscope equipped with a fluorescent lens.

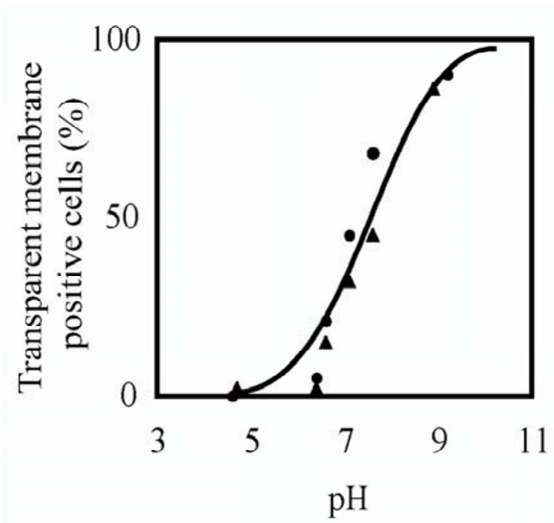

Figure 2. pH-dependence of the morphologic changes of Japanese cedar pollen. Japanese cedar pollen was suspended in 25 mM sodium-phosphate buffer (*triangles*) or 25 mM potassium-phosphate buffer (*circles*) with various pH values at 25˚C for 10 min. Samples were analyzed under a microscope with a phase contrast lens. The number of pollen grains with morphologic changes was counted.

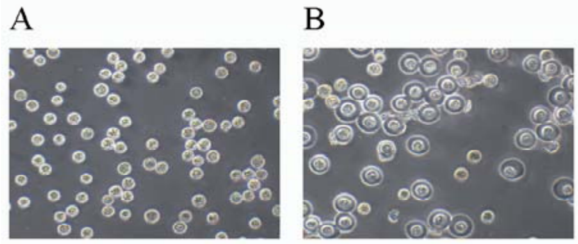

Figure 3. Morphologic changes of Japanese cypress pollen in weak alkaline solutions. Japanese cypress pollen was suspended in distilled water (A) or 1 M $NaHCO_3$ (B), incubated at 25˚C, and samples were observed under a microscope.

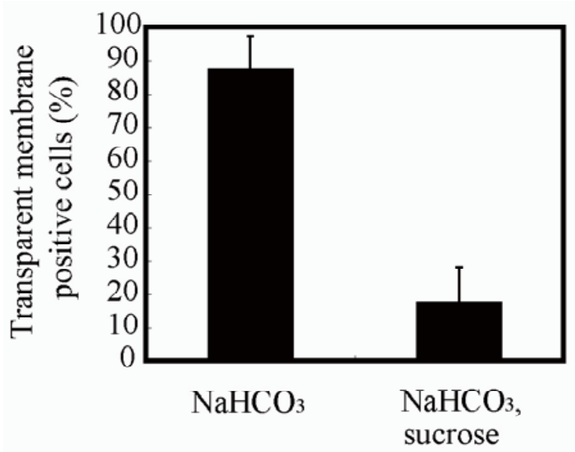

Figure 4. Inhibitory effect of hypertonicity on the morphologic changes of Japanese cedar pollen. Japanese cedar pollen was suspended in 1 M $NaHCO_3$ or 1 M $NaHCO_3$ containing 1 M sucrose and incubated at 25˚C for 10 min. Samples were observed under a microscope. The number of pollen grains with morphologic changes was counted.

Suppression of cedar pollen release from blossoms by spraying with weak alkaline solutions

In general, mature pollen grains are in a dried state when released from the anthers in response to the oscillation of branches caused by wind. Pollen grains that undergo morphologic changes lose their outer coats, which are necessary for floating in the wind and for protecting the interior contents from the environment. The altered morphology of cedar pollen suspended in weak alkaline solutions might therefore render them unsuitable for scattering, and such pollen might be easily destroyed in the environment. We evaluated whether spraying the branches with weak alkaline solutions decreased the amount of cedar pollen released from the anthers. First, pollen release was inhibited when sodium bicarbonate solutions were sprayed onto cedar blossoms (Figure 5A). Next, we sprayed phosphate-buffered solutions of various pHs onto cedar blossoms and examined the effect on pollen release. The amount of released pollen decreased in a pH-dependent manner (Figure 5B). Cedar pollen is covered by a waxy material that protects it against the invasion of weak alkaline solutions. We examined the effect of detergents that are generally added to agrochemicals against insects and pathogens. The addition of NP-40 to a 1 M sodium bicarbonate solution suppressed the pollen release from cedar blossoms in a dose-dependent manner (Figure 6). Therefore, the addition of detergents allowed for penetration of the waxy material and suppressed pollen release from the anthers.

Discussion

Suppression of the dispersal of cedar pollen by spraying with weak alkaline solutions

Pollinosis is a clinically important allergic disease caused by airborne pollen from various plants.

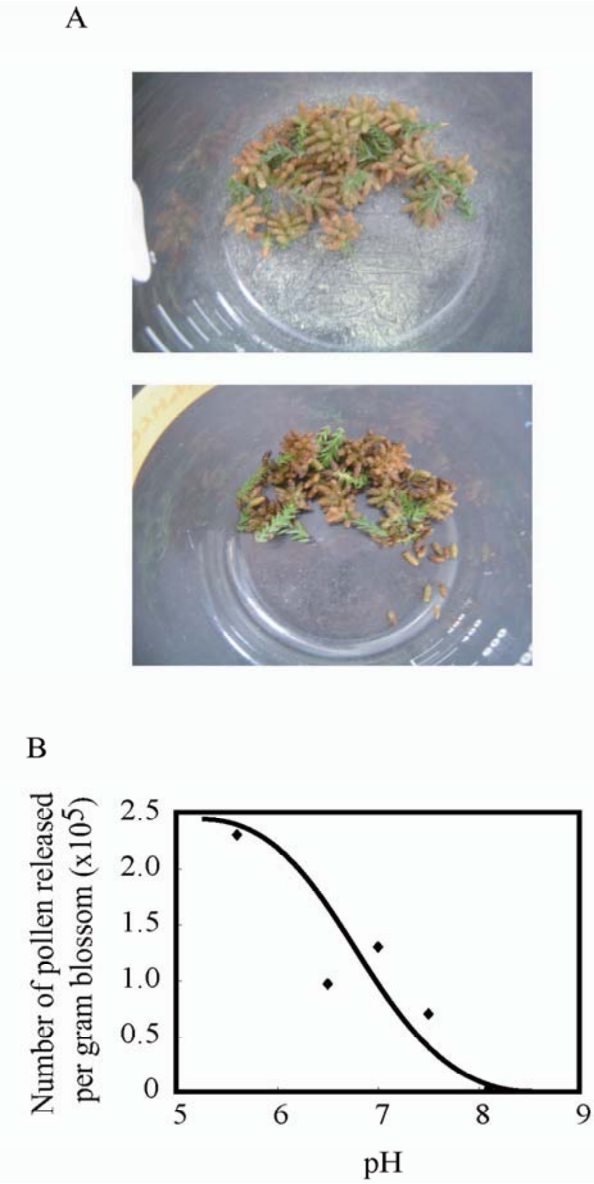

Figure 5. Suppression of pollen release from cedar blossoms by spraying with weak alkaline solutions. (A) Japanese cedar blossoms were sprayed with 10 mL of distilled water (*top*), or 1 M $NaHCO_3$ (*bottom*), and dried at 37˚C for 10 h in 1-L beakers. Release of yellow-colored pollen was observed in (*top*), but not in (*bottom*). (B) Japanese cedar blossoms were sprayed with 10 mL of 0.1 M sodium phosphate buffer with various pHs. After drying on a paper towel for 3 h, cedar blossoms were placed in a 50-mL plastic tube, and agitated vigorously with a vortex mixer. Released pollen grains were suspended in distilled water, and counted under a microscope.

