An Agricultural Research Service scientist in Oxford, Mississippi, is working toward developing new herbicides by focusing on a molecular pathway that not only controls weeds in soybean fields, but might also have helped shape our nation’s history.

Franck Dayan, a plant physiologist with the ARS Natural Products Utilization Research Unit in Oxford, is an expert on a class of weed killers known as “PPO herbicides,” which choke off the weed’s ability to make chlorophyll. His efforts are increasingly important because weeds are beginning to develop resistance to glyphosate, the world’s most widely used herbicide, and alternatives are needed.

Much of Dayan’s work focuses on a class of ring-shaped pigment molecules known as porphyrins (pronounced POR-fer-ins) that “bind” or react with different metals and perform vital functions in both plants and animals. Chlorophyll is a porphyrin that binds magnesium, giving plants their green pigment and playing a pivotal role in photosynthesis. Heme is a porphyrin that binds iron as an essential step in supplying oxygen to animal blood cells.

One of the key steps in porphyrin synthesis is performed by an enzyme (protoporphyrinogen oxidase, or PPO), and its disruption can cause problems in plants and animals. In humans, disruption of the PPO enzyme is associated with a congenital disease known as “porphyria,” with symptoms that may include light sensitivity, seizures, and neuropsychiatric problems. Scholars have argued that a case of porphyria in King George III may have contributed to the colonies’ struggle for independence. (See sidebar.)

In plants, PPO herbicides work by disrupting the enzyme’s production of porphyrins, causing harm to the plant. PPO herbicides have been around for almost 40 years and are specifically designed so that they only disrupt PPO enzyme activity in plants and not in humans. “With these herbicides, we are able to intentionally and specifically disrupt plant PPO enzyme activity and do it in a way that cannot possibly have any effect on enzyme activity in humans,” Dayan says.

At the ARS Natural Products Utilization Research Unit in Oxford, Mississippi, support scientist Susan Watson extracts a sample of pigments from leaf tissue for high-performance liquid chromatography analysis by plant physiologist Franck Dayan.

Franck Dayan observes wild-type and herbicide-resistant biotypes of pigweed (Palmer Amaranth) as Mississippi State University graduate student Daniela Ribeiro collects plant samples for DNA analysis.
Dayan recently published a report on the molecular mechanism that can trigger resistance to PPO herbicides in a common weed. Understanding the resistance mechanism should lead to better herbicides.

**Working in the Weeds**

Since the mid-1990s, glyphosate use in crop fields has been so successful that interest in research and development of alternative weed killers had been on the wane. Many experts considered it too difficult to come up with an herbicide that could match glyphosate for cost and effectiveness, Dayan says. But with weeds developing resistance to glyphosate, interest in PPO herbicides is picking up. Herbicides have also become essential tools in modern agriculture, increasing the ability to control weeds to a point where growers are better able to adopt environmentally friendly practices, such as no-till cropping systems.

“Glyphosate still plays a dominant role in weed control in soybeans and other crops, but with glyphosate resistance, there is renewed interest in herbicides that inhibit the PPO enzyme,” Dayan says.

Scientists recently showed that waterhemp (*Amaranthus tuberculatus*), a common weed, developed resistance to PPO herbicides by deleting an amino acid known as “glycine 210” from the PPO enzyme. Such an evolutionary mechanism is unusual. Enzymes and proteins are made up of amino acids, but when a plant develops resistance to a weed killer, it is usually because one amino acid in an enzyme is substituted for another—not deleted. “This was the first time that resistance caused by a deletion was ever seen,” Dayan says.

Dayan examined the consequences of this amino acid deletion on the PPO enzyme by conducting protein-modeling studies of waterhemp. “The question was, How did the deletion of this amino acid allow the plant to become resistant?” says Dayan.

To find the answer, he and his colleagues overlaid the genetic sequence of the enzyme in the resistant waterhemp plants on the genetic sequence of a related enzyme that has a known structure, in this case, the PPO enzyme from tobacco plants. They also compared the molecular structure of enzymes from PPO-susceptible waterhemp to the structure of enzymes from resistant waterhemp. Using that information, they developed a computer-generated, three-dimensional version of the enzyme in the resistant plant.

The work, published in the journal *Biochimica et Biophysica Acta*, confirmed that an evolutionary change in a single enzyme—the deletion of an amino acid—caused structural changes in the enzyme-binding site and allowed waterhemp to become resistant to the herbicide. While the structural changes were too insignificant to affect most of the plant’s physiological functions, they did disrupt the PPO enzyme production of porphyrins and caused the enzyme-binding site to become enlarged so that the herbicide did not bind as well.

“The place where the herbicide binds on the enzyme is a key,” Dayan says. Knowing the shape of the binding site will help scientists design herbicides with a different shape that would bind more effectively.

Understanding porphyrins has a practical benefit because of their role in the development of herbicides. But the ubiquitous presence of these ring-shaped molecules, Dayan says, serves as an example of the unified nature of life on Earth. In an article coauthored with his daughter, Emilie Dayan, and published in the May-June 2011 issue of *American Scientist*, he writes, “They attract little attention, but you find them throughout the plant and the animal kingdom, and life couldn’t exist without them.”—By Dennis O’Brien, ARS.

**King George’s Porphyrin Problem**

Disruption of the PPO enzyme in humans is rare but is known to cause porphyria, a group of congenital diseases that, in one form, known as “variegate porphyria,” can cause symptoms that include temporary paralysis of limbs, sensitivity to light, seizures, hallucinations, and other neuropsychiatric problems. Symptoms can appear intermittently throughout someone’s life.

Agricultural Research Service plant physiologist Franck Dayan notes in *American Scientist* that porphyrins form pathways that “serve as the assembly line for the most abundant pigments in nature.” Because pigments are involved, people with porphyria may also excrete purplish tint in the urine and feces.

Dayan recounts how several experts have found historical evidence that King George III, monarch of England from 1760 until his death in 1820, had the disease, periodically suffering from abdominal pains, paralysis of the arms and legs, wine-colored urine, and psychiatric problems that eventually forced him into confinement. Some experts have argued that the American Revolution may be partially attributed to the king’s illness because it contributed to his stubbornness in dealing with the colonies.

The king’s illness was portrayed in the 1994 film, “The Madness of King George.”

This research supports the USDA priority of promoting international food security and is part of Crop Protection and Quarantine (#304), an ARS national program described at www.nps.ars.usda.gov.

Franck Dayan is in the USDA-ARS Natural Products Utilization Research Unit, Room 2012, University of Mississippi, Oxford, MS 38677; (662) 915-1039, franck.dayan@ars.usda.gov.©.