

Alternaria spreads by means of fungal spores called conidia (inset), assembled in long chains (above) that are easily dispersed through the air.

Alternaria, Allergies, and Asthma

Exploring a local fungus that can trigger attacks

by Susan McGinley

sthma sufferers have flocked to Arizona's deserts for more than 100 years to find relief from their symptoms. Yet the exploding number of asthma cases—it is now the most common chronic respiratory disease of children in the United States—include more cases in Arizona as well. In 2002, 31 million Americans (11 percent) were diagnosed with asthma; 8.9 million of these were under 18 years of age. Current asthma-related healthcare costs are estimated at \$14 billion annually. Coupled with 14.7 million missed school days and 11.8 million missed work days annually that are asthma-related, the total societal costs are enormous.

Why the increase in Arizona? There may be multiple factors, many of which are not yet known. However, one factor is simple population demographics. Not only are more people with asthma moving here, their children are also genetically predisposed to developing respiratory problems that include asthma. These demographics result in Arizona being cited as one of 15 states with the highest reported incidence of asthma. Scientists at The University of Arizona are studying local allergens to help doctors diagnose asthma triggers in the desert.

While irritants such as cigarette smoke can trigger attacks in almost anyone with asthma, allergens will cause attacks only in people who are actually allergic to them. Unfortunately, about 80 percent of all persons with asthma are sensitized to at least one allergen. In addition to controlling asthma symptoms, part of the treatment involves skin prick tests to determine the specific pollens, molds, mites, animal dander and other agents people are allergic to. In this procedure, the skin is pricked with a needle and then a small amount of specific allergen is applied. A raised bump indicates an allergic reaction.

Barry Pryor, a mycologist in the Division of Plant Pathology and Microbiology, part of the Department of Plant Sciences in the University of Arizona College of Agriculture and Life Sciences, is collaborating with allergy researchers in the UA College of Medicine Arizona Respiratory Center to determine sensitivity to desert strains of a widespread mold called *Alternaria*.

"The Arizona Respiratory Center has determined that sensitivity to *Alternaria* spores is the single factor that most significantly correlates with the onset of childhood asthma in the Tucson area," Pryor says. "After following childhood cases for over 20 years, this was one of the findings that really piqued their interest in *Alternaria*."

Alternaria is one of the most common fungi worldwide, found literally everywhere, both indoors and outdoors. It produces long chains of spores that are easily dispersed through the air. "There is really no way to get away from it — it can live almost anywhere," Pryor says. "It breaks down leaf litter and other organic matter and is an important part of the ecosystem." Yet it's also a notable plant pathogen that produces very toxic secondary metabolites in addition to human allergens. Some of these toxins may have a bioactive relationship to asthma that compound the effects of allergens.

In addition, *Alternaria* fungi are diverse with numerous strains unique to different environments worldwide. "*Alternaria* produce many different proteins that function as allergens, and the production varies among different strains," Pryor says. "You may be sensitive to certain proteins from one strain and not to different proteins of another strain. The skin prick test we've been using, which is standard for much of the medical industry, is prepared from one strain recovered from onion in Colorado."

Pryor hypothesized that skin prick tests using strains of *Alternaria* found in and around Tucson, Phoenix and other Arizona population centers would be a more accurate prediction of allergy and asthma incidence in Arizona. "Why would someone want to use skin prick tests not prepared from local fungal populations?" Pryor asks. "Wouldn't this provide better information to local asthma sufferers?"

To study this, Pryor obtained \$150,000 in funding from the Arizona Disease Control Research Commission. His particular emphasis has been on determining *Alternaria* sensitivity in children. He chose 25 different sites in and around Tucson, including undisturbed natural desert and urban areas, where he took samples of soil and leaf litter. In the laboratory he found *Alternaria* species in all samples, and then ran more tests to characterize their morphology (physical form), genetic diversity and immunological properties. Most of the isolates were found to be in or closely related to two *Alternaria* species, *A. alternata* and *A. tenuissima*.

The next step was to find out if local asthma sufferers were sensitive to these strains. In the laboratory he extracted proteins from each strain and mixed or hybridized them with blood sera from children who tested positive for sensitization to *Alternaria*. In this process, immunoglobins (IgE) in the children's blood sera react to specific proteins by binding them.

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"We run the proteins through a gel to separate them into bands, and then transfer the bands to a thin membrane using an electrical charge," Pryor says. "We then put the membrane in a tray, add the blood sera, and the IgE molecules bind and attach to the proteins that they are specific to. That's what immunoglobins do, their binding is very specific." After washing the membrane to get rid of all unattached IgE and blood sera, the researchers were able to get an allergen "fingerprint" for each strain and then group the strains based on allergen profiles.

The study population included four groups: 1) children who were asthmatic and had tested positive for *Alternaria* sensitivity; 2) asthmatic children who tested negative for *Alternaria* sensitivity; 3) non-asthmatic children who tested positive for *Alternaria* sensitivity; and 4) non-asthmatic children who tested negative for it. Pryor's study used blood sera collected from these children as part of routine allergy testing performed at the Arizona Respiratory Center.

"Arizona strains showed considerably different allergen profiles than the standard strain used by the industry," Pryor says, "which supports our hypothesis that these strains may be unique in their ability to affect asthma." In addition, *Alternaria* sensitive asthmatics had IgE specific to the same proteins as did Alternaria sensitive non-asthmatics with one exception.

"The blood sera from asthmatics had IgE specific to one additional *Alternaria* protein that the non-asthmatic sera did not react to," Pryor says. His lab is currently trying to isolate

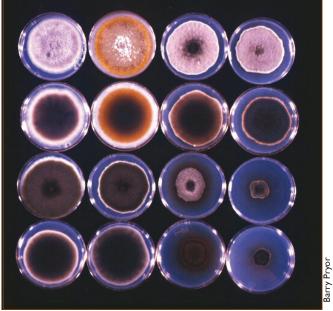
and characterize this unique protein. Testing of additional strains from the Tucson area will be ongoing for the next two years in an effort to uncover other unique allergens (proteins) for further analysis.

Depending on results, he will look at developing more representative allergen preparations specific for people living in southern Arizona. He notes that finding unique allergens in Tucson that are different from those used in the standard tests for Alternaria would open the door to commercial development of region-specific tests with a wider representation of *Alternaria* strains.

"We're looking at allergen profile diversity," he says, "and it does seem that there are some unique allergens produced by *Alternaria* recovered in Arizona compared to standard *Alternaria* isolates. The bottom line is to develop a skin prick test that is specific and more suitable to people living in the Southwest deserts, rather than commercially developed tests using extracts for allergens derived elsewhere."

Pryor's huge collection of *Alternaria* from the Tucson area, along with a large collection of blood sera at the Arizona Respiratory Center, provides a unique opportunity to learn more about how allergies work and how to control them.

"It's a great template for studying the mechanisms of allergy and asthma development," he says. "We can look at interactions between specific fungal strains and the exact people who have been sensitized to these strains. We can then characterize unique allergens in the laboratory and examine the corresponding chemical signaling in the immune responses to see if we can correct or change these responses during an attack. The collaboration is really exciting—a bridge between plant pathology and medical mycology."



Culture plates show diversity of Alternaria recovered from Tucson area.

CONTACT

Barry Pryor 520-626-5312 bmpryor@email.arizona.edu