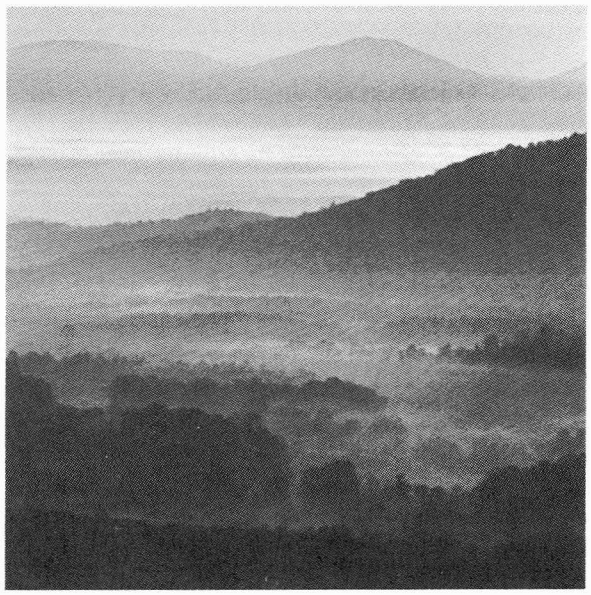
sip NEWSLETTER

society for invertebrate pathology

VOLUME 25, NUMBER 2
May 1993

XXVI ANNUAL MEETING
SOCIETY FOR INVERTEBRATE PATHOLOGY
GREAT SMOKIES RESORT
AND CONFERENCE CENTER
ASHEVILLE, NORTH CAROLINA, USA
AUGUST 1-6, 1993



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THE PROGRAM

A copy of the Program and Abstracts for the meeting will be included along with this issue of the Newsletter. Although a few extra copies will be available at the meeting, they are to be distributed only to registered non-members. PLEASE REMEMBER TO BRING YOUR COPY OF THE PROGRAM AND ABSTRACTS WITH YOU TO THE MEETING!

ADDITIONAL REGISTRATION DETAILS

As indicated in previous issues of this Newsletter, our 1993 Annual Meeting of the Society will be held in Asheville, North Carolina at the Great Smokies Hilton Golf Resort and Conference Center. The 130 rooms we have booked with the hotel, however, have proven to be insufficient and arrangements have been made with the Best Western Asheville Central Motel to accommodate late registrants. This motel is located only 0.9 mile from the Hilton and a free shuttle van will be available to carry participants back and forth between the two hotels as necessary. Excellent accommodations are also available at the Best Western and registrants assigned there should experience little inconvenience in participating in the meeting. Registrants staying at the Best Western will benefit from the lower room charge of $49.00 per night (flat rate for up to 4 persons). Arrangements have been made with the Hilton to route your reservations directly to the Best Western once all rooms are reserved at the Hilton. One night's advanced deposit is required payable by check or credit card. Cancellation should be made 48 hours prior to arrival for full refund. For registrants who might wish to contact the Best Western Motel directly, their phone number is 704-253-1851. Please inform them that you are a member of the SIP to receive the group rate.

Members planning to attend this meeting are reminded to register prior to June 30 in order to avoid a late registration fee of $45.00. While T-shirts and tickets for the Chimney Rock Excursion may be purchased at the meeting, advanced registration for these items will be helpful in the planning activities of the Local Arrangements Committee. In addition, please note that

July 1 is the cut off date for registration at the Hotels. After this date, rooms can be reserved only on a space-available basis. And, while there are numerous hotels and motels in the Asheville area, shuttle service will only be available between the Hilton and Best Western Central. Participants registering at other motels will require their own means of transportation to and from the Hilton. None are located within walking distance of the Hilton.

TOURIST INFORMATION

The Asheville area is a popular base for family vacations, and there are activities available to accommodate all tastes. For nature lovers there are several beautiful areas offering swimming, fishing, camping, hiking, horseback riding, and picnicking. These include the Great Smoky Mountains National Park, the Appalachian Trail, the Pisgah and Nantahala National Forests, Grandfather Mountain, and Chimney Rock Park. Three lakes in the Asheville vicinity offer good fishing, and there are miles of trout streams. Most of North Carolina's majestic waterfalls are located here, and outfitters can provide full or half day whitewater rafting trips over Class I through V rapids on the French Broad, Ocoee, Chattooga, Nolichucky, and Nantahala Rivers. The Blue Ridge Parkway, a 470 mile scenic route, winds along the highest ridges of the Great Smoky Mountains. Some points of interest along the parkway include Craggy Gardens and Mount Mitchell, the highest peak east of the Mississippi River. Closer to the meeting site are the arboretum and botanic gardens on the University of North Carolina-Asheville campus.

If more civilized activities are preferred, Asheville offers a variety of shops, restaurants, galleries and museums. These include the Pack Place museum complex and historic Biltmore Village, a community of shops, restaurants, galleries, and museums. There are several Art Deco buildings in the Asheville historic district and walking tours are offered by the local preservation society. Also of historic interest are the Carl Sandburg Home, the Thomas Wolfe House, the Smith-McDowell Home Museum and Heritage Center, and the Cradle of Forestry, where scientific forestry was first practiced and taught in the U.S. In the evenings you may want to visit one of Asheville's clubs or restaurants to enjoy traditional bluegrass music and dancing.

Asheville is known for traditional mountain crafts and offers a variety of shopping in Biltmore Village and in the downtown area as well as in several commercial galleries. Contemporary and traditional folk art can be seen and purchased at the Southern Highland Handicraft Guild museum and shop. There is also a museum, craft co-op, and theater on the nearby Cherokee Indian Reservation. Bargain hunters won't want to miss the River Ridge Factory Outlet Center. The Asheville Area Visitor's Bureau can provide you with information on attractions and services available to make your visit a memorable one. Their telephone number is (704) 258-6109 or toll-free (800) 257-5583, or stop by 151 Haywood Street in Asheville.

