National Historic Chemical Landmarks
Chemists and Chemistry that Transformed Our Lives

Isolation of Phytochrome

U.S. Department of Agriculture, Agricultural Research Service,
Beltsville Agricultural Research Center

American Chemical Society

One of the most characteristic features of plant growth is the tendency shown by various species to flower and fruit only at certain periods of the year. In midwinter, the brilliant color of poinsettias are reminders of the season; in spring we expect to see the unfolded blossoms of dogwood; as summer approaches, poppy, rhododendron and iris begin flowering; in the autumn, chrysanthemum herald the approaching end of the growing season.

The thought at once suggests itself that the underlying cause of flowering at a particular season must be purely internal, else the vagaries of the weather and other conditions would seriously upset the cycle. It has long been known, also, that light is indispensable.

—Adapted from W. W. Garner and H. A. Allard, Yearbook of the Department of Agriculture, 1920

For thousands of years, humankind has recognized that plants follow predictable cycles of development through the seasons. The regular phases of seed germination, stem and leaf growth, and flowering repeat with little variation in a given species year after year. How is this possible?

"Of the many intricate and beautiful control mechanisms living organisms have evolved to optimize their survival in a variable and changing environment, none is more elegant than the phytochrome system of plants."

—Warren L. Butler

The explanation came from one of the 20th century's great discoveries in plant science: detection of the elusive pigment phytochrome, whose action determines how plants are able to regulate their growth and development processes by detecting light and darkness. This finding resulted from a more than 40-year effort by researchers at the U.S. Department of Agriculture.

DISCOVERY OF PHOTOPERIODISM

In 1918, scientists at the USDA were puzzled by a pair of questions facing crop growers: Why did Maryland Mammoth tobacco plants, a desirable commercial variety, fail to produce flowers at the end of summer like other tobacco? And why did soybean plants mature at the same time, despite staggered planting by farmers wishing to prolong their harvest?

Harry A. Allard (1880–1963), a botanist, and Wightman W. Garner (1875–1955), a chemist and plant physiologist, investigated these problems. Aware that a particular plant could exhibit differences in development when grown outside its natural habitat, the two reasoned that an environmental factor must trigger growth behaviors. They explored soil moisture, temperature and other possible explanations before settling on light as the most likely cause.

The scientists tested the impact of day length on tobacco and soybeans. A control group of plants was placed outdoors and received natural light—close to 14 hours at that time of year—while a test group was placed outdoors for seven hours and kept in darkness for the remainder of the day. They found that the tobacco grown under test conditions matured sooner, flowering and developing seeds, while the control plants continued to make only leaves. Likewise, the soybean test group developed seed pods more quickly than the control group.

Allard and Garner concluded that "of the various factors of the environment which affect plant life, the length of the day is unique in its action on sexual reproduction." They announced their findings in the *Journal of Agricultural Research* in 1920, calling the phenomenon photoperiodism in reference to behavior that is impacted by the relative length of day and night. They also proposed a new classification system for plants based on photoperiodic traits. Plants like lettuce and poppies, which flower when day length exceeds about 12 hours, were called long-day plants; those such as chrysanthemums and poinsettias, which flower when day length is less than 12 hours, were called short-day plants.

PHOTOPERIODISM IN ACTION

Starting in 1936, the USDA launched a project to look more closely at the nature of photoperiodism, led by botanist Harry A. Borthwick (1898–1974). Borthwick tested Allard and Garner's assumption that a critical period of light was responsible for flowering and found that, actually, the duration of darkness was the controlling factor. In long-day plants, a brief period of light during the dark period encouraged flowering, while in short-day plants, a brief period of light during the dark period inhibited flowering.

Borthwick and his colleague Sterling B. Hendricks (1902–1981), a chemist, devised an experiment to gauge plants' reactions to different wavelengths of light. Using a prism to cast different colors on an array of plants, they found that red light was a strong inhibitor to flowering. Similar findings were seen in several plant types, both short- and long-day, and the scientists reasoned that the same mechanism was found across species.

In a another lab, botanists Eben H. (1889–1967) and Vivian K. Toole (1906–2003) found the same relationship between seed germination and light. The Tooles shared previous research which showed that red light of a specific wavelength, 670 nanometers (nm), produced optimal germination. Light in the far-red region, at the edge of the visible spectrum near 700nm, was the strongest inhibitor to germination. Moreover, the reaction was reversible—red and far-red light could be flashed alternately 100 times or more, but only the final flash determined a plant's response. The exchange gave Borthwick and Hen-

Harry Borthwick (left) and Sterling Hendricks separate light into its various wavelengths to study the effect of each on plant development.

dricks important clues and a better test to work with, because germination tests could be conducted more quickly than tests on mature plants.

