

2008 Meeting of WERA-060

12 May 1:30 pm – 13 May 12:00 pm 2008

Hilton Hotel, Fort Collins, CO

Attendees (physically present): Mark Whalon, Carol Mallory-Smith, Bill Dyer, Sarah Ward, Scott Nissen, Andy Wyenandt, Meg McGrath, and Tom Holtzer (Administrative Advisor).

Additional participants (connected by teleconference):

Blair Siegfried, Mark VanGessel, Gerald Holmes, and Tim Dennehy.

Tim Dennehy coordinated and funded the teleconference connection to permit participation of committee members unable to travel to the meeting. All members were greatly appreciative of this opportunity to increase involvement, and are very grateful to Tim for making this possible, but acknowledged that interaction was best amongst those physically present.

Meeting Notes:

Committee Business Activities:

Mark Whalon led a discussion about the Global Arthropod Pesticide Resistance Database (APRD) and the Resistant Pest Management (RPM) Newsletter (now in its 14th year) which are housed on a server at Michigan State University. These originated from a committee on resistance that preceded WERA060. These resources are well used. APRD has almost 10,000 cases.

Andy Wyenandt was elected to be the next WERA060 Chair and Mark Whalon the Secretary/Chair-elect, to serve as Chair in 2010.

The next WERA060 meeting will be held around (e.g. immediately before, during, or after) the Sixth International IPM Symposium to be held in Portland 24-26 March 2009. Carol Mallory-Smith will handle local arrangements. The 2010 meeting will be held in Washington, DC, and arranged by Mark Whalon. This will provide an opportunity to meet with USDA CSREES staff, EPA staff covering pesticide resistance and others, as was done the last time this committee met in DC.

Possible opportunities were discussed for WERA060 to sponsor a symposium or discussion session on pesticide resistance during an up-coming scientific conference. This is a committee objective.

Committee Discussion:

Most of the meeting was devoted to informal presentations and discussion of research and extension activities pertaining to pesticide resistance and its management. Exchange of information across disciplines is the primary objective of the committee. Researchers participating in the 2008 meeting cover all three major pest disciplines (insects, weeds and pathogens). Several committee members are involved in distance learning courses

covering pesticide resistance, mostly for single disciplines. These activities were also discussed. There is a need for effort on a cross-discipline course, which WERA060 members together could achieve. Bill Dyer volunteered to lead an on-line discussion among committee members about developing an online course dealing with Pesticide Resistance and its Management.

Several resistance themes cutting across disciplines arose during the discussion. One was predicting resistance. There have been cases where field resistance was predicted to occur and it still hasn't, and cases where resistance was not predicted to occur but it did. For example, insects were found surviving on crops genetically engineered with Bt toxin gene soon after these transgenic crops were commercialized. Since these insects were highly resistant, there was concern that control failure would soon occur. However, several years later these transgenic crops continue to be an effective tool for managing insects. Insect pests tolerating high concentrations of neonicotinoid insecticides were found several years ago, resulting in great concern about the future of this new class, but they are no longer found. On the other hand, the herbicide glyphosate (Round-up) was thought to have a low risk for resistance developing. However, the selection pressure from multiple applications per season to Round-up Ready crops has resulted in resistance in some weeds.

Other topics covered included: Procedures for testing pests for resistance. Documenting its occurrence especially in commercial fields where an integrated management program is implemented. Determining impact of resistance on control. Cost of resistance. Similarities and differences in resistance management practices across disciplines. Predicting resistance. Resistance mechanisms. Laboratory versus field resistance. Funding resistance research. Working with industry on resistance issues. Challenges of getting information to growers. Andy Wyenandt shared tables he has been involved with developing that have resistance risk of fungicides for specific vegetable crop diseases.

2007 State Reports for WERA-060

Bill Hutchison
University of Minnesota

**GEOGRAPHIC EXPANSION OF A CORN EARWORM MIGRATION AND
RESISTANCE MONITORING NETWORK: EASTERN U.S.**

In cooperation with Dr. Shelby Fleischer and Information Technology (IT) colleagues at Pennsylvania State University (PSU), and funding from the NC IPM Center and the Insecticide Resistance Action Committee (IRAC), we expanded the "PestWatch" on-line tool for monitoring flights and migratory phenology of the corn earworm (CEW),

Helicoverpa zea in 2007. Prior to 2007, the PestWatch tool was limited to use in the Northeastern U.S. PestWatch is a spatially explicit and temporal database of migratory lepidopteran trap captures (www.pestwatch.psu.edu).

In 2007, via increased cooperation from private sector, industry representatives, as well as extension staff, over 70 cooperators input data from 545 trap sites in 28 states. There was a total rewrite of the software in 2007, primarily to enable this geographic expansion, improve data quