Phytochemistry 69 (2008) 1454–1456

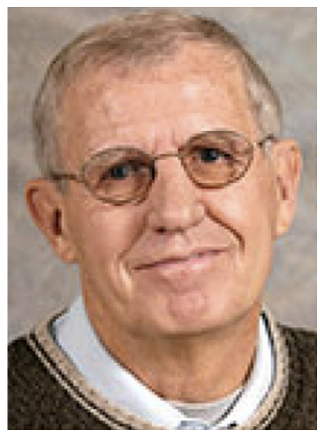
Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Obituary

Clarence A. "Bud" Ryan



Clarence A. "Bud" Ryan, Charlotte Y. Martin Professor of Biochemistry and a Fellow of the Institute of Biological Chemistry, Washington State University, died on October 7, 2007, at the age of 76. Bud was widely known for his work on the regulation and function of plant proteinase inhibitors in defense against insect herbivores. In addition to being an excellent scientist, Bud was a cherished mentor to dozens of students and postdocs, and he was dedicated to promoting plant biology at the university, national, and international levels.

Bud was born on September 29, 1931, in Butte, Montana, the second of four children. When Bud was 10 years old, the family moved to Helena, Montana. Bud became interested in chemistry at Cathedral High School and, after graduation, decided to enroll at Carroll College in Helena. He paid his way through college by cleaning a bar and working as a ticket taker at the local movie theater. As a starter for the conference-winning basketball team for two years, Bud would maintain his passion for the game—both as a participant and a spectator—for the rest of his life. Bud received his Bachelor's degree in chemistry with a minor in bacteriology from Carroll College in June, 1953.

After college, Bud worked for the Montana State Highway Laboratory and as a cabbie at night to pay off his college loans. During this time, he met Patricia Meunier, the love of his life, at a local barn dance. Bud and Pat were married the following May, in 1954.

Bud entered the graduate program at Montana State University in 1954. He conducted his thesis research, entitled "A New Transglucosidase Found in Potatoes", in the laboratory of Dr. Kenneth Goering, and in June 1959 he obtained his Ph.D. degree in Chemistry. After graduation, Bud received several job offers to work in industry. With a young family (Bud and Pat's children, Jamie, Steven, and Janice, were born while Bud was in graduate school), a career in industry seemed to be the best route for the Ryan family.

But by this time Bud had been bitten by the research bug. Relying on intuition, as he did so often during his life, Bud decided to pursue an opportunity to do postdoctoral work. He first studied with Dr. T.E. King at Oregon State University and then at the USDA Western Regional Laboratory in Albany, California, in the laboratory of enzymologist Dr. A.K. Balls. It was in Albany where Bud came across a research article describing how potato peels inhibit cholinesterase activity. Bud wondered what the plant chemical might be and whether it would inhibit the esterase activity of trypsin and chymotrypsin. Always quick to improvise, Bud ran down to the grocery store and purchased a bag of potatoes and, within six months, had crystallized the first chymotrypsin inhibitor from plants. Based on this discovery, Bud received a Career Development Award from the NIH to further study the properties of the inhibitor and to purify other inhibitors. The course for Bud's long and productive career in plant biology was set.

Bud and his family moved in 1964 to Pullman, Washington, where he accepted an assistant professor position in the Department of Agricultural Chemistry at Washington State University (WSU). In 1966, a fourth child, Joseph Patrick (Joe Pat) Ryan, was born. Bud would spend the rest of his career at WSU. His decision to remain at WSU was influenced by the stimulating research environment in the Department of Agricultural Chemistry which, in 1980, was changed to the Institute of Biological Chemistry (IBC). Throughout his career, Bud played a prominent role in shaping the Institute into a world-renowned research facility.

Bud's early success in characterizing the biochemical properties of proteinase inhibitors (PIs) led him to investigate factors that control their accumulation in plants. He discovered, for example, that chymotrypsin inhibitor I accumulates transiently in potato leaves as a temporary storage protein, before protein resources are allocated to the developing tubers. Extending his studies on

doi:10.1016/j.phytochem.2008.02.023

PIs to other plant species, Bud observed the occasional and somewhat unpredictable accumulation of PI-I in tomato leaves, and suspected that the expression of the inhibitor was influenced by environmental conditions. Upon investigating this phenomenon more closely, Bud and Terry Green (a postdoc in the lab) found that tissue damage inflicted by Colorado potato beetles resulted in massive accumulation of PIs in potato and tomato leaves. This landmark discovery, which was published in *Science* in 1972, suggested that wound-inducible PI expression makes the host plant less nutritional and perhaps lethal to invading insects. The paradigm that plants, rather than being passive victims of insect assault, respond dynamically to herbivory through the production of defensive compounds pervades much of the molecular plant-insect interaction research to this day.

The 1972 Green and Ryan *Science* paper also reported that wounding of a single leaf causes PI expression in undamaged aerial tissues of the plant. This discovery implied that plants possess an intercellular communication system in which signals generated at the wound site are propagated to distal leaves to warn of impending danger. Wound-induced systemic expression of PIs has been adopted by many laboratories as a model system for studying long-distance signaling in plants.

Tragically, in 1976, Bud and Pat's oldest son, Steven, died in a scuba diving class accident. Later the same year, Joe Pat was killed when the car he was riding in was struck by a drunk driver. Somehow, Bud and Pat persevered through these terrible events.

