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## Message from Li Tian, PSNA President

Dear PSNA colleagues,

Starting the New Year off with heavy winter storms has given me a greater appreciation for the remarkable resilience of plants supported by the various phytochemicals they produce. Now, the arrival of spring brings both the blossoming of plants (showcasing the colorful side of phytochemicals), and the blossoming of many of our young members who are graduating after delving into the fascinating world of phytochemistry.

Congratulations to all and I wish you the very best as you embark on the next chapter of your professional journey. Along this line, please take a moment to check out the recent publications by our young members highlighted in this newsletter. These public-



ations truly demonstrate their talent and dedication. While we celebrate the success of our voung members, my congratulations also go out to Dr. Mark Berhow for the welldeserved recognition of a PSNA Lifetime Membership Award. Dr. Berhow, your contributions to our society have been numerous and invaluable, and your passion and commitment have inspired us all.

# Phytochemical Society of North America

Phytochemical Society of The (PSNA) America North is a nonprofit scientific organization whose membership is open to with anyone an interest in phytochemistry and the role of plant substances in related fields. Annual membership dues are U.S. \$100 for regular members and \$30 for student members. Annual meetings featuring symposium topics of current interest and contributed papers by conference participants are held throughout the United States. Canada, Mexico. **PSNA** and

### President's message continued...

Over the past few months, I'm pleased to have had the opportunity to connect with some of you during my PSNA President's Office Hours. It has been wonderful getting to know you, learning about your work, and exploring how our society can support you. I look forward to meeting more of you virtually during my Office Hours in May and June, and in-person at our annual conference at Michigan State University (MSU). Our local organizing committee, led by Dr. Bjoern Hamberger, has been working tirelessly to bring together a fantastic lineup of keynote speakers. There will also be plenty of opportunities for contributed talks and poster presentations. Furthermore, the Young Members' Committee has planned several interesting and informative workshops. To learn more about what's in store for the MSU meeting in July, I encourage you to check out the program details in this newsletter. Don't miss out on this exciting event!

The annual conference serves as a crucial platform for our members to connect, but there are other ways in which we can foster collaboration. For example, as we share a common interest in phytochemistry, we could explore opportunities for joint research through visits to each other's labs and by working on grant proposals, manuscripts, and conference organization together. Additionally, we can collaborate in teaching and learning through approaches such as Collaborative Online International Learning (COIL), which enables us to overcome geographical barriers and work together on learning projects. I recently had the opportunity to develop a COIL module that centers around the United Sustainable Nations Development Goals (UNSDGs) and focuses on plant natural and traditional products medicine, which was supported by a three-way collaboration Tecnológico among de Monterrey, Shanghai Jiao Tong University, and UC Davis. I would be happy to share my COIL experience with you.

As my term of President is drawing to a close, I would like to extend a warm welcome to Dr. Dhirendra Kumar, who will serve as the President of the PSNA for 2023-2024. Dr. Kumar has been a dedicated supporter of our society for many years, having previously served as the Treasurer for six years and played a crucial role in ensuring the financial health of our society. Currently, Dr. Kumar is a Professor and Chair of the Department of Biological Sciences at East Tennessee State University. His leadership skills will be invaluable as he quides our society into the coming year and beyond. Thank you for your continued support for our society. Have an enjoyable and productive spring season!

Sincerely,

Li Tian PSNA President (2022-2023)

meetings provide participants with exposure to the cuttingedge research of prominent international scientists, but are small enough to offer still informality and intimacy that are conducive to the exchange ideas. This newsletter is of circulated to members to keep them informed of upcoming developments meetings and within the society, and to provide forum for the а exchange of information and you like ideas. lf would additional information about the PSNA, or if you have material that you would like included in the newsletter, please contact the PSNA Secretary or visit our website www.psnaat online.org. Also check the PSNA website for regular updates.

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The PSNA is an all-volunteer organization which depends on its membership to run the organization. We appreciate the time and effort these volunteers are putting in to keep the organization up and running. As a member, please consider volunteering to serve on one of these committees. The PSNA can always use more help!



### PSNA EXECUTIVES 2022-2023

#### President

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Mark Berhow (member, USDA ARS NCAUR)

Mark Lange (member, Washington State University)

Nikola Kovinich (member, York University)

# **PSNA ELECTION 2023**

Leading us into the future...



# DEAR PSNA Members,

We are pleased to announce that the nomination process for the PSNA President position for 2024-2025 has been completed, and we have a candidate who has expressed her willingness to serve. We thank all those who participated in the nomination process. To ensure a fair and transparent nomination process, we followed a procedure that included both self-nominations and nominations by others through a survey sent out to all active PSNA members. The Advisory Council carefully reviewed all the nominations, and after reaching out to the candidates for their willingness to serve, we are excited to announce that Dr. Sangeeta Dhaubhadel has been nominated as the sole candidate for the position of PSNA President for 2024-2025. Dr. Dhaubhadel is currently a Research Scientist in the Science and Technology Branch (London) of Agriculture and Agri-Food Canada and has served as the Secretary of PSNA since 2021. Dr. Dhaubhadel will be running against any potential write-in PSNA member candidates that are proposed by the electorate. The election will commence on May 26 and will be open to June 11. All active members will receive an invitation to vote. We encourage all society members to participate in the election process and help decide the PSNA President for 2024-2025.

#### Dr. Sangeeta Dhaubhadel (sangeeta.dhaubhadel@agr.gc.ca)

Dr. Sangeeta Dhaubhadel is a Research Scientist at Agriculture and Agri-Food Canada and an Adjunct Professor in the Department of Biology, Western University, Canada. She received her early education in Nepal and her undergraduate and master's degrees in India. She conducted her Ph.D. research (1996-2001) in the Department of Plant Sciences (Biology), Western University, where she discovered the first direct evidence on the mechanism of brassinosteroidinduced thermotolerance in plants. In 2001, Dr.



Dhaubhadel completed a short postdoctoral fellowship at Agriculture and Agri-Food Canada-London, where she studied the routes of isoflavonoid accumulation in soybean seeds and demonstrated that the total accumulation of isoflavonoids in soybean seeds is a result of both in vivo synthesis within the seed and transport from maternal tissues. Since 2002, she has continued her work as a principal investigator at Agriculture and Agri-Food Canada-London. Her research interest lies in the area of expression and regulation of genes involved in the synthesis of plant natural compounds. The current research in her laboratory focuses on identifying and characterizing the factors involved in the biosynthesis of phenylpropanoids with a long-term goal to develop strategies and technologies to accelerate the selection of genotypes with disease resistance and value-added quality traits in pulses and oil seed crops. She serves as a board member of Plant Canada, an umbrella organization of Canadian Plant Science Societies.

Dr. Dhaubhadel received the Arthur C. Neish Young Investigator Award of the PSNA in 2009. She has been a member of the society since then, and has presented her research at several PSNA meetings. She was vice-chair of the 'Future Meeting Committee' of PSNA for the 2018-2021 period. Currently, she serves as Secretary of the society (2021-2024). Dhaubhadel states, "I will continue to engage young members' participation in the society and promote diversity and equity in PSNA. One of the main goals of my role will be to help PSNA membership grow across North America".

# 62ND ANNUAL MEETING OF THE Phytochemical society of North America



The 62nd annual meeting of the Phytochemical Society of North America (PSNA) will be held in the heart of Michigan, East Lansing at the Kellogg Conference Center on July 16-20, 2023. This long-standing conference brings together researchers with interest in the chemistry and biochemistry of plant natural products, their Synthetic Biology and their effects on plant and animal physiology and pathology, and their agricultural, pharmacological, and industrial utilization.

# SUE RHEE

#### Director of Plant Resilience Institute, MSU



Plenary talk: Dr. Rhee is a renowned scientist with diverse and interdisciplinary experience ranging from plant development to plant metabolism, bioinformatics, genomics and computational modeling.

#### Symposia Sessions and Keynote Speakers

SYMPOSIUM I: Plant Synthetic Biology: Dr. Yasuo Yoshikuni- Lawrence Berkeley National Laboratory

SYMPOSIUM II: New Technologies, Analytics & Pathway Discovery: Dr. Sankarganesh Krishnamoorthy-Pacific Northwest National Laboratory

SYMPOSIUM III: Gene Discovery & Functional Genomics: Dr. Dae-Kyun Ro- University of Calgary

SYMPOSIUM IV: Metabolic Engineering: Dr. Argelia Lorence- Arkansas State University SYMPOSIUM V: Botanical Medicine, Flavors, & Fragrances: Dr. Cory Harris- University of Ottawa

SYMPOSIUM VI: Ecology & Plant-Environment Interactions: Dr. Andrea Glassmire- Louisiana State University

SYMPOSIUM VII: Chemistry, Food Security, & Nutrition: Dr. Jessica Cooperstone- The Ohio State University

SYMPOSIUM VIII: Indigenous Connections: Dr. Isabel Desgagné-Penix- University du Quebec a Trois-Rivieres

SYMPOSIUM IX: Computational Biology: Dr. John Morgan- Purdue University

#### Workshops Art-Plant Fusion Industrial Career, Fellowships, & Grants Insights CV Improvement & Mock Interviews Social Session & Excursions

#### Deadlines:

Registration: Early-bird June 19, 2023; Regular after June 19, 2023 Abstract submission: May 30, 2023 For more information, please visit the <u>conference website</u>

#### **Update from Young Members Committee**

The Annual Meetings of PSNA offer plenty of opportunities to young members interested in learning about plant chemistry and biochemistry, networking with old and new colleagues, and having fun. Three unique workshops, held this year at PSNA 2023, are specifically designed for the professional advancement of young members. A "Fellowship writing" workshop has been designed to assist young members in writing their first fellowship proposal.

Another workshop will focus on "Succeeding at Interviews" for career advancement. This year's new workshop will focus on "Art & Science" and proposes valuable tools for preparing illustrations, infographics, and videos to showcase research results.

The "Women's Breakfast" will provide time for our female scientists to gather together and discuss issues of gender balance and discrimination and why not advantages of being a woman in science. Finally, our young members want to attend the "Trivia night," a traditional gathering to meet old and new friends and have fun together!

### PSNA EXECUTIVES 2022-2023

#### **Current PSNA Executive Committee Members**

#### President: Li Tian

Research interest: My research group is interested in understanding how phytonutrients (e.g. phenolics) are made in plants using molecular, genetic, and biochemical tools. We also examine how accumulation of phytonutrients in plants is controlled by different factors under various environmental conditions. Our long-term goal is to apply the knowledge obtained from these investigations to improve the nutritional value and agronomic performance of crop plants.

#### President Elect: Dhirendra Kumar

Research interests: Understanding the salicylic acid-mediated biotic and abiotic signaling pathway in plants. Most of the current research in my lab is focused on the characterization of the SABP2-interacting proteins. We hope to develop stress resistant crop plants with less dependence on pesticides and other chemicals.

#### Past President: Dorothea Tholl

Research interests: My research group investigates the metabolism, function, and biosynthetic evolution of plant and animal specialized metabolites (primarily volatile terpenes) in intra- and inter-specific interactions. Applied aspects of our research include determining the metabolism and function of volatile aroma compounds and defense metabolites in root crops and engineering insect pheromones in plants for developing novel pest management strategies.

#### Secretary: Sangeeta Dhaubhadel

Research interests: Seed quality and defense-related traits in legume crops such as soybean, pea and common bean. Our research goal is to understand the molecular mechanisms underlying the synthesis of specialized metabolites involved in those traits and identify the regulators that control the synthesis/accumulation of these beneficial compounds in legumes.

#### Treasurer: Philipp Zerbe

Research interests: functional genomics, metabolomics, biochemical and genetic approaches to investigate the biosynthesis, regulation and function of specialized terpenoid metabolites in bioenergy, food and medicinal plants with the goal to develop resources for crop optimization and natural product engineering.

#### Editor-in-Chief, Phytochemistry Reviews: Reinhard Jetter

Research interests: Reinhard Jetter's research group is studying the surface waxes of various model plants and crops, spanning a wide range of metabolites from fatty acid derivatives to terpenoids, phenolics and polyketides. Current projects focus on the chemical analysis of the complex wax mixtures and the characterization of key enzymes involved in their formation.















# **Publication Highlights**

#### Tuan-Anh Minh Nguyen

Tuan-Anh Minh Nguyen is a Ph.D. student at Department of Chemistry, University of British Columbia, Kelowna. He obtained his BSc from the University of Science (Vietnam National University at Ho Chi Minh) and his MSc. from UBC. Anh is currently pursuing the Ph.D. program in Dr. Thu-Thuy Dang's lab to study the alkaloid biosynthesis in medicinal plants.

Dang's group (UBC, Canada) recent work on Frontiers in Plant Science reported the discovery and characterization of new cytochrome P450 enzyme from Mitragyna speciosa (kratom) which could convert a secoyohimbine-type alkaloid corynoxeine to the spirooxindoles isocorynoxeine and 3-epi-corynoxeine with different stereogenic centers. This is the first report of a plant cytochrome P450 enzyme that creates spirooxindole alkaloid scaffold, an alkaloid class that includes many compounds with significant bioactivities and chemical scaffolds that continues to challenge synthetic organic chemists.

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Spirooxindole alkaloids from plants include compounds with an extensive range of biological activities such as anticancer. antibiotics, and anti-hypertension. Although hundreds of tetra- and pentacyclic spirooxindole alkaloids have been discovered and monoterpene indole alkaloid biosynthesis has been studied extensively, no enzymes underlying the oxidative rearrangement of corynanthe-type alkaloids to spirooxindoles and other oxindole alkaloids have been reported. The

discovery has provided an answer to the historic question of spirooxindole biosynthesis in plants and highlights the versatility of cytochrome P450 enzymes in alkaloid scaffolding. It also paves the way for green chemistry and biotechnology approaches in providing access to this valuable yet challenging scaffold.

#### Jie Lin

Jie Lin is a Ph.D. student in Dr. Nik Kovinich's lab at the Department of Biology, York University, Toronto, Canada. She obtained her BSc from Southwest Agriculture University (Chongqing, China) and her MSc from Hunan Agricultural University. Jie's Ph.D. research is aimed at understanding the transcription factors that regulate phytoalexin biosynthesis in plants.

Jie Lin, together with other students in the Kovinich lab, recently published a study in MDPI Plants. The study used transcriptomics to understand why two essential transcription factors (TFs) are insufficient to activate the entire phytoalexin biosynthetic pathway in soybean. By comparing transgenic lines that overexpress the TFs GmMYB29A2 or GmNAC42-1 to plants that were treated with a pathogenderived molecule that fully elicits phytoalexin biosynthesis, Jie identified key biosynthesis genes that were not upregulated the TFs. She also identified a group of TFs that were upregulated by the pathogen elicitor, but not by the overexpression of GmMYB29A2 or GmNAC42-1. The TF GmHSF6-1 directly activates the transcription of several phytoalexin biosynthesis genes that are not regulated by GmMYB29A2 or GmNAC42-1. The study provides important insight into how genetic engineering can be used to unlock the biosynthesis of phytoalexin molecules that are of tremendous value to plant protection in agriculture and as scaffolds for pharmaceutical development.

#### Your Publication Highlights in the PSNA Newsletter

The PSNA newsletter (also shared on Twitter and Facebook) highlights your recent publications and features first authors that are current PSNA members. Interested? Then, please send us a brief non-technical summary of your paper including the title and authors, and a publication link and graphical abstract or image, if possible. In addition, provide a photo and a brief statement including the first author's affiliation and research interests. Please send your contributions (text as word document; images as pdf or jpg files) by email to Sangeeta Dhaubhadel (sangeeta.dhaubhadel@agr.gc.ca) or Armando Alcazar Magana (alcazara@oregonstate.edu).

We look forward to hearing from you!







# Citrus, Soybeans, and Mustards: My Phytochemical Research Career

#### **Mark Berhow**

After completing my PhD in Dr. Bruce McFadden's lab in the Biochemistry and Biophysics Program at Washington State University, I accepted a post-doctoral position at the USDA ARS Fruit and Vegetable Chemistry Laboratory in Pasadena, California. I became a research chemist and began work on plant natural product analysis.

#### The USDA Fruit and Vegetable Chemistry Laboratory-a Brief History

In 1910 the Citrus By-Products Laboratory was established by the Chemistry Bureau of the USDA on South Mission Road in Los Angeles at the request of the California Fruit Growers Exchange (now Sunkist Growers). The name was changed to the Los Angeles Fruit and Vegetable Chemistry Laboratory (FVCL) in 1921. The emergence of citrus as a major agricultural crop in California led the University of California to establish the Riverside Citrus Experiment Station in 1914. The USDA sent its first scientist to California in 1904, leading to the eventual establishment of a collaborative research program between the USDA and the Citrus Experiment Station.

The research group at the FVCL worked on projects aimed at evaluating and enhancing citrus products: juice, vinegar, butter, preserved peel products and concentrates. The Citrus By-Products research group developed the Brix:acid maturity tests for citrus fruits that are still used today. Other early research projects included: the use of ethylene gas as a greening agent; development of maturity standards for avocados, walnuts, and other fruits; dried fruit and vegetable products; determination of the composition of lemon oil; and the development of the Davis colorimetric test for the measurement of flavonoid content in grapefruit juice. In 1947, a fire badly damaged part of the Los Angeles Laboratory, and subsequently a new facility was built on surplus government property in Pasadena.

The new building was dedicated on April 14, 1949, as a field station of the Western Regional Research Laboratory in Albany, California. Significant projects that originated here include the invention of modern thin-layer chromatography, the characterization of citrus flavonoids, the development of dihydrochalcone sweeteners, the discovery of a new class of plant biochemical regulators, and the elucidation of the chemistry and biochemistry of citrus limonoid bitter compounds.

Citrus contains a wealth of phenylpropanoid and terpenoid compounds, some of which impact the flavor and taste of citrus fruit. I worked with Dr. Shin Hasegawa, an expert in citrus limonoids, both in the identification of these compounds in various species and cultivars, and in the elucidation of biosynthesis of these compounds in developing fruit. Limonoids are of interest to the citrus industry as several are bitter in taste and are the cause of delayed bitterness in naval orange juice. Shin showed that there was a natural pathway, that converts the precursor limonoic acid to non-bitter limononate glucoside, which prevents the formation of limonin (limonoate D-ring lactone) in Valencia oranges. Shin worked out that this was due to the longer maturation time of the Valencia, which remains on the tree a full 11 months before harvest, allowing for the natural debittering process to occur. The navels are on the tree less than 6 months, which is not enough time for the bioconversion to occur, so the juice from navels turns bitter in a few hours. By the beginning of the 1990s, I was involved with some of the work leading up to the identification of the gene involved in this biotransformation. To facilitate this research, I went to the lab



of Dr. Mitsuo Omura at the Japan's Ministry of Forestry, Fisheries, and Agriculture's Fruit Tree Research Station, Okitsu Branch, for 6 months to learn biotransformation techniques and plant regeneration methods. I was also involved in some of Shin's chemotaxonomy work on the limonoids of various citrus species. I thoroughly enjoyed my collaboration with Shin and regretted when it finally came to an end as will be noted below.





My main research interest was the further elucidation of the control of the pathway for the synthesis of flavanone and flavonol glycosides in citrus. At FVCL, I was fortunate to work with a premier authority on citrus flavonoids—Dr. Robert Horowitz. Bob did all his academic work at UCLA, receiving his PhD in the lab of Dr. Theodore Geissman, and Bob was an invaluable resource for the work I did at Pasadena.

In an interesting connection, Dr. Geissman was one of the founding members of the Phytochemical Society of North America in 1975 and was a fellow life member. He was a pre-eminent plant natural products chemist and an authority on plant flavonoids, and wrote one of the first texts on plant flavonoids in 1962.

Along with my fellow colleagues Dr. Karl Vandercook, Dr. John Manthey and Dr. Brent Tisserat, we began research aimed at trying to modulate accumulation of the bitter flavonoids in grapefruit, especially the flavanone glycoside, naringin. Naringin, the neohesperidoside of the flavanone naringenin, is the principal cause of the bitter taste of grapefruit. Naringin and its close structural relatives, narirutin (naringenin rutinoside) hesperidin (hesperetin rutinoside) and neohesperidin (hesperetin neohesperidoside), are among the major flavanone glycosides accumulated in key economic citrus cropsoranges, lemons, limes and grapefruit. Limes, lemons and oranges only accumulate rutinosides, which are tasteless, while grapefruit accumulates both the bitter neohesperidosides and the rutinosides. The difference between the neohesperidosides and the rutinosides is the linkage of the rhamnose monosaccharide to the glucose monosaccharide attached to the flavonoid; 1-2 for the neohesperidoside (2-O-alpha-Lrhamnosyl-D-glucopyranose) versus 1-6 for the rutinoside (6-O-alpha-L-rhamnosyl-Dglucopyranose). The 1-2 rhamnotransferase gene comes from the pummelo, one of the three progenitors of the various citrus species, the other two being the mandarin and the citron. An extra twist to this taste chemistry is that if neohesperidin is chemically converted to dihydrochalcone, it becomes neohesperidin dihydrochalcone, a compound that is 1,550-2,000 times sweeter than sucrose. An interesting set of chemicals indeed!

I worked on determining the site of biosynthesis of flavonoids in grapefruit, which occurs in the leaves and fruit, and is derived from simple precursors such as acetate. It was clear from this work that the flavonoids were biosynthesized during the plant cell division stage of developing leaves and fruit, and addition of a malonic acid at the end of the synthetic pathway targets at least naringin for accumulation in the cell vacuoles. As the cells elongate during the later part of the organelle development, the concentrations of these compounds are progressively diluted. We theorized that applications of plant growth regulators during fruit development and maturation might modulate the accumulation of the bitter flavanone disaccharides. As it turned out, only applications of gibberellic acid had a measurable effect—making the fruit larger, and likely just enhancing the dilution of the naringin concentrations. We also were involved in the chemotaxonomic analysis of other citrus phenolics.

I think my most interesting research occurred while working with some citrus callus cultures. Nearly all citrus callus cultures developed from wound tissue are white and are generally devoid of any secondary metabolite accumulation. However, during my 1991 visit to the Okitsu lab I found one callus line derived from Citrus aurantifolia 'Mexican lime' that







formed bright yellow cultures. These cells had been in continuous culture for over 8 years. This intrigued me. as I guessed that this callus was accumulating phenolic compounds. Back in Pasadena, I continued to maintain these cultures, along with a colorless marsh grapefruit callus culture as a control. We purified three major compounds from the Mexican lime callus identified as: kaempferol 3-O-B-rutinoside; kaempferol 3-O-B-D-glucopyranoside-6"-(3-hydroxy-3-methyl-glutarate); and kaempferol 3-O-B-D-glucopyranoside-6"-(3-hydroxy-3-methyl-glutarate)-7-O-B-D-glucopyranoside.

HPLC examination of extracts from leaf and fruit tissues from mature Mexican lime plants did not detect the presence of any of these compounds. Acylated flavonoids are not routinely found in citrus species. There are probably acylated flavonoids present in citrus, but their levels are likely very low in healthy mature tissues.

I thought this culture system was an excellent model system for the study of what occurs with wounding in plants. The kaempferol glycosides seem to be only accumulated in cells developed during the wounding process. I began examining the effect of known plant growth regulators and other compounds that were known to be released during wounding process. Growing the cultures in the dark stops the accumulation of the acylated kaempferol glycosides, and returning the cultures to light reinitiates this accumulation. The addition of phytohormones has a profound effect on the accumulation of the acylated kaempferol glycosides. Auxins, gibberellic acid, cytokinins, and abscisic acid have a stimulatory effect, while the cytokinin 6-

benzylaminopurine has an inhibitory effect. Abscisic acid was stimulatory at all levels examined, which was interesting! The abscisic acid effect was enhanced by the addition of either indoleacetic acid or gibberellic acid.

Classical elicitors, such as chitin, arachidonic acid, and autoclaved fungal mycelial preparations, did not seem to have an effect; neither did the plant "secondary messenger" salicylic acid. Interestingly, carbohydrate fractions prepared from macerated Mexican lime leaves stimulated the formation of additional flavonoids in the Mexican lime cell cultures. This could be mimicked to a lesser degree by the addition of partially-digested fractions prepared from polygalacturonic acid

# Effect of various compounds added to the culture media on flavonoid accumulation in Mexican lime callus

Compound/Classification	Effect
no light	_
Benzyladenine/PGR-cytokinin	_
Gibberellic acid/PGR	+
Abscisic acid/PGR	+
Napthaleneacetic acid/PGR-auxin	+
2,4 Dichlorophenoxyacetic acid/PGR-auxin	NE
Zeatin/PGR-cytokinin	+
Zeatin riboside/PGR-cytokinin	+
Indoleacetic acid/PGR-auxin	NE
Indolebutyric acid/PGR-auxin	NE
Salicylic acid/signal	NE
Acetylsalicylic acid/signal	NE
Arachadonic acid/elicitor	NE
Chitin/elicitor	NE
Fungal extracts/elicitor	NE
Leaf extracts/elicitor	+
Pectin digests/elicitor	+

and citrus pectin. Addition of these oligosaccharide fractions to Marsh grapefruit callus cultures—which do not normally produce any phenolic compounds—induced them to form low levels of flavonoids. including hesperidin.

This callus culture could have been used as a model for examining ways to alter the accumulation patterns of flavonoids in citrus fruit. Despite several attempts to get this callus work published in refereed journals, my manuscript was not accepted. I finally wrote it up for a book chapter published by ACS in 1998.

In February of 1994 the Pasadena laboratory was put on the federal closure list, along with several other USDA

research facilities. The Pasadena lab was officially closed in November of 1994, ending a long and successful research program. In October of 1994 I was transferred to the USDA ARS National Center for Agricultural Utilization Research in Peoria, Illinois.

+ increase in flavonoid production; - decrease in flavonoid production; NE no effect

#### The USDA, ARS, National Center for Agricultural Utilization Research

The agricultural industry in the late 1930s faced large food and feed surpluses and low profits for farmers. Through an act of Congress, four Regional Research Laboratories were established by the United States Department of Agriculture, which were charged with conducting scientific research to find new uses for US agricultural commodities. Operations at the Peoria lab (originally named the Northern Regional Research Center) began on December 16, 1940, with a mission to develop new uses for corn, wheat, and other Midwest crops. The lab is famous for its work on the development of new products from microbial and agricultural production, including the mass production of penicillin and the NRRC microbial culture collection. The Center has had a



distinguished research record that has resulted in the development of many products of benefit to the public including penicillin, dextran blood extender, guar gum, corn sweeteners, xanthan gum, cellulosic liquid absorbents, soluble fiber fat replacers, biofuels, and many others. In addition, the research has supported the development of new crops, including soybeans, guayule, field pennycress and cuphea.

The discovery of penicillin and the recognition of its therapeutic potential occurred in England in the 1920s; the development of the process to mass-produce the drug occurred in the US at the Peoria laboratory, using corn-steep liquor liquid culture. Ironically, after a worldwide search, it was a strain of penicillium from a moldy cantaloupe in a Peoria market that was identified and developed to produce large amounts of penicillin. As a result of research efforts conducted around-the-clock, penicillin was available in quantity to treat Allied soldiers wounded on D-Day. Often called the "Wonder Drug" because of its effectiveness, one of the true wonders of penicillin was the short development time from recognition of its value to mass availability. Large-scale commercial production of penicillin opened the era of antibiotics and is recognized as one of the great advances in modern medicine. As a result of their work, two members of the British group were awarded the Nobel prize. Dr. Andrew J. Moyer from the Peoria lab was inducted into the U.S. Inventors Hall of Fame, and both the British and Peoria labs were designated as International Historic Chemical Landmarks. Microorganisms are a key component of today's biotechnology because they are nature's wrecking and building crews; they decompose practically everything in the organic world, permitting basic elements to be used in new ways. Thousands of culture samples were gathered from around the world by 1950, which was the basis for the NRRC microbial collection. More than 80,000 strains of microbes, including some 20,000 bacteria, are classified, stored, and maintained in Peoria. About 60,000 additional strains make up the microbial collection of bacteria, molds, and yeasts. The NRRC collection is unquestionably the largest that is accessible to the public, and is widely considered to be among the largest and most useful collections in the world.

Of course, I was only involved peripherally in the microbial work at the lab! After a bit of time to find my place in these new research programs, I became a part of the new crops research and new uses program at NCAUR. My role soon became one of "what is in this?" in collaboration with researchers at the lab and around the world. NCAUR research programs had a reputation for expertise in natural products analysis and availability of an outstanding scientific equipment collection. NCAUR had mass spectrometers, NMRs, HPLC systems, prep chromatography systems, etc., that allowed me to further develop and refine natural product identification and analysis. A key component of my research was developing collaborations with researchers for the biological and biochemical evaluation of extracts



and purified natural products. I could do the chemistry, but not the biology that I needed done. Collaboration was essential to show the impact of these natural products I was working on in plant, pest, animal, and human systems.

My first foray into the "what is in this" jungle was a collaboration with the University of Illinois at Urbana-Champaign. An extract from soybean processing had a "chemoprotective" effect in preventing DNA damage caused by arylamines, which are potent carcinogens. Isoflavones were present in this extract, but none of them were as effective as the extract. What else was in this material that caused this effect? We fractionated the extract and found, to no surprise, that the extract also contained a mixture of soy saponins. Little work had been done to fully characterize the saponins found in commercial soy processing products due to the lack of standards. We modified quantitation methods and produced purified soy saponins which were demonstrated to have the chemoprotective effect. This led to many other collaborations that demonstrated the potential role that plant saponins and isoflavones might play in the mitigation or prevention of human cancers. As can be seen below the composition of saponins in soy and legumes can be quite complex. This work led to many collaborations over the rest of my research career.



Soyasapogenol A: R1 = OH, R2 = OH Soyasapogenol B: R1 = H, R2 = OH; Soyasapogenol E: R1 = H, R2 = O I would be remiss if I did not mention how important interactions with researchers at the University of Illinois were for my research. These led to several outstanding collaborations on a variety of plant natural products including the saponins, isoflavones, anthocyanins, glucosinolates, furanocoumarins and other phenolics. I am extremely grateful for all the collaborations and access to the facilities this afforded me. I also had appointments as an adjunct professor in the Department of Crop Science and the Department of Food Science and Human Nutrition for over 20 years.

As my new research project developed, I worked closely with my fellow scientist and friend my fellow scientist and friend Dr. Steven Vaughn. Steve was interested in understanding how natural products that might be used in plant nutrition and pest control could affect crop and horticulture production. We both developed a keen interest in the glucosinolates found in the Brassicaceae or mustard family. We published several interesting papers characterizing the chemical and biological degradation of these compounds and their effect on plant heath and plant disease systems, work which is still ongoing today.

The analytical work was greatly enhanced using the (then) new ion trap mass accurate mass spectrometer, which provided not only mass spectra accurate to 1 ppm or less, but reproducible mass ion fragmentation data which allowed for nominal structural identification. This allowed us to generally identify alucosinolates, flavonoid glycosides, triterpene glycosides, and other acylated conjugates with a single chromatographic run. We used this approach to rapidly identify potential compounds for study and to focus any purification efforts on unknown compounds of interest. We analyzed mustard family seeds using phytochemical analysis to support the ARS new crop program. This contributed to the development of new uses for seed meals



from emerging oil seed crops such as camelina, carinata, Lesquerella, various mustards and field penny cress (Thalaspi arvense). Commercial availability of this type of analysis is not generally available, and we developed several successful collaborations for research on the use of these seed meals as animal feeds, fish meals, biological control agents, and potentially in enhancing human nutrition.

I was especially interested in the structural similarity of the camelina glucosinolates (and their isothiocyanate degradation products) to those found in broccoli. Sulforaphane, the isothiocyanate degradation product of glucoraphanin (a C-4 methylsulfinyl glucosinolate), was shown to be involved in inhibiting several processes in humans that lead to the induction of certain types of cancer. This is part of one of the few anti-cancer health claims allowed by the FDA for broccoli, specifically broccoli sprouts. Unfortunately, the concentration of glucoraphanin in broccoli florets is quite low, higher in sprouts, and highest in seeds. Broccoli (and the other varieties of Brassica oleracea) can contain up to 20 other glucosinolates that may or may not be of benefit. Camelina seed meals, in contrast, contain only three glucosinolates—the C-9, C-10, and C-11 methylsulfinyls—each with longer aliphatic chains than glucoraphanin. The concentrations of these compounds are much higher in camelina seed meals than those of glucoraphanin in broccoli sprouts or florets. Initial work on the isothiocyanate forms of these compounds also showed promise in modulating the processes that lead to cancer, but more work needs to be done. Camelina is currently grown for oil for biofuels and oil enriched in omega-3 fatty acids, but new uses are needed to increase the value of the seed meal press cake. Could consumption of camelina seed meal have a cancer prevention effect as well?

I have analyzed many other phytochemicals over the years through contacts here at the Peoria Ag lab, including some work with Dr. Brent Tisserat on the effect of high CO2 concentration on the accumulation of phytochemicals in plants and plant tissue culture, as well as with other researchers on insect attractants, repellants, and antifeeding compounds found in plants. One my interests has been on reliable and rapid nondestructive analytical methods for determining phytochemical composition and concentrations in agricultural raw and processed products, like the Star Trek tricorder! Near Infrared Spectroscopy (NIRS) has been used for bulk analysis for many years for total oil, moisture, fiber, protein content. We have worked on developing the use of this instrumentation by developing calibrations that parse out fine chemical component analysis, such as the total glucosinolates in mustard seeds, or the isoflavone composition in soybeans. I think we have made good strides in showing this type of fine analysis is possible with NIRS and that this technology could have potential future applications for finer pre-grading of raw agricultural materials to be targeted for specific consumer products with defined natural product compositions. Exciting times lie ahead!

Along the way I have a chance to work with many interesting scientists, both at the Pasadena Lab and here at the Peoria Ag Lab. I even met my wife Dr. Renée Wagner as a fellow mass spec collaborator. I have had a chance to interact with school groups touring the lab and (hopefully) sparked some interest in the STEM sciences. Several high school, undergraduate and graduate students have spent some time in my lab, and I am grateful for the help they have provided me in the arduous task of sample preparation and analysis. I hope they benefited from the time in the lab and the experience helped them in a successful career.

And finally, I absolutely must acknowledge how important my membership in the PSNA has been to my career. I first attended my first PSNA meeting in Pullman in 1998, and I was struck by the fact that these researchers had the same interests as I. Unlike when I attended the meetings of the larger scientific societies where I seemed to be out of place, the PSNA members were talking about research on phytochemicals— their biosynthesis, accumulation, and biological effects—just the things I was working on. I had found my scientific home. Though the PSNA I learned about a wide range of research projects and efforts, heard exciting talks, and saw outstanding posters. I ALWAYS came back to the lab from a PSNA meeting rejuvenated and excited about science, with several new ideas to try. I was able to meet and talk to giants in phytochemical research, which really provided inspiration to continue my work. Many of the researchers and students I met at these meetings have become lifelong friends. The PSNA was then, as it is now, an all-volunteer organization, with no paid employees. The members volunteer to serve as the officers and the meeting organizers and planners. It was soon apparent to me that if I wanted the PSNA to continue to flourish, I would have to do my part to help. My first opportunity came when Daneel Ferreira asked if I would consider hosting and organizing a PSNA meeting, which I did in Peoria in 2003. I then went on to serve on the executive committee in various roles as secretary, newsletter editor, and president, even volunteering to head up another meeting at the University of Illinois in 2015. I know that my activities with the PSNA have contributed to my career and I hope that I have contributed to the success of the PSNA.

I would highly encourage any PSNA member to do more than just pay your dues! Get involved and help the PSNA thrive. It will pay you back!

My career as an active ARS research chemist came to a close, as I retired on December 31 of 2022 as a salaried USDA employee after 39 years of service. I plan on being active as a collaborator with ARS and hopefully some of you, and look forward to seeing you at future PSNA Meetings.

# An Appreciation from Zhi-yan (Rock) Du: Recipient of the 2022 PSNA-TPJ Early Career Award

It's such an honor to receive the 2021-2022 PSNA-TPJ early career award, together with Monica Borghi, Narayanan Srividya, and Thu-Thuy Dang. In August 2020, I started my lab in the Department of Molecular Biosciences & Bioengineering at University of Hawai'i at Mānoa, a hard time during the COVID-19 pandemic. It was very challenging to establish a new lab and



research team for the past two years, and this award is certainly an exciting energizer for my research. Mahalo!

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I am very grateful for the recognition of my previous work on plant lipid metabolism. With the new lab and research team, I will keep exploring insights into plant lipid dynamics and functions and expand my research on synthetic biology and applications of valuable lipid products. As an awardee, I am offered a chance to write a review for this highly reputed journal. My review will be focused on "metabolic engineering for lipid production in microalgae." My lab is developing microalgae as super factories to produce food, nutrient, medicine, and energy products, for the potential crisis from the pandemic, wars, and global climate change. I feel blessed to live and work in the tropical paradise, where I can find abundant algal resources in Hawai'i. It's also shocking to know that the rainbow state is dangerously dependent on food imports. According to a recent Sea Grant report, Hawaii has as low as four to five days of food, or up to eleven days by some other reports, in its supply chain at any given moment. I hope to contribute to the food and energy security of Hawai'i and beyond.

I remember working hard for almost three years to publish my first Plant Journal research paper in 2013, almost ten years ago. It's such a journey from a graduate student to an independent Pl. I am excited to meet everyone at the PSNA conference, share my experience with the students and junior faculty, and learn from the senior members. I can't wait to attend the coming conference at Michigan State University, where I did my postdoc research. It will be great to see friends and colleagues during the beautiful, comfortable season in Michigan. No need to dig my car out of the snow, Mahalo Ohana!

# An Appreciation from Alexandra Dickinson: Recipient of the PSNA 2022 Arthur Neish Young Investigator Award

Receiving the Arthur C. Neish young investigator award last year was a great honor for me. This is a very exciting time to be a young investigator studying phytochemistry. Plants have a vast, unexplored chemical landscape. This presents many challenges, but poses a wealth of exciting opportunities and discoveries. Research this field has enormous potential to benefit society in the field and the clinic. Therefore, I deeply appreciate the support that the Phytochemical Society of North America (PSNA) provides to early career scientists who are making important advances in plant chemistry. Receiving this award, named for a pioneer in phytochemistry, has been inspiring and has helped advance my career. Most importantly, it gave me the opportunity to connect with the broader phytochemistry community, and I look forward to many more interactions in the future!



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fields.



PSNA 2022 photo courtesy of Crystal Founds, Program Coordinator, Virginia Tech Translational Plant Sciences Center.