

FLORAL GUARDIANS

These tiny, curious organs help keep plants from dying of thirst. Finding out how they do it is this lab's specialty.

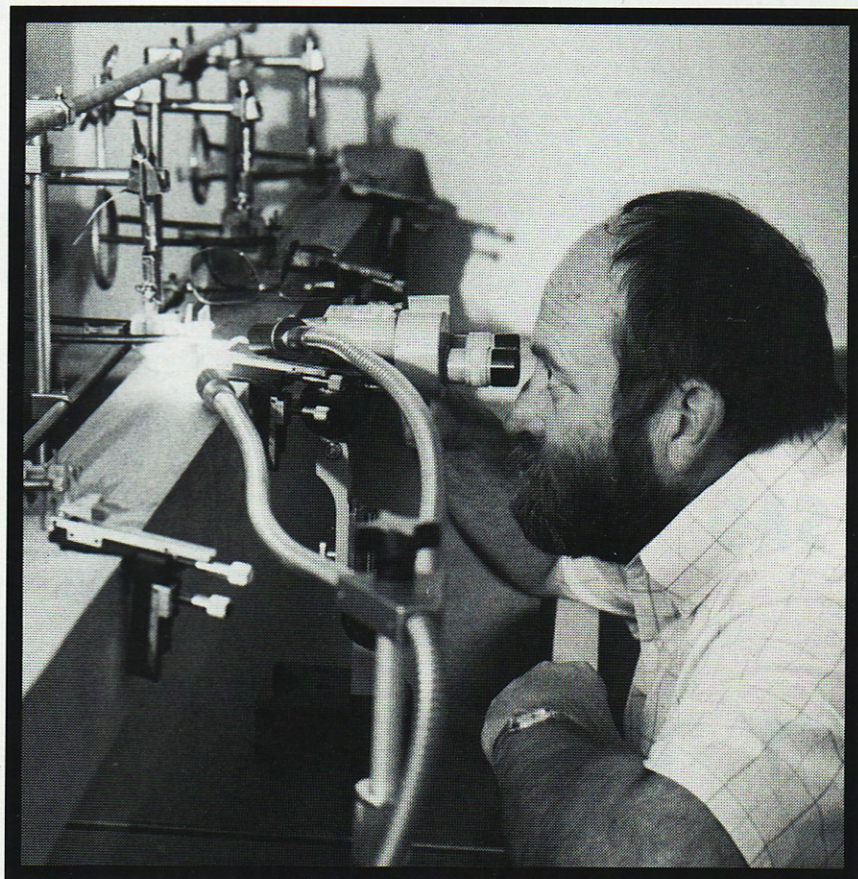
On an average day, Florida farmers douse their crops with upwards of three billion gallons of water, nearly half of it sucked from the ground. Such massive, costly irrigation schemes are replicated wherever agribusiness is found, from the sunny valleys of California to the windy plains of the Midwest.

The upshot? Florida's sweetwater wells are primed for invasion from the sea; California's San Joaquin Valley—the nation's richest farming region—has sunk more than 30 feet in the past 40 years; and the Ogallala Aquifer, the nation's largest underground water reservoir and the creator of the Midwest's vast corn and wheat belts, is slowly being pumped dry.

Experts say that the "Great American Water Crisis" is not a matter of if, but when. They prophesy that only the cleverest management and conservation techniques can prevent a waterborne catastrophe in this country. Water managers must look beyond conventional approaches to economizing water usage, they argue. Otherwise, the U.S. will almost certainly lose one of its last remaining trumps in foreign trade—vast, easily replenishable stores of food.

The challenge to find new ways to reduce agriculture's share of America's annual water consumption—now estimated at a whopping 70 percent—has found its way into scores of campus-based research programs throughout the country. One of these is at Florida State, in the labs of Dr. William Outlaw (Ph.D., Georgia). For the past 12 years, Outlaw has worked to understand the fundamental physical, chemical and mechanical processes that control the primary way plants breathe and give up moisture to the atmosphere.

Guard cells in *Difffenbachia*



DR. BILL OUTLAW

control a plant's need for water, says Outlaw. Should such a method ever be developed for crops, the savings in irrigation water—and in the energy used to supply it—could be substantial.

Outlaw chiefly studies the guard cells of the *vicia faba* plant, a bean grown commercially in China and in the Mediterranean. The plant's long history as a model for botanical studies provides Outlaw with extensive background information about its physiological characteristics.

The methods developed at FSU permit biochemists to study guard cells individually, a feat of considerable singularity. A typical guard cell is about a thousandth of an inch long and weighs only six nanograms—about one-billionth the weight of a nickel.

