THE BUZZ

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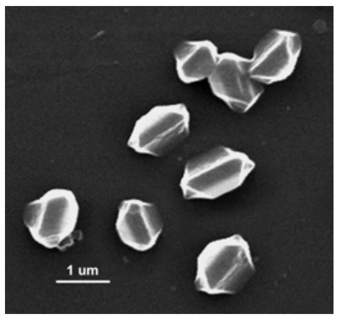
IMPROVING HUMAN HEALTH USING PATHOGENS OF INSECTS

By Brian Federici

Infectious diseases continue to be the most significant cause of human morbidity and mortality worldwide. This is despite significant advances in medicine over the past century including the discovery of antibiotics to cure bacterial diseases and the development of insecticides for controlling the mosquito vectors of malaria, Dengue fever, and filariasis. The World Health Organization estimates that more than 400 million people suffer from malaria, with approximately two billion people living in regions of the world where this disease is endemic. Dengue fever and filariasis, though not as significant, still cause disease in hundreds of millions of people in India, Africa, and many countries in Southeast Asia. While these diseases primarily affect people living in less developed tropical and subtropical countries, people highly industrialized in temperate regions are not immune to vector-borne infectious diseases, a good example being the current outbreak in the United States of West Nile fever, a viral disease transmitted by mosquitoes. Moreover, globalization of the world economy with its concomitant increase of international trade and travel is expected to increase the spread of both the causative agents of infectious diseases and their vectors during this century. In addition to vector-borne infectious diseases, the public is increasingly concerned about the safety of food and water, the principal reason being the ongoing reports that these are contaminated with chemicals, the most notorious being synthetic chemical insecticides and herbicides.



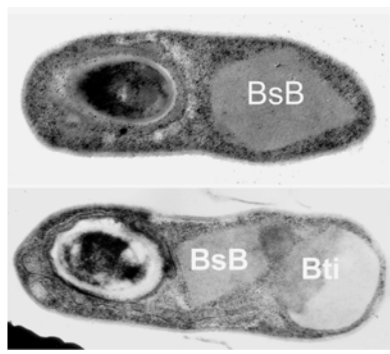
Crystals of the *Bacillus sphaericus* mosquitocidal protein produced by genetically engineering the encoding gene for high levels of synthesis in *Bacillus thuringiensis*. These crystals are tenfold larger than those produced by wild type strains of *B. sphaericus*.

Given the significance of these problems, the research carried out by my group focuses on basic and applied studies of insect pathogens, especially viruses and bacteria, with the aim being to use these to develop more environmentally safe tools for managing insect pest and vector populations. One of our major goals is to develop pathogens as effective insecticides that will reduce the use of chemical insecticides. In the past, we contributed to the development of several bacteria and viruses that have been commercialized and are used to control vectors and caterpillar pests. For example, through collaborative work with Mir Mulla's group, we carried out many of the initial studies on *Bacillus thuringiensis* subspecies *israelensis* (Bti) that led to its use as the most successful bacterial insecticide for controlling mosquitoes and blackflies. As a member of several technical advisory committees for the World Health Organization, Mir and I, based in part on the research of our students including **Lerry Lacey** (PhD, 1980) and **Jorge Ibarra** (PhD, 1986), helped facilitate the development of Bti for use in the Onchocerciasis Control Program in West Africa during the 1980s. This program resulted in extensive control of larvae of the blackfly vectors of a nematode worm that caused blindness in many regions of tropical West Africa. Bti is now used throughout the world to control numerous nuisance and vector mosquito and blackfly species. With respect to viral insecticides, we developed several baculoviruses to for controlling caterpillar pests. The most successful have been the nuclear polyhedrosis virus (NPV) of the beet armyworm (*Spodoptera exigua*), and the granulosis virus (GV) of the grape leaf skeletonizer (*Harrissina brillians*), the latter developed in collaboration with Vern Stern, who retired several years ago. The beet armyworm NPV, based in part on research carried out by **Wendy Gelernter** (PhD, 1984) is used both as a commercial product (Spodex) in the U.S. and Europe, as well as in many developing countries, where it is produced by "cottage industries." The grape leaf skeletonizer GV is used by grape growers in several regions of California to control the larvae of this highly destructive pest. This is one of the most remarkable viruses used for insect control. A single treatment of about 5 grams of virus per acre gives virtually total control for a period of three to five years. Ironically, this virus is so effective that it has not been commercialized, as in the absence of routine application, there is no way for industry to make a reasonable profit. Thus, the grape leaf skeletonizer GV is used as an augmentative biological control agent, provided free to growers as needed by UC Cooperative Extension personnel.

Naturally occurring bacteria and viruses used to control agricultural pests have suffered serious economic setbacks due to the development of insect-protected transgenic crops. Most transgenic crops currently produced in the U.S. are based on insecticidal proteins of *B. thuringiensis*. These crops, mainly Bt cotton and Bt corn, have been so successful that they have eliminated the major markets for bacterial and viral insecticides, as most of these were developed and marketed to control the caterpillar pests of cotton, corn, and vegetables. This has caused an upheaval in the bacterial and viral insecticide businesses. Few viral insecticides are currently marketed in the U.S., and major producers of bacterial insecticides have sold their Bt businesses to Japanese chemical companies, Abbott Laboratories to Sumitomo Chemical, and Sandoz to Mitsui Chemical. There remain important markets in which microbial insecticides can compete, but their efficacy must be improved. In addition, though transgenic plants are not a threat to mosquito control agents such as Bti, improving the efficacy of these would benefit their use throughout the world for controlling nuisance and vector mosquitoes. Transgenic mosquitoes incapable of transmitting the pathogens that cause diseases such as malaria hold potential for use in the long term, but just how and when these will be available remains uncertain, as their use is plagued by a variety of technical and ethical issues.

Advances in the field of molecular biology, especially the development of recombinant DNA techniques and their application to practical problems, commonly known as genetic engineering, have provided an avenue to extend our previous research with naturally occurring pathogens of insects. Because bacteria are cheaper to produce, and so much easier to manipulate with recombinant DNA technology than viruses, our research over the past decade has focused on the development of improved bacterial insecticides, with emphasis on recombinant bacterial insecticides for control of mosquitoes. In addition, we are extending this basic technology to control sucking insect pests such as aphids and whiteflies directly through the plant.

One of our main projects is to develop a series of recombinant bacterial insecticides for mosquito control based on Bti and *B. sphaericus* (Bs). The latter is another bacterial insecticide recently registered for mosquito control. Bti is effective against many mosquito species, but even the best formulations only last for two weeks or so in many typical habitats in which mosquito larvae breed. In contrast, Bs formulations often persist for as long as six weeks, and they are more effective than Bti in highly polluted waters. However, Bs has a limited target spectrum, and resistance to it has developed quickly in regions where it has been used extensively, the reason being its insecticidal properties are due to a single two-component toxin. In contrast, no resistance has developed to Bti over more than twenty years of use. One reason for this is that Bti consists of four insecticidal proteins, one of which that has the remarkable property of delaying resistance to the others. Thus, we are using recombinant DNA technology to develop strains of Bti and Bs that will combine the best properties of both. Different recombinants are being developed for use against major mosquito and blackfly species, with an emphasis on tailoring these strains for improved efficacy in specific habitats. Heading up the research on this project is Assistant Research Entomologist, **Hyun-Woo Park** (PhD, 1999). Hyun-Woo is being assisted by post-doctoral fellow **Dennis K. Bideshi** (PhD, 1998), SRA **Jeffrey J. Johnson**, and Professor **Eleazar Barboza Corona**, a faculty member from the University of Guanajuato, Mexico, currently on sabbatical leave in my laboratory. In addition, **Katie Poole,** a new graduate student, has recently joined this project. Our recombinant Bti/Bs project is being carried out in collaboration with members of William Walton's group, especially Dr. Margaret C. Wirth and Joshua Jiannino, who develop and maintain mosquito strains resistant to various Bti and Bs insecticidal proteins, and assist in analysis of the recombinant bacteria we produce.



Recombinant strains of *Bacillus thuringiensis* (Bt) that produce large amounts of the *Bacillus sphaericus* (Bs) mosquitocidal protein (BsB). The top panel shows production in a Bt strain engineered to produce the *B. sphaericus* toxin alone. The bottom panel shows production in a strain of *B. thuringiensis* subsp. *israelensis* (Bti) engineered to produce the Bs toxin along with the normal complement of mosquitocidal proteins (Bti) produced by wild type strains of this subspecies. Transmission electron micrographs.

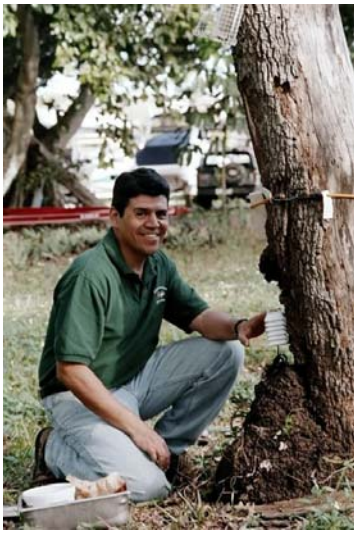
One of the first recombinants we developed through this project uses Bti as the host strain. In addition to the normal complement of insecticidal proteins that Bti produces, this strain also synthesizes a high quantity of the Bs protein. In laboratory trials, this Bti/Bs recombinant is tenfold as effective as the parental Bti or Bs strains against *Culex* mosquitoes, the major vectors of many of the viruses that cause encephalitides, including West Nile Virus, as well as the nematode worms that cause filariasius. We have obtained permission from the U.S. and California Environmental Protection Agencies to undertake preliminary field trials of this virus through a collaboration with Mir Mulla. Initial results from these trials have confirmed that this Bti/Bs strain is much better than the parental strains from which it was derived. We are currently researching the development of additonal Bti strains, and strains for Bs that produce Bti proteins and are much less prone to the development of resistance. This research is being supported by grants from the NIH, USDA, and UC Mosquito Research Program.

Whereas we are no longer involved in the development of viral insecticides, we do continue to do basic research on viral phylogenetics and evolution. This research is led by post-doctoral fellows **Dennis K. Bideshi** and **Yeping Tan**. The goal of this research is to determine the origin of large double-stranded DNA viruses that attack insects, as well as how the polydnaviruses originated and evolved. Polydnaviruses compose a large group of virus-like particles produced in the reproductive track of certain endoparasitic wasps. The particles are injected into insect hosts during oviposition and suppress the host's immune system, thereby contributing significantly to parasitic success. Most polydnavirus researchers consider the particles produced by the wasps to be viruses. In contrast, our view is that these are no longer viruses, but rather are wasp organelles that evolved by symbiogenesis between the DNA viruses and wasps, the same evolutionary process by which, for example, mitochondria and chloroplasts evolved from bacteria. We are currently testing this hypothesis through a collaboration with the Dr. Yves Bigot's group at the University of Tours, in Tours, France. This research is supported by NSF and the French National Science Foundation.

Arguably the most successful advance in pest control technology of the past two decades is the development of the insect protected Bt crops noted above. The successful development of this technology during the latter part of the last century is the forerunner of a major shift in the way we will control many insect pests and plant diseases in the future, in other words, directly though the plant using molecular technologies. Knowledge of plant molecular biology, recombinant DNA techniques, and the development of novel insecticidal peptides are coming together to yield new and environmentally safe ways to control these pests. To explore this new and exciting field, we have recently initiated a new project in my laboratory aimed at combining knowledge of plant molecular biology and insect midgut physiology to control sucking insects directly through the plant. Our objective is to identify key midgut and salivary proteins in sucking insects and block their function by engineering plants to produce short peptides derived from antibodies that bind to and interfere with the function of these proteins. This research is being led by post-doctoral fellow **Cynthia Levesque** with the assistance of **Michael Diaz**, and is supported by the Tomato Commission, Cotton Incorporated, the USDA, and UC BioSTAR program. We expect to add one or two additional postdoctoral fellows to the project over the next year.

During the early part of the 20th century, UCR Entomology pioneers such as Professor Harry S. Smith contributed to the development of pathogens as biological control agents though their research and teaching. A century ago no one could have foreseen the enormous changes in pest control technologies yet to come, which included the development of synthetic chemical insecticides, pheromones, and insect resistant plants. While often controversial and not without undesirable consequences, few would question the overall benefits of these technologies. The next phase in this evolutionary process will be the expanded use of molecular technologies to control insect pests. Our goal is to contribute to the development of these new, more environmentally safe technologies though basic research that has an applied orientation. Emphasizing basic research is important because, as history has shown, it is impossible to envision from which discoveries the most effective pest control technologies will arise. Nevertheless, there is little doubt that improved knowledge of the molecular biology of plants, insects, and their pathogens will contribute to the development of these technologies in the decades to come.

ALUMNUS FEATURE – DR. BRIAN CABRERA



At UCR I studied the effects of heat on the behavior and water relations of the western drywood termite, *Incisitermes minor* (Hagen). I received my M.S. in 1993 and my Ph.D. in 1998 under the guidance of Dr. Mike Rust. Working in his lab was a rewarding experience, both personally and professionally. In November 1998, I left the desert hills and smog of Riverside for the plains and a post-doc at the University of Nebraska, Lincoln. There I studied overwintering in subterranean termites and found they can survive exposure to 0°C for at least one week and escape lethal freezing most likely by overwintering below the frost line. Having been born and raised in Los Angeles, I also learned that paying a little extra for good cold weather clothing kept me from becoming moribund during the windy Nebraska winter.

In February 2000 I began a dual post-doc at the University of Minnesota, St. Paul and UC Berkeley. I worked on the chemical ecology of a deathwatch beetle, *Hemicoelus gibbicollis*. Preliminary results indicated females produce stegobinone, the same sex pheromone of two other anobiids, the furniture beetle, *Anobium punctatum*, and the drugstore beetle, *Stegobium paniceum*. We also described a new braconid species, *Heterospilus luridostigmus* Marsh, which we collected from our beetle rearing boxes. What is noteworthy about this wasp is it emerged from wood from an infested patio deck illustrating that undescribed insect species exist in our own backyards. We also isolated stegobinone from another anobiid, *Ptlinus basalis*. Electro-antennogram detection studies by Dr. Allard Cosse (USDA-ARS, Peoria, IL) showed antennae of male *P. basalis* responded positively to this compound. I am planning further research on the chemical ecology of wood-destroying beetles in collaboration with Dr. Steve Seybold (U.S. Forest Service, Davis, CA).

In October, 2000 I left the damp cold of Berkeley for the sub-tropical climate of Fort Lauderdale, FL where I am currently an Assistant Professor and Extension Specialist at the University of Florida, Ft. Lauderdale Research and Education Center. I have a 70% extension, 25% research, and 5% teaching appointment. My extension program includes educating homeowners and the pest control industry on common household insect pests and their management. I also provide training for identification of major insect pests, especially termites. Currently, I am working on a training manual for structural fumigation. I will also be involved in providing educational material and programs for the Spanish-speaking citizens of Florida. As part of my research I am testing termite baits for eliminating Formosan subterranean termite colonies and am beginning laboratory evaluations of several different treatments for controlling a unique and localized infestation of a nasute termite, *Nasutitermes costalis* (Holmgren) in nearby Dania Beach, FL. This is the first record of a "higher" termite established outside of its endemic range. This past summer, I assisted with teaching the University of Florida's first-ever termite biology class.

Living in South Florida is quite an experience – from botched elections to hurricane watches, high humidity and biting flies, awesome thunderstorms, and a diverse flora and fauna (including gators, iguanas, and geckos). But I always return to California at least once a year and usually squeeze in a trip out to ol' Riverside.

HONORS AND AWARDS

Alex Raikhel has been awarded a ten-year, $4.3 million research merit grant from the National Institute of Health to support continuing research into the genetic and molecular mechanisms regulating egg development and maturation in mosquitoes.

Four Entomology Department faculty were named 2002 Fellows of the American Association for the Advancement of Science - **Mike Adams** "for pioneering contributions to our knowledge of the biological chemistry and actions of ion channel-specific toxins from venoms, and discovery of ecdysis-triggering hormones in insects." **Alex Raikhel** "in recognition of pioneering research that defined the hormonal and molecular genetic pathways for mosquito vitellogenesis and led to generation of the first transgenic mosquitoes refractory to plasmodium transmissions." **Nelson Thompson** "for pioneering research on the nutrition and nutritional biochemistry of parasites and parasite-invertebrate host interactions involving parasitic Hymenoptera, insect parasitic nematods and schistosomes." **Mike Rust** "in recognition of seminal contribution to our understanding of the biology and control of major fleas, and for outstanding administrative service." Mike also received the 2002 PCT/Syngenta Leadership Award. **Rick Redak** received the 2002 Researcher of the Year Award from the California Association of Nurserymen. **Miriam Cooperband** has been awarded the 2002 Herbert Kraft Scholarship for $2000 from CNAS at UCR. **Brad Mullens** gave the plenary address to the European Veterinary Entomologists (COST 833), Bari, Italy in September, 2002.

FAREWELL TO MARSHALL

By Bill Carson

In November, 2002 we said our farewells to Marshall Holman. This was both a happy and a sad occasion. Happy, because for Marshall this is a very positive career move that will enable him to advance much farther in the purchasing field, and sad for all of us here in Entomology because we will be losing such a wonderful staff member and good friend.

It is difficult to overstate how important and valuable Marshall has been to us over the years (18, in fact). I'm sure that each and every one of us has at one time (and probably many) been the recipient of an extra favor or service that he would always cheerfully provide.

A small measure of how much he has meant to us was shown at the large and unprecedented going away party given in his honor on November 8th. Good luck Marshall!

IN MEMORIUM

Dr. Glenn Carman joined UCR's Department of Entomology in 1943 as a junior entomologist. He became Professor of Entomology in 1963, holding this position until his retirement in 1981. From 1963 to 1968, he served as Chair of the Entomology Department.

His academic degrees were a bachelor of science in entomology and zoology from Iowa State University in 1932, a master of science in entomology from Iowa State in 1936, and a doctorate in economic entomology from Cornell University in 1942.

His honors included recognition by UCR as one of the most important contributors to the California citrus industry during its "A Celebration of Citrus: Past, Present and Future" gala in March 2000. He was cited for his research on the biology and control of major citrus pests, his service as president of the California Quality Control Council, and his role as co-editor of volumes four and five of "The Citrus Industry," considered the definitive series of scientific books about citrus fruits. He also was elected a fellow of the American Association for the Advancement of Science in 1962 and received a Entomological Society of America's Recognition Award for Contribution to Agriculture in 1978. Sponsored by the CIBA-GEIGY Corp., the award included an all-expense paid tour of major agricultural sites in Europe.