By the late 1980s, Bud was focusing his research on understanding the signal transduction pathway leading to PI gene expression in wounded tomato leaves. But where should one start in such a project? Brady Vick and Don Zimmerman, researchers at the USDA Agricultural Research Service in Fargo, ND, had recently elucidated the biosynthetic route of an interesting linolenic acid derivative called jasmonic acid (JA). At that time, JA's function was unknown, but experiments being conducted in Japan, Germany, and the US suggested that it had various biological activities, including stimulation of the accumulation of "jasmonate-inducible proteins" of unknown function.

Fortunately, Bud had a nearly photographic memory of previous experiments conducted in the lab, and recalled an early unpublished experiment by Mary Kay Walker–Simmons showing that linolenic acid treatment could stimulate PI production. Maybe linolenic acid was metabolized to JA to induce PIs? The first sample of JA to come into Bud's lab was found on the WSU campus. The compound was sprayed onto tomato plants and, the following day, the result was spectacular: MeJA had captured the laboratory record for a compound that induces the highest level of PI accumulation. It thus became clear that JA is an important regulator of plant defense responses. These early experiments performed in Bud's lab played an important role in transforming JA from a relatively obscure phytochemical to a full-fledged member of the plant hormone family.

Interestingly, the inducing effect of MeJA appeared to spread from the sprayed plant to nearby control plants, presumably as a result of MeJA's volatility. This observation immediately raised the exciting possibility that plants able to produce MeJA might be able to stimulate *PI* gene expression in neighboring tomato plants. Indeed, this prediction was confirmed in a now classic Ryan lab experiment in which MeJA emitted from sagebrush (*Artemisia tridentata*) induced PI expression in nearby tomato plants. Although this experiment was conducted in an artificially closed system, the unequivocal demonstration of interplant communication sparked intense excitement within the community of plant biologists and ecologists. The role of plant volatiles in mediating plant-to-plant communication under natural conditions remains a highly active research area to this day.

Well before the discovery of JA and MeJA as endogenous regulators of PI expression, Bud's lab had been engaged in a long-term study to identify PI-inducing factors from tomato leaves. This effort relied on a simple bioassay in which fractionated leaf extracts were tested for PI-inducing activity in a immunodiffusion assay. In 1991, after tens of thousands of such bioassays, this work paid off with the discovery of systemin, which was the first peptide signal identified in plants. Bud's group would go on to publish a series of influential papers describing systemin's role in the wound response of tomato plants.

In 1999, at the age of 68, Bud "retired" from university administrative and teaching duties, only to take a more active role in his research on plant peptide signaling. During this period, Bud and his colleagues would discover several new classes of bioactive peptides, including hydroxyproline-rich glycopeptides (HypSys), rapid alkalization factors (RALFs) that regulate root development, and a small polypeptide (Pep1) from Arabidopsis involved in the control of defense responses against pathogen attack. As was the case for tomato systemin, these novel peptides are derived from proteolytic processing of larger precursor proteins. These pioneering contributions to our understanding of peptide signaling in higher plants show that Bud remained fully engaged at the cutting-edge of plant biology research, even after "retirement".

Bud received numerous honors for his outstanding contributions to science, including election to the US National Academy of Sciences (1987) for his early work on proteinase inhibitors and plant-herbivore interactions. He was the first faculty member from WSU elected to the Academy. Bud received the Stephen Hales Prize from the American Society of Plant Physiologists (1992), and was a member of both the academic and athletic halls of fame at Carroll College. In recognition of his outstanding and long-term contributions to plant biology and service to the Society, Bud was named a member of the inaugural class of ASPB Fellows in 2007.

To many of us who were fortunate enough to work with Bud, he will always be remembered as a wonderful mentor and colleague, whose enthusiasm for science was contagious. With modesty and a steady stream of humor, Bud created a stimulating research environment in which students and postdocs could thrive. As the jasmonate and systemin stories were unfolding in the late 1980s and through the 1990s, the work often carried on at a fast pace and with a sense of excitement and camaraderie. The "hot" results of the day were typically discussed around the coffee pot, and new experiments were planned. The atmosphere that Bud created made his laboratory an exceptional place in which to conduct research.

Bud was in good health at the time of his death. He was an avid golfer and fisherman. Until the last two years, he was still playing basketball with the noon group at the gym. When he finally retired from basketball, he took up a new sport, ice skating. Proving it is never too late to learn something new, Bud and Pat took skating lessons and hit the ice before work twice a week.

Bud Ryan is survived by his loving wife Pat, his two daughters, Jamie Ryan and Janice Thrall (Terry) and two special granddaughters, Kymberly and Haleigh Thrall. The family suggests that contributions be directed to the Steve & Joe Pat Ryan Memorial Fund, Carroll College, 1601 N. Benton Ave., Helena MT 59625-0002.

Edward E. Ted Farmer
Plant Molecular Biology, University of Lausanne, Lausanne CH-1015, Switzerland
E-mail address: Edward.Farmer@unil.ch

Gregg A. Howe
Plant Biology, Michigan State University, East Lansing, MI 48824-1312, USA
E-mail address: howeg@msu.edu

Greg Pearce
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA
E-mail address: pearce@wsu.edu

Andreas Schaller
Institut für Physiologie und Biotechnologie der Pflanzen, Universität Hohenheim, D-70593 Stuttgart, Germany
E-mail address: schaller@uni-hohenheim.de

Available online 18 March 2008