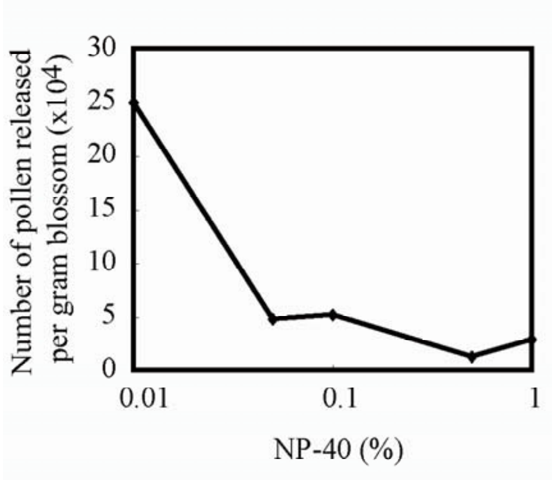

Figure 6. Influence of NP-40 on pollen release from cedar blossoms sprayed with a sodium bicarbonate solution. Japanese cedar blossoms were sprayed with 1 M $NaHCO_3$ containing various concentrations of NP-40. Blossoms were dried on paper towels at 25˚C for 3 h, and agitated vigorously in a 50-mL tube with a vortex mixer. Released pollen grains were suspended in distilled water and counted under a microscope.

The number of patients suffering from pollinosis is increasing, especially in industrialized cities. In Japan, cedar pollinosis was first reported in 1964, and Cryj1, a major allergen of cedar pollen, was discovered in 1983. Since then, there have been a number of attempts to overcome the disease, such as the development of medicines that inhibit inflammatory mediators released from activated mast cells (*28*), vaccination and desensitization against pollen allergens (*29-31*), and the generation of new races of cedar that release less pollen. Unfortunately, because these drugs and remedies often have unwanted side effects (*32,33*), and because planting new strains is time-consuming and costly, these attempts have not been successful. Therefore, the establishment of an inexpensive, simple method to suppress pollinosis is highly desirable. In this report, we suggest that 1) cedar pollen undergoes morphologic changes in weak alkaline solutions, and 2) spraying cedar blossoms with alkaline solutions induces morphologic changes of the pollen inside the anthers, resulting in a decreased amount of pollen released from the blossoms.

The transparent membrane covering the cytosolic component of pollen is made of pectin (*34,35*). As pectin contains acidic sugars, water molecules are easily trapped on the surface. Thus, cedar pollen that undergoes morphologic changes in weak alkaline solutions has increased adhesiveness. Furthermore, pollen lacking the external wall might be easily destroyed by environmental changes in temperature or humidity, and by physical force. Therefore, treatment with weak alkaline solutions is expected to be useful for suppressing the dispersal of cedar pollen. We propose that by reducing the amount of scattered pollen by spraying cedar forests with weak alkaline solutions, such as sodium bicarbonate solutions, the release of allergens might be decreased. Spraying a harmless alkaline solution, such as sodium bicarbonate, onto paved roads might also be effective to prevent the pollen from scattering back up into the air. Because cedar blossoms are covered with a waxy substance that repels water, detergents in the sprayed solution will facilitate the induction of morphologic changes of the pollen inside the anthers.

Mechanism of disruption of the external walls of cedar pollen in weak alkaline solutions

The external walls of cedar pollen function as barriers against physical force or environmental changes. The walls are extremely rigid, and only severe conditions such as acetolysis by strong acid can break the structure. In contrast, cedar pollen that enters the nasal cavity of humans seems to readily release allergen molecules. This process in the nasal cavity is considered to be triggered by the high pH of the mucosal surface. Moreover, as the changes in pollen morphology are inhibited by adding sucrose to the alkaline solutions, the process is suggested to involve water absorption into the wall structure due to osmotic pressure. Cellulose is a major constituent of the external walls of cedar pollen. *D*-glucose, a structural unit of the external walls, possesses hydroxyl groups at the C2, C3, and C6 positions, which are involved in hydrogen bonding with other oxygen molecules of cellulose fibrils. These hydrogen bonds might be weakened in alkaline solutions, as the hydroxyl groups of glucose undergo deprotonation. As a result, the loosened fibrils of the external walls might become permeable to water molecules. Further studies are required to understand the mechanism of the sharp pH-threshold for the morphologic changes of cedar pollen.

Morphologic changes of other types of pollen that cause pollinosis

Cypress is another anemophilous gymnosperm that causes pollinosis. The structure of cypress pollen is similar to that of cedar pollen, and it is covered by a cellulose wall. Cypress pollen undergoes the same morphologic alterations as cedar pollen in a weak alkaline solution, which suggests that spraying weak alkaline solutions onto other types of gymnosperms might help to minimize pollinosis in humans.

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