ATHLETIC FACILITIES

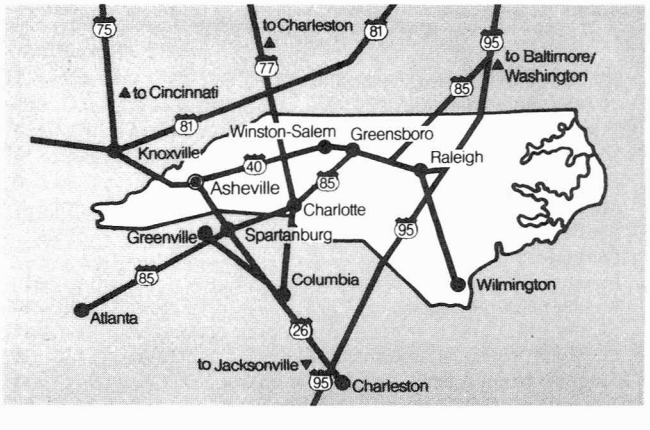
Two swimming pools are located on the grounds of the Hilton along with four outdoor and four indoor tennis courts. Tennis fees will be $7.00 per hour for the outdoor courts; green fees for the golf course are $26.00 for guests of the Hilton and include a golf cart. There is no charge for use of the swimming pools. Volleyball facilities are also available on the Hotel grounds.

WEATHER AND DRESS

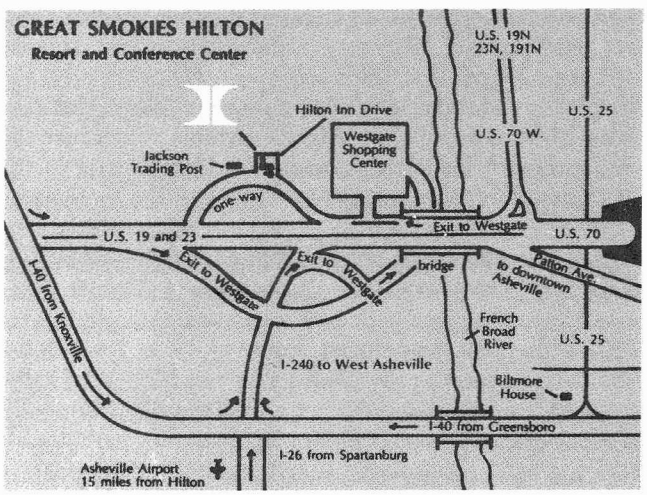
At an approximate elevation of 2000 feet, Asheville typically experiences mild summer weather with highs in the upper 70s to low 80s and lows in the high 50s to low 60s. Casual dress will be in order during the day; a sweater or wind breaker may be necessary in the evening. An occasional evening thundershower can be expected, and an umbrella or rain coat may be useful. Since the meeting will take place under the common roof of the Hilton, adverse weather clothing should only be necessary if the weather is unexpectedly cool in the evenings or on the Chimney Rock excursion. Families planning to tour the Smoky or Blue Ridge Mountains may wish to bring along heavier jackets or sweaters to wear at higher elevations, especially at night.

TRANSPORTATION

CAR: Located in the heart of the southeastern United States, Asheville is within 500 miles and a day's drive from such cities as Tampa, FL, Mobile, AL, Jackson, MS, St. Louis, MO, Chicago, IL, Cleveland, OH, and Philadelphia, PA. The city is the focal point of a number of Interstate highways and connections can be readily made from all directions as shown below:



The Hilton Inn is located on the west side of Asheville adjacent to the Westgate Shopping Center near the intersections of U.S. 19 and 23 and I-40 and 26. Entrance to the Hotel is a bit tricky from the highways and careful attention must be paid to highway signs providing specific directions to the Hotel itself. When traveling from the south or west, take the marked turnoffs to the Hilton and the Westgate Shopping Center as shown on the map below. These exits will bring you directly into the Westgate Shopping Center parking lot proper where a sign will direct you to make a U-turn to skirt the outer edge of the parking lot and onto an exit lane from which you will follow signs to Hilton Inn Drive and to the Hotel itself. Travelers coming from the east will also take the exit to the Hilton and Westgate Shopping Center but will miss the Shopping Center by following subsequent directions to the Hilton as also shown on the map below. If you miss the proper exits the first time, do not be discouraged - you will be in good company with most first-time visitors to the Hilton! Parking is free at the Hilton.



AIR: When arriving by air, participants should fly directly into the Asheville Regional airport if at all possible. The airport is serviced by three major airlines: U.S. Air, Delta, and American. Non-stop flights to Asheville are available from five major hubs: Charlotte, Atlanta, Raleigh-Durham, Nashville and Cincinnati. Participants may also consider flying into the nearby Spartanburg - Greenville, S.C. airport. Asheville can then be reached by rental car in about a pleasant, 1-hour drive.

Rental cars are available from major rental companies (Avis, Hertz and Budget) at the Asheville Airport as well as from a number of other regional rental agencies throughout the city.

As the Hilton does not provide van service to or from the airport, plan to take a taxi (about $20.00) or limousine (about $12.00) to the Hotel. The airport is about 15 miles south of Asheville on I-26, and a one-way trip takes only 15 to 20 minutes.

CAMPGROUNDS: There are many good camping grounds near Asheville as might be expected due to its proximity to the Great Smokies. A few close to Asheville are listed below:

Bear Creek RV Park and Campground - on South Bear Creek Road, just off NC South with easy access to I-40 and I-26. Phone: (704) 253-0798.

KOA Asheville West - Route 5, Chandler, NC. Phone: (704) 665-7015.

Big Cove Campground - 13 miles west on I-40, exit 37, 1 1/4 miles on US 19 and 23. Phone: (704) 667-9375.

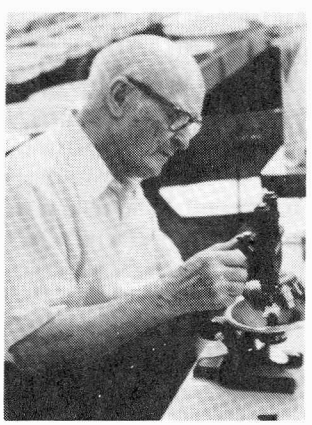
Tanglewood Lakes KOA Campgrounds - 10 miles east on US 70, off I-40, exit 59. Phone: (704) 686-3121.

TAPS RV Park - 1327 Tunnel Road in Asheville, Phone: (704) 299-0277.