SEARCH FOR PHYTOCHROME

As the various scientists collected information about the relationship between light and plant development, interest grew in identifying the chemical that produced the photoperiodic response within plants. Until this point the researchers were studying physiological processes, not chemical reactions. The question of how light triggers a response in plants on a molecular level remained.

By this time, enough was known about the phenomenon to hypothesize traits of the still-undiscovered substance. Because it responded to visible light, it had to be a pigment. Hendricks proposed that a single pigment was responsible, and that it was photo-reversible, meaning that it could change between one form responding to red light and another responding to far-red. Because it responded to these wavelengths, the pigment was likely to be in the green-blue range, the complementary colors to red. Further observations caused Hendricks to reason that the substance would be intensely colored, yet present in minute concentrations. Because of its scarcity, the pigment must act like a catalyst—a chemical that encourages other reactions. In a biochemical context, that meant that it was probably an enzyme, most likely a protein.

Hendricks and Borthwick worked with instrumentation experts Warren L. Butler (1925–1984) and Karl H. Norris (*1921) and agricultural biochemist Harold W. Siegelman (1920–1992) to identify the pigment. Butler and Norris designed a highly sensitive tool called a spectrometer that could detect weak light absorption by such a pigment. Numerous plants were tested, each test looking for the expected absorption in the red and far-red regions.

Finally, in 1959, tests on turnip seedlings showed the expected spectral reversibility. Siegelman then ground the turnip tissue and boiled it, after which it no longer reacted. This confirmed Hendricks's prediction that it was a protein. Siegelman was soon able to isolate the protein, which was indeed blue-green in color.

Others had doubted the existence of such a pigment, whose properties were unlike any other known at the time. One critic notably dubbed it a "pigment of the imagination." However, the detection of a photoreversible pigment that controlled development of plants by detecting light and darkness appeared in the *Proceedings of the National Academy of Sciences* that December. It was later named phytochrome for the Greek words for plant and color.

RESEARCH IMPACT

The discovery of photoperiodism led to important changes in the production of commercial plants, even before phytochrome was identified. Categorizing crops by their photoperiods—either short-day, long-day or day-neutral—became standard practice, and it enabled crops to be chosen for the suitability of their response to the light of a specific area. For example, soybeans are now available in a variety of maturity groups, each suited to a particular latitude, ranging from Canada to the southern U.S. and throughout the world.

Growers capitalized on the discovery of photoperiodism in the 1920s and developed methods to produce plants during seasons in which they don't grow naturally. Many popular seasonal flowers such as chrysanthemums and poinsettias could be made available throughout the year by manipulating their exposure to light and darkness.

Further exploration of phytochrome responses may provide scientists with the ability to create new breeds of plants that borrow their relationship to light from one another. For instance, phytochrome enables plants to detect if they are growing in shade. This can result in several responses, some of which are advantageous in crop plants and some of which are not. Modification of the phytochrome response, either through conventional breeding or genetic engineering, could produce plants that respond favorably, thereby improving crop yields.

Isolation of Phytochrome
A National Historic Chemical Landmark

The American Chemical Society designated the isolation of phytochrome by the U.S. Department of Agriculture, Agricultural Research Service, as a National Historic Chemical Landmark in a ceremony at the Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, on October 21, 2015. The commemorative plaque reads

> *In 1959, researchers at the USDA Beltsville Agricultural Research Center first isolated phytochrome, a light-sensitive pigment found throughout plant species. Phytochrome allows plants to regulate many growth and development processes by detecting light and darkness. For example, some flowers bloom based on changes to day length over the course of their growing season in a phenomenon known as photoperiodism. Understanding the role of phytochrome in plant development allows scientists to produce crops for seasons and latitudes not previously possible, both by manipulating the environment through lighting controls and by breeding plants with desirable photoperiodic traits.*

About the National Historic Chemical Landmarks Program

The American Chemical Society established the National Historic Chemical Landmarks program in 1992 to enhance public appreciation for the contributions of the chemical sciences to modern life in the United States and to encourage a sense of pride in their practitioners. The program recognizes seminal achievements in the chemical sciences, records their histories, and provides information and resources about Landmark achievements. Prospective subjects are nominated by ACS local sections, divisions or committees; reviewed by the ACS National Historic Chemical Landmarks Subcommittee; and approved by the ACS Board Committee on Public Affairs and Public Relations.

The American Chemical Society is a nonprofit organization chartered by the U.S. Congress. With more than 158,000 members, ACS is the world's largest scientific society and a global leader in providing access to chemistry-related research through its multiple databases, peer-reviewed journals, and scientific conferences. Its main offices are in Washington, D.C., and Columbus, Ohio.

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