Using microtechniques developed elsewhere, but honed to unprecedented levels of precision at FSU, Outlaw is able to cut out individual guard cells from freeze-dried leaves, weigh them, and then extract and analyze their contents. The methods don't permit much carelessness—the entire content of a guard cell represents a volume of only one ten-millionth of a drop of water.

Scientists have never agreed on exactly what causes guard cells to swell or contract, the mechanism by which stomata open and close, Outlaw said. What's clear is that the plant manufactures a hormone called abscisic acid, which plays an integral role in the process. For example, when the acid is extracted and then sprayed on a leaf, the stomata instantly close. And when a leaf is deprived of water, the acid builds up in its guard cells, swelling them shut.

But aside from these rather perfunctory observations, no one knows much about the acid's role in guard cell function. Does it work only on the outside of the guard cells, or on the inside? Do the cells manufacture it on their own, or do they import it when needed?

A technique developed in Outlaw's lab permits the measurement of abscisic acid in guard cells to a level 100 times lower than any other research group has reached. What Outlaw has found so far has led him to suspect that the compound acts both externally and internally on guard cells.

He also believes that the acid may somehow be tied to the phenomenon of

stomatic memory, in which the pores "remember" times of stress, such as drought. "If you desiccate a plant, its stomata won't act normally for days. There's a memory there that says 'Let's be careful with water.'" "We think that perhaps during stress, this compound may be built up in the cell, perhaps from some internal physiological compartment."

Another technique Outlaw developed allows him to examine the enzyme activity of a single guard cell with uncanny sensitivity. Using this and other methods, Outlaw's lab is the first to provide conclusive evidence that photosynthesis occurs only to an insignificant extent in guard cells. The question had been a point of contention among biochemists for years.

Conventional thinking said that guard cells go about their photosynthetic chores just as other cells do—making sugars and starches, drawing in water and carbon dioxide as raw materials. This would naturally result in the cells' swelling.

But Outlaw's analysis showed that a key protein necessary for photosynthesis is practically nonexistent in guard cells. The find reinforced Outlaw's suspicions about the guard cell's special nature. "These cells are simply marching to an entirely different drummer from other cells. In every way, they seem to be different."

The basic mechanism by which guard cells operate—essentially an osmotic phenomenon involving potassium—has been understood in some detail only since 1984, Outlaw said. Scientists working in West Germany developed special techniques which led to the discovery of the biochemical pathway by which supplies of potassium get quickly shunted back and forth through the guard cell's membranes. The rapid changes in osmotic pressure, induced by the presence or absence of potassium, is responsible for the cell's familiar swelling and contracting.

But what triggers this process? How does a plant respond to drought, or sunlight, or any other environmental condition that may warrant the closing or opening of its stomata? Do guard cells act strictly on their own or are they sent messages? If so, from where?

Fundamental, even arcane questions certainly, but of great interest to science, says Outlaw. And in the face of this country's frightening water woes, it may in fact be impossible to underestimate the importance of answering such questions if scientists are ever to achieve some measure of biological control on agriculture's terrific—and costly thirst for water.

—Frank Stephenson

Outlaw is one of the world's leading authorities on *stomata*, the tiny openings found mostly on the underside of the leaves of all plants. Stomata (from the Greek word *stoma*, meaning "mouth") are literally a plant's nose and mouth combined. Depending on the species of plant, these microscopic organs may number as many as 100 per square millimeter of a leaf's surface.

It is through its stomata that a plant receives most of the carbon dioxide it needs to live on and to drive its complex photosynthetic machinery. The pores are regulated by pairs of sausage-shaped cells appropriately called guard cells.

Guard cells act as a plant's chief gatekeepers for controlling the critically balanced ebb and flow of carbon dioxide gas and water. It's a deceptively complex task. Every time the cells open to receive carbon dioxide, they inadvertently allow moisture to escape. The cells spend their entire lives performing an amazingly delicate balancing act, capturing just enough carbon dioxide to feed the photosynthetic furnace while keeping the plant from drying out.

Since the early 1800s, botanists have recognized stomata's role in a plant's uptake of carbon dioxide and in regulating water loss. But the process by which the pores function has never been completely understood.

Only during the past two decades have there been laboratory methods of sufficient precision developed to permit close-up looks at the heart of the process—the guard cells themselves. Outlaw and his doctoral students are the architects of several of the most refined and exacting methods for studying guard cells ever devised.

If scientists succeed in fully understanding how guard cells function, then someday it may be possible to precisely