INFORMATION NUMBERS

The phone number of the Hilton in Asheville is (704) 254-3211 or 1-800-733-3211. Tourist information can be obtained from the Asheville Travel and Tourism Office - (704) 258-6109 or 1-800-257-5583. Additional meeting or registration details can be obtained by contacting Wayne M. Brooks at: (919) 515-3771, FAX - (919) 515-7746.

1993 FOUNDER'S LECTURE
Asheville, North Carolina
John Nathaniel Couch:
A Founder of Entomological Mycology



John Couch
(photo by Marshall Laird
courtesy of C. Bland)

Howard Whisler
(photo by David Freeman)

In 1917, a young pre-med student at Trinity College (now Duke University) transferred to the University of North Carolina at Chapel Hill to complete his preparation to medical school. But his basic curiosity about the natural world soon brought him under the spell of the celebrated mycologist, Professor William Coker, and he moved thenceforth into the world of molds and mushrooms. The excellence of his thesis studies on the sex-life of water molds earned him a rare postdoctoral grant to study with the geneticist Blakeslee at Cold Spring Harbor and then an opportunity to accompany the Johns Hopkins Botanical Group's foray to Jamaica in 1926. During this two month expedition, John Couch became entranced with the conspicuous molds decorating the stems and branches of the tropical vegetation. Further study revealed that these fungi were living in a complex association with scale insects, a fact that signaled the start of a brilliant career in entomological mycology which continued into the late 1980's. Now, the genera, *Septobasidium, Coelomomyces, Lagenidium, and Culicinomyces* all evoke thoughts of John Nathaniel Couch.

The insect-related fungi were obviously at the heart of his research interests, but he also found time to make major contributions in developmental mycology (Oomycetes), bacteriology (Actinoplanaceae) and the biology of the lower fungi. All this while chairing the Department of Botany of the University of North Carolina for 16 years and serving as Editor of the Journal of the Elisha Mitchell Scientific Society for 15 years. An appointment to an endowed chair as the Kenan Professor of Botany, membership in the National Academy of Science, and numerous other honors, never lessened his devotion to teaching and his desire to be in the lab. Indeed, Charles Bland tells us that at age 88, during one of their last conversations, Couch remarked: "Now that we have gotten *Coelomomyces* out of the way, we need to get started on a revision of the genus *Septobasidium*!"

It is highly appropriate that our Annual Meeting in Asheville, North Carolina is recognizing the contributions of Professor Couch and his students to the general field of entomological mycology. This year's Founder's Lecture will be presented by **Howard Whisler**, Professor of Botany, University of Washington, Seattle. His own research interests extend to the Trichomycetes, Laboulbeniales, Lagenidiales, Entomophthorales and *Coelomomyces* and provide a base for appreciating Dr. Couch's influence on our discipline. Although he was not a student of J.N. Couch, their joint interest in *Coelomomyces* resulted in an extended and warm collaboration on the biology of fungal parasites of mosquitoes.

Burk, W.R. & Charles E. Bland, 1989. John Nathaniel Couch, 1896-1986, Mycologia 81: 181-189.

EDITORIAL
Time for SIP to "Reach Out and Touch Someone"

I just returned from the Beltsville Symposium XVII, Pest Management: Biologically Based Technologies, co-chaired by Barbara Leonhardt and SIPer, Jim Vaughn. The key speakers painted a very bright future for biological control with predictions that Integrated Pest Management with Biological Methods as the key component would be the norm by the year 2000. The topic of microbial control of insects was well covered by SIPers Pat Vail, Marty Shapiro, Ann Hajek, George Poinar, Bruce Carlton and Dave Fischhoff. Others covered the areas of microbial control of plant pathogens, microbial control of weeds, and use of microbials as plant promoters as well as use of semiochemicals to control insects, and arthropods to control weeds and arthropods. It was an excellent opportunity to learn what the "others" were doing. What was very evident is that the area of use of microbials as plant growth promoters or as controls of plant pathogens has made significant progress in the last few years and it is a very rapidly expanding area of research. What was disconcerting, though, was the conspicuous absence of SIPers; of over 350 registrants, under 25 were members of SIP. Could this be because the announcement for this Symposium did not appear in the SIP Newsletter? I doubt it!

There is a tremendous amount that we can learn through interdisciplinary work. Our past president Don Roberts recognized this over a decade ago and co-organized in 1983, with plant pathologist James Aist, a Rockefeller Foundation sponsored conference entitled "Infection Processes of Fungi" in order to "afford the opportunity for in-depth discussions on comparative studies by pathologists from the two host groups" (i.e. plants and arthropods). In the introduction chapter of the book "The Fungal Spore and Disease Initiation in Plants and Animals" (G.T. Cole and H.C. Hoch (Eds), Plenum Press, New York, NY. 1991) which Don entitled "Fungal-Host Interactions - Opportunities for Interdisciplinary Research" he wrote "With few exceptions, there is little contact between students of animal and plant mycoses. This book was conceived as one tool to facilitate comparing basic knowledge, current problems, and methods of research on fungal infective units and early disease events between the two host groups. If the book succeeds in its goals, it will stimulate cross-disciplinary collaboration and incite significant progress in mycoses of both host groups."

I think it is time for SIP to take a more active role to bridge the gap between invertebrate pathologists, plant pathologists and others involved in various disciplines of microbe-host relations. The field is moving ahead so rapidly, it is time we get involved lest we be left in the

dust. We could do this through back-to-back meetings with other organizations or organize special symposia at our meetings featuring the "others" research. What do you think? Letters to the Editor are welcome.

Mark Goettel
Assistant Editor

MICROBIAL CONTROL NEWS

Division on Microbial Control Activities at the Annual Meeting in Asheville.

Be sure to attend the annual business meeting and workshop of the Division on Microbial Control to be held in Asheville. The meeting is scheduled for Monday night with the business meeting first and the workshop following immediately thereafter. The business meeting will feature election of new officers as well as discussion of workshop and symposia topics for next year. The workshop for this year is entitled "Recent Activities in Product Registration" and will feature reports from various companies and other sources with products that are or will soon be registered for use. The workshop will be moderated by Ann Hajek and Michael McGuire.

This year the Division is sponsoring a symposium entitled "The use of microbial insecticides in crop protection: where are we, where are we going?" organized by George Soares and Mark Goettel. This will feature speakers from around the world and should be an informative session.

Michael McGuire
Chair, Division on Microbial Control

The Directory of Industries Involved in the Development of Microbial Control Products.

Supplement No. 1 was mailed to all SIP members with the last SIP Newsletter (vol. 25, no. 1, Feb. 1993). This supplement contains updated information and new listings. **The original directory (1991) is now sold out.** We are planning to publish an updated version in January, 1994. Deadline for submission of updates or new listings will be 1 December, 1993.

Mark Goettel
(see page 12 for address and Fax. No.)

Color Slide Atlas of Microbial Control

There are still about 100 copies of the slide atlas available. It consists of 200 slides on various aspects of microbial control projects, application techniques, bioassay, production, and formulation. The 200 slides come in a box and each slide is cross referenced to a 28-page legends.

We had at first restricted advertising of the atlas to the SIP Newsletter to allow SIP members time to purchase it. However, we have started to advertise outside of the Society in order to move our inventory. Therefore, if you intend to purchase a copy, please do so soon in order to avoid disappointment.

The slide atlas can be ordered by sending a cheque, money order or international bank draft (drawable on an American bank) in the amount of US $50.00 (add $5.00 for overseas air mail) to Dr. Ann Hajek, Boyce Thompson Institute, Tower Road, Ithaca, NY 14853-1801, USA (fax 607-254-1242; internet Ann_Hajek@qmrelay.mail.cornell.edu).

EPA Seeks Input for Proposed Changes to Experimental Use Permits and Notifications for Microbial Pesticides

On January 22, 1993, the U.S. Environmental Protection Agency published an article entitled "Microbial Pesticides; Experimental Use Permits and Notifications; Proposed Rule" in the Federal Register (40 CFR Part 172, pp. 5878-5902). The summary of this article is as follows "EPA proposes to amend its experimental use permit regulations for pesticides to clarify the circumstances under which an experimental use permit is presumed not to be required. As part of that amendment, EPA proposes to implement a screening procedure that requires notification to the Agency before initiation of small-scale testing of certain microbial pesticides. Three options for defining which microbial pesticides would be subject to the notification requirement are discussed. The Agency will review notifications to assess the potential for adverse impacts on human health or the environment and will then determine whether to require an experimental use permit. This notification scheme would implement provisions of the Agency's policy statement of June 26, 1986, with modifications."

Environmental Use Permits have long been a contentious issue. This is an opportunity to let your thoughts be known. If EUP's affect your work, be sure to obtain a copy of this article and make your comments known to EPA.

Although the comment period is "officially" closed, Fred Betz invites interested parties to "feel free to send your comments in for the docket - public record." Mail your comments to Frederick Betz, Acting Chief, Science Analysis and Coordination Staff, Environmental Fate and Effects Division, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460, Washington, D.C. 20460. Tel. 703-305-6307; Fax. 703-305-6309.

Bt Management Working Group

The *Bt*MWG is an international group of 13 companies that are developing improved *Bt*-based products for plant protection, using a variety of approaches. These approaches include traditional strain optimization, genetic improvements resulting in new combinations of insect control proteins, and novel delivery methods of *Bt*, such as transgenic plants and endophytic bacteria.

Founded in 1988, the group is committed to fostering the judicious use of *Bt*-based products, funding research to address both the potential for development of resistance to *Bt* and to develop strategies that will minimize or prevent resistance. Over the past four years we have provided $250,000 in funding, nearly 100% of our budget, for these research projects. In addition, we sponsored an insect resistance symposium at the annual meeting of the Entomological Society of America in December, 1991. At this symposium, scientists that we supported presented their research results.

Due to the diversity of *Bt*-based products, the *Bt*MWG anticipates wider use of biological pest control tactics and wishes to take a proactive approach to minimizing the threat of insect resistance to these products. *Bt* products must be wisely used in IPM programs. Technical representatives from all member companies, working as a team, meet regularly to review scientific issues related to the preservation of *Bt* as a biocontrol agent.

"The members of the *Bt*MWG recognize the importance of *Bt* as a valuable pest control resource. Investment in programs to assess and manage resistance risks represents an important component of stewardship of *Bt* products to ensure their continued value as safe and effective tools for crop protection," states Sue MacIntosh, Chair of the *Bt*MWG.

Industrial participants in the *Bt*MWG include:

Abbott Laboratories	Ecogen, Inc.
Bactec Corporation	E I DuPont de Nemours & Co.
Ciba-Geigy	ICI Agrochemicals/ICI Seeds
Crop Genetics Intl.	Monsanto Company
Mycogen Corporation	Novo Nordisk Entotech, Inc.
Plant Genetic Systems	Pioneer Hi-bred Intl., Inc.
Sandoz Crop Protection	

Scientists who have received funding through the *Bt*MWG are:

1990-91

Dr. I.A. Deloof, Zoölogisch Instituut, Leuven, Belgium

Drs. Peter Dunn & Arthur Aronson, Dept. Biological Science, Purdue University

Dr. Bruce Tabashnik, Dept. Entomology, University of Hawaii

Dr. Galen Dively, Dept. Entomology, University of Maryland

Dr. William McGaughey, USDA-ARS, Manhattan, KA

1992-1993

Drs. Anthony Shelton & Richard Roush, Dept. Entomology, Cornell University

Dr. Fred Gould, Dept. Entomology, North Carolina State University

Dr. David Heckel, Dept. Biological Sciences, Clemson University

To become a corporate member or for further information please contact: Sue MacIntosh, Chairperson, *Bt*MWG at Novo Nordisk Entotech, Inc., 1497 Drew Avenue, Davis, CA 95616 or Marnix Peferoen, Plant Genetic Systems, J. Plateaustrast 22, B-9000, Gent, Belgium for more information.

CALL FOR PROPOSALS

During 1991-1993, the *Bt*MWG has funded *Bt* research projects dealing with *Bt* mode of action and resistance totalling $120,000.

Our current funding period will end Dec. 1993. Proposals are now being solicited for the next funding period (Jan. 1994-Dec. 1994). Successful proposals will be funded for one year, with the subsequent year's funding contingent upon submission of a satisfactory progress report by Sept. 1994.

Investigators currently funded by the *Bt*MWG are also invited to submit new proposals or requests for extension of ongoing projects. These will be given equal consideration to new project proposals. Successful proposals will address one or more of the following topics (these are not listed in order of priority):

1) MONITORING: There is a need to develop a better understanding of variable susceptibility and baseline "R" (gene frequency) levels of key pest population in various agricultural areas. Methods for the detection of *Bt* resistance in field populations are needed. Standardized bioassay method for measuring *Bt* resistance levels need further development to provide reliable methodology at lower cost, greater throughput, and applicability to more researchers.

2) BASIC RESEARCH: More information is needed to better understand the mechanism of resistance in pests

such as armyworms, loopers, etc. Does *Bt* resistance in other lepidopteran pests develop in the same way as observed for diamondback moth? Cross-resistance is not well understood. Studies are needed to determine the potential for cross-resistance to develop among various *Bt* products/isolates having differing toxin compositions. What differences exist in the ability of various pest species to inherit genes for *Bt* resistance, and how genetically stable are these resistant populations?

3) *Bt* UTILIZATION STRATEGIES: Which strategies are best for using *Bt* effectively and at the same time reducing the potential for the build-up of resistance? (i.e. - refugia?; rotations?; combinations?; high dose/low dose applications?) Data from controlled field testing is needed to validate proposed models and theories.

The deadline for submission of proposals is Sept. 1, 1993. Proposals should be no longer than 10 pages and must include a clear statement of the objectives of the study relating to one or more of the topics above, an outline of proposed methods to include existing facilities and capabilities to accomplish the objectives, and a proposed budget and timeline. Abbreviated curricula vitae of the principal investigator(s) should be included with the proposal. Any number of unique proposals may be submitted by the same researcher.

Send proposals to:
Dr. Brian E. Melin
Abbott Laboratories
CAPD, D-91M
1401 N. Sheridan Rd.
N. Chicago, IL 60064.
(708) 937-8060

MICROSPORIDIA DIVISION WORKSHOP
"Biological Significance of Spore Polymorphism in Microsporidia."

We plan to explore the role of spore polymorphism as a determinant of microsporidian host or tissue cross infectivity. Members of the Society wishing to participate should come prepared to comment on or give their interpretation of recent research findings in the area of microsporidian spore structure and function. Dr. Hidetoshi Iwano (Nihon University, Japan) has agreed to discuss his recent findings on dimorphic spore development in *Nosema bombycis*. T. Kurtti will describe work with *Nosema furnacalis*, finding evidence for polymorphic spore differentiation. Other participants will be recruited.

SPECIAL FEATURE
Biotechnology Industry's Interest Grows in Baculovirus Expression Systems

Reprinted from *Genetic Engineering News* 15 March, 1993 by permission. Author: Vicki Glasner.

(**Note from your Editors:** We will occasionally reprint a short article of general interest, such as this one on baculovirus technology. We welcome submissions from the membership.)

Although the most common systems for cloning foreign genes and expressing recombinant proteins employ *E. coli*, mammalian cells or yeast, the biotechnology industry is showing a growing interest in commercial applications of baculovirus vectors for protein expression in insect cells.

While the main advantage of *E. coli* is the relative ease of making a recombinant vector and the high yield of protein (in grams/liter quantities) from these prokaryotic expression systems, *E. coli's* primary disadvantage lies in its limitations in producing eukaryotic proteins. Many proteins do not fold correctly, and *E. coli* lacks the enzymes to perform the post-transnational modifications that are required for biological activity of may eukaryotic proteins. Mammalian expression systems offer a high level of post-transitional processing, but many provide much lower yields of protein (micrograms per liter to 300-400 milligrams per liter) than are possible with *E. coli* or yeast.

In many ways, baculovirus systems offer the advantages of both *E. coli* and mammalian cell culture -- good yields (1-500 mg/L), and sufficient post-translational modification to produce recombinant proteins that are antigenically, immunogenically, and functionally similar to the original protein.

A large variety of viral, fungal, bacterial, plant and animal genes have been cloned in insect cells using baculovirus expression vectors. The insect cells contain the post-translational modification enzymes necessary to perform the glycosylation, fatty acid acetylation, alpha amidation, N-terminal acetylation, phosphorylation, cleavage of signal sequences, and some intron splicing.

With respect to glycosylation, baculovirus was once thought to be incapable of this function, but experience later showed that if proteins are not harvested until late in the infection process, such enzymatic processing is achieved. Expression of two or more proteins from a

single vector, or coinfection with multiple viral vectors enables the synthesis of dimers, heterodimers, and oligomeric complexes. The recombinant proteins produced are soluble and have undergone proper folding and disulfide bridge formation.

Commercial Applications

Baculovirus vectors broke through a major regulatory barrier in 1987 with the first FDA approval for testing of a baculovirus-produced product -- VaxSyn, a gp160 HIV vaccine made by MicroGeneSys (Meriden, CT). VaxSyn is now in Phase III clinical trials as a therapeutic vaccine. It is being given to HIV-positive patients to evaluate its effectiveness in attenuating the course of the disease, says MicroGeneSys president Frank Volvovitz. The company also intends to explore its potential as a prophylactic vaccine -- to test its ability to block infection in a HIV-negative population. The U.S. military is currently testing the gp160 vaccine in more than 600 HIV-positive individuals in Phase II trials.

(Note: VacSyn was withdrawn from a comparative clinical trial in March, 1993 -- Science 259:1821.)

MicroGeneSys now has two other baculovirus-based protein products in clinicals; a p25 HIV vaccine in Phase I trials, and a malaria vaccine, which has completed Phase I trials.

American Bio-Technologies, Inc. (Cambridge, MA) uses a baculovirus expression system to produce HIV-1 as well as HIV-2 recombinant proteins for immunodiagnostic and research applications. Enzon, Inc. (Piscataway, NJ) recently announced that the FDA had granted Orphan Drug status for its compound PEG-glucocerebrosidase for the treatment of the genetic disorder Gaucher disease. The National Institute of Mental Health developed the recombinant glucocerebrosidase using a baculovirus expression system, and Enzon applied its proprietary drug delivery technology to produce the final therapeutic protein. Enzon is also applying this technology to recombinant alpha galactosidase, developed in a baculovirus expression system to produce a drug for the treatment of the inherited metabolic disorder Fabry's disease.

Baculovirus Vectors

The term baculovirus describes a group of rod-shaped DNA viruses that infect insects. The virus particles contain a double-stranded, circular genome that can range in size from 80-150kb. The fact that baculoviruses infect only insect cells makes them safer to use than the adenoviruses or retroviruses traditionally used in mammalian cell culture. Although the baculovirus will fuse with mammalian cells, it cannot replicate, and therefore poses no risk to human handlers. Although insect cells can grow at room temperature, they are still grown in incubators or in warm rooms at 27° C because temperature fluctuations can adversely affect protein expression. The cells do not require carbon dioxide, and they grow rapidly in monolayer or suspension cultures.

The baculovirus most commonly used to express foreign genes is *Autographa californica* nuclear polyhedrosis virus (AcMNPV), originally isolated from the alfalfa looper caterpillar. AcMNPV is so named because during its reproductive cycle it produces both extracellular viral particles and occluded viral particles. The latter exist as polyhedral occlusion bodies in the host cell nucleus, in which multiple viral progeny are incased in a polyhedrin protein matrix. This protective fortress enables the virus particles to survive the death and disintegration of the infected insect as well as destructive environmental agents. On ingestion by another caterpillar, the polyhedra dissolve in the gut, releasing virus particles and repeating the lifecycle of infection and replication.

In the laboratory, AcMNPV is typically grown in cell cultures of *Spodoptera frugiperda* (the fall armyworm). In cell culture, the 29kDa polyhedrin protein is amply produced under the control of a strong promoter. The polyhedrin gene, as well as other genes used in making the occluded form of the virus, such as the p10 gene, are not needed for infection and replication of AcMNPV in cultured cells. This allows for the insertion of a foreign gene in place of the polyhedrin gene.

Construction of recombinant baculoviruses is generally performed in two steps because of the large size of the viral genome. First, the target gene is introduced into a plasmid transfer vector between a baculovirus promoter, usually the polyhedrin promoter, and the polyadenylation signal. The transfer vector and parental baculovirus DNA are then cotransfected into cultured insect cells. The baculovirus sequences surrounding the target genes recombine with the viral DNA yielding a viral genome that contains the target gene in place of the polyhedrin coding sequence.

Improvements in the viral DNA and transfection procedure have made these expression systems more "user-friendly," by dramatically increasing the percentage of recombinants from 1-2% to 75-95%, and reducing the

total time for production of recombinant virus from 4-6 weeks to 7-10 days.

The MaxBac baculovirus expression system from Invitrogen (San Diego, CA), BaculoGold kit from PharMingen, and BacPAK6 system from Clontech (Palo Alto, CA) all use a linearized baculovirus DNA, which greatly increases the percentage of recombinant versus wild-type plaques. Since the linear viral DNA cannot replicate unless it recombines with the transfection vector, the great majority of plaques will be recombinant and will contain the target gene.

MaxBac was the first baculovirus kit on the market, when about four years ago Invitrogen obtained an exclusive license to market the technology from Max Summers, Ph.D., at Texas A&M University. The MaxBac kit contains all the materials needed to purify recombinant virus, including the insect cells, transfection vectors, cell growth media, controls, a liposome-based transfection system, and PCR primers which amplify a segment of the polyhedrin coding sequence, for analysis of plaque purity.

Invitrogen's pBlueBac III transfer vector contains the β-galactosidase gene, which in the presence of chromogenic substrate, simplifies identification of recombinants based on their blue color. The company offers a new insect cell line -- High Five cells derived from *Trichoplusia ni* -- which, it claims, provide higher levels of expression than *Spodoptera frugiperda*.

BaculoGold and BacPAK6 expression systems achieve high percentages of recombinants by using linearized parental baculovirus DNA that contains a lethal deletion. Restriction of the parental DNA removes a segment of a gene essential for viral replication, which flanks the polyhedrin expression locus. Only recombinant viruses, in which the transfer vector provides the missing segment of the essential gene, with the concomitant transfer of the foreign gene, are able to replicate and produce viable virus. This high rate of recombination reduces the time-consuming task of performing plaque assays to screen for and purify recombinant virus.

Future Considerations

Several improvements are needed before insect cell systems can compete on a large scale with *E. coli*, mammalian cell, and yeast systems in the commercial arena. These include improved expression vectors that contain new cloning sites or provide higher recombination frequency with fast and easy identification and purification of recombinant virus, stable insect cell lines, lower-cost materials, more experience with growing large quantities of insect cells in bioreactors, and greater familiarity with the regulatory issues inherent in scaling up to commercial production.

New insect lines may offer improved growth properties or contain different modification enzymes that offer specific advantages in post-translational processing. There is also a need for lower-cost culture media specifically formulated to improve propagation of insect cells on a commercial scale. Experience with growing insect cells on a commercial scale will help in optimizing conditions for growth in the bioreactor, downstream processing, and purifying the recombinant proteins.

Production of baculovirus-infected insect cells is now performed as a batch process, because the virus kills the cells in the process of infection and replication. Work is underway to perfect transfer vectors that contain a secretion signal sequence short-circuiting the lytic lifecycle of the virus and enabling the recombinant protein to be secreted from the cells, and protein production to continue without reinfection.

LABORATORY PROFILE

Insect Virus Laboratories, Wuhan University, Loujia Hill, Wuchang. Whan, Hubei 430072, P.R. China.

The laboratory was founded in 1956 by the late Professor Gao Shangyin (H. ZangYing Gaw), and research fields of the laboratory have been extended during the last decades. The staff has increased from two to sixteen, including one Chair Professor (Qi Yipeng) Vice Professors (5), Lecturers (4) and other assistants and technicians. Professor Qi was accredited as a Ph.D. teacher by the National Education Committee. There are around thirty undergraduates each year, almost half of them pursuing study leading to the Masters degree. Two Ph.D. candidates and one Postdoctoral are also working in the laboratory. The major emphases of research in the laboratories include identification and characterization of pathogens, genetic engineering, and *Bacillus thuringiensis* and viral pesticides.

Recent publications of importance include:

Yipeng, Qi, et al., Construction of a new transfer vector carried polyhedrin gene of insect baculovirus. Vth International Colloquium on Invertebrate Pathology and Microbial Control Proceedings and Abstracts, 265. 1990.

Yipeng, Qi. Expression and regulation of genes for insect baculoviruses. Chinese J Virol 6(4) 383-90, 1990.

Yipeng, Qi, et al., Cloning and expression of polyhedrin gene from insect virus in E. coli cells. Acta Microbiologica Sinica 31 (1) 25-31, 1991.

Zhang, Shengjia, Yipeng, Qi. Physical mapping and partial sequences of polyhedrin gene in Buzuura supperissuria NPV genome. Chinese J Virol 7(1) 42-48, 1991.

Ma, Xiawen, Yipeng, Qi. Construction of expressive vector for B. subtilis and cloning and expression of Hepatitis B virus e gene. High Tech Lett 4, 1992.

Yipeng, Qi, et al. Construction of genetical engineering insecticide expression Bt endotoxin protein recombinant in SF cells. ibid. 6, 1992.

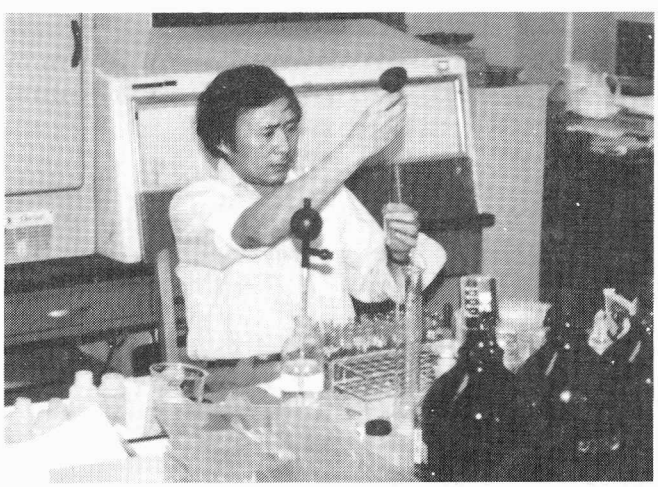
Konzo, Kanda, Yeping, Yan and Keio Aizawa. A novel phage genome integrated into a plasmid in Bt strain AF101. J of General Microbiol 135 3035-41, 1989.

Books:

Yipeng, Qi, et al. Principle and method of gene engineering. Sichaun U Press, 1990.

Insect Virology:

Yeping, Tan and Tao Tao. Wuhan Press, 1991.



Dr. Qi working at the bench.

From left to right: Ku Zhu, H. Dulmage, Mrs. Dulmage (USA), L. Padua (Philippines), Keio Aizawa (Japan), and Tan Yeping.

INVERTEBRATE PATHOLOGY IN THE NEWS
(We welcome submissions)

Nematodes: Biosys Inc of Palo Alto, California announces the development of a new type of trap for cockroaches which utilizes insect-parasitic nematodes. Inside the device, the nematodes live on a moistened pack of roach-attracting fiber. When roaches go inside, the nematodes invade the roaches, infect them with symbiotic bacteria, and multiply within the roach. Biosys is also developing products for soil-inhabiting insects.

Also news of nematodes: Agricultural Genetics Co. of England announces the discovery of a nematode which selectively infects slugs and snails.

The Asian Gypsy Moth, a recent invader into the Tacoma, Washington and Portland, Oregon (USA) regions, is being targeted for eradication by the US Department of Agriculture and the British Columbia and Agriculture Canada forestry agencies. A Bacillus thuringiensis product, "Foray", produced by Novo Nordisk, is the chosen agent in this program.

MEMBERS ON THE MOVE

Dr. Ming-Guang Feng has returned to China after spending several years as a PhD student and post-doctoral fellow in the United States and Canada working on microbial control of the Russian wheat aphid and grasshoppers. He has joined the faculty at the China National Rice Research Institute as an associate professor. His new research will focus on development of fungi for control of homopteran insects on various crops. Feng is keen on pursuing international cooperation and welcomes SIP members to establish joint projects with him or to visit his laboratory in China. His new address is: Department of Plant Protection, China National Rice Research Institute, Hangzhou, Zhejiang 310006, P.R. China.

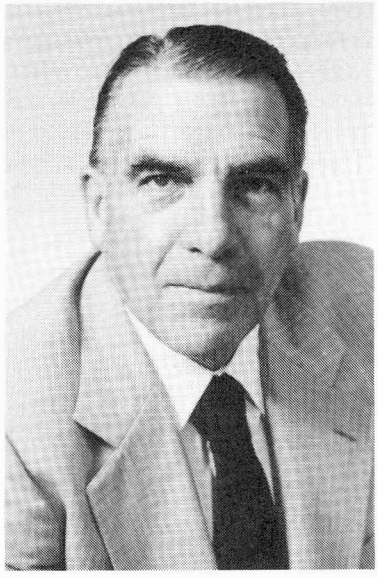
Dr. John Vandenberg, Research Entomologist with the USDA Agricultural Research Service (ARS), was transferred in April to the Plant Protection Research Unit in Ithaca, New York. John will serve as Lead Scientist for the group, well known to many SIP members, studying entomogenous fungi as biological control agents of pest insects. From 1987 to 1993, John served as Research Leader of the ARS Bee Biology and Systematics Laboratory in Logan, Utah. The focus of John's research will remain on fungal pathogens of insects, but the emphasis will turn from the diseases of beneficial insects to those of pestiferous ones. John's new address is: Plant

Protection Research Unit, USDA-ARS, Plant, Soil & Nutrition Laboratory, Tower Road, Ithaca, NY 14853, USA. Tel. 607-255-2456; Fax. 607-255-2459.

Dr. Nancy C. Hinkle has accepted the position of Extension Veterinary Entomologist at the University of California, Riverside. Her address is:

Dr. Nancy C. Hinkle
Department of Entomology
University of California
Riverside, CA 92521
Phone 909-787-2422.

OBITUARY



Dr.William G. Yendol

Dr. William G. Yendol, Professor of Entomology at Pennsylvania State University, died on March 20, 1993 at the age of 62. Dr. Yendol received his Bachelor of Science degree in agriculture from California State University and the Master of Science and PhD degrees from Purdue University. He served in the US Army during the Korean Conflict. He joined the Penn State faculty in 1965, where his research centered on microbial control of the gypsy moth. Dr. Yendol was a member of the Entomological Society of America, was President of the Eastern Branch of the ESA, and was a member of the Society for Invertebrate Pathology as well as several other professional organizations. He received the Purdue Research Foundation Scholarship, the AIBS-EPA Biological Control Task Force Group award, and the ESA J.E. Bussart Memorial Award.

Dr. Yendol is survived by his wife, Maureen, a brother, two children, and two grandchildren. Memorial contributions may be made in his name to the Entomological Research fund of the Entomology Department at Pennsylvania State University.

POSITION DESIRED

Dr. Thomas C. Cheng, recently retired from the Medical University of South Carolina, is seeking a full- or part-time position in the Washington, DC area. Dr. Cheng is a broadly-based invertebrate pathologist, and was Editor of the Journal of Invertebrate Pathology for over 20 years. Dr. Cheng may be contacted at:

Route 1, Box 58
Broad Run, Virginia 22014.
Phone 703-349-2405 or 703-347-9182.

GRADUATE RESEARCH ASSISTANTSHIP

M.S. or Ph.D. to study the ecology of a virus released for classical biocontrol in a marsh ecosystem. Stipend $12,191 plus out-of-state tuition waiver. Please contact James R. Fuxa, Dept. of Entomology, Louisiana State University, Baton Rouge, LA 70803. Phone (504) 388-1836.

MEETING ANNOUNCEMENTS

Bioremediation Risk Assessment Workshop
Duluth Entertainment and Convention Center
Duluth, Minnesota
June 17-18, 1993

Sponsored by the US Environmental Protection Agency, Environment Canada and the US Department of Agriculture. This meeting will bring together researchers from Canada, the USA and several European countries, and University collaborators from all over the world. Speakers will provide insight into regulatory oversight of environmental releases of biotechnology products and results will be presented in the areas of exposure to, effects of, and risk control of biotechnology products applied to the environment and agriculture. For further information contract:

Ms. Barbara Wireman, Meeting Coordinator
Technical Resources Inc.
c/o US EPA Environmental Research Laboratory
1 Sabine Island Drive
Gulf Breeze, Florida 32561-5299
Phone 904-934-9241
Fax 904-934-9388

Second Canberra *Bacillus thuringiensis* Meeting
Canberra, Australia
21-23 September, 1993

The Second Canberra *Bt* Meeting will review all aspects of research on *Bt* by means of invited and contributed papers and posters, with particular emphasis on work relating to the Australian region.

Symposia	Speakers
Keynote Address	Brian Federici (U of California, Riverside)
Mode of Action	Barbara Knowles (Cambridge Univ.)
Resistance	Jeroen van Rie (Plant Genetic Systems, Belgium)
	Fred Gould (North Carolina State Univ.)
Conventional Bt	Richard Milner (CSIRO Entomology, Australia)
	Victor Rajakulendran (NSW Dept. of Agriculture, Australia)
	Dudley Pinnock (Waite Institute, Australia)
	Brian Young (Spraysearch, Australia)
Transgenic Bt	Bruce Carlton (Ecogen, USA)
	Danny Llewellyn (CSIRO Plant Industry, Australia
	Derek White (AgResearch, New Zealand)
	Rebecca Harcourt (CSIRO Plant Industry, Australia)
Production	Keith Osborne (University of NSW, Australia)
	Bala Devisetty (Abbott Laboratories, USA)

Symposia	Speakers
New Opportunities	Chris Chilcott (HortResearch, New Zealand)
	Hidetaka Hori (Kubota Corp., Japan)

Fora
Evaluation of Transgenic Plants
Registration Issues
Social Issues
Role of the Bt Working Group

Abstracts of all papers will be provided at the meeting. Text of invited speakers' presentations will be published as Proceedings, and posted to delegates after the meeting.

Registration and Abstract forms due by July 5, 1993 to:
Second Canberra *Bt* Meeting
c/o Dr. David Dall
CSIRO Division of Entomology
GPO Box 1700
Canberra ACT 2601
Australia
Tel. 61 6 246 4123
Fax. 61 6 246 4000

INVERTEBRATE CELL CULTURE SOURCE BOOK: A DIRECTORY OF RESEARCHERS AND CELL LINES

The Invertebrate Division of the Tissue Culture Association is creating a directory of scientists active in invertebrate cell culture. You are invited to participate in this project. The directory will include a listing of invertebrate cell lines currently available for research. The directory will serve as a source book for persons active in invertebrate cell culture and also for those seeking expertise in invertebrate cell culture or considering the use of invertebrate cells in their research. The directory will be made available to all participants and other interested parties. If you wish to be included in the directory please send your name, mailing address, telephone and fax numbers, research interests related to invertebrate cell culture, and cell lines available to other researchers (include invertebrate, genus/species, line code, reference) to Dr. Timothy J. Kurtti, Department of Entomology, 1980 Folwell Ave., University of Minnesota, St. Paul, MN 55108.

Newsletter Editor:
Dr. Elizabeth W. Davidson
Department of Zoology
Arizona State University
Tempe, AZ 85287-1501
Phone: 602-965-7560
FAX: 602-965-2519
Bitnet: ATEWD@ASUACAD

Assistant Newsletter Editor:
Dr. Mark Goettel
Agriculture Canada Research Station
Box 3000, Main
Lethbridge, Alberta
T1J 4B1 CANADA
Phone: 403-327-4561
FAX: 403-382-3156
Internet: GOETTEL@ABRSLE.AGR.CA

FASEB:
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, MD 20814
Attn: Debbie Stoutamire
Phone: 301-530-7120
FAX: 301-530-7049

Deadline for the next Newsletter: October 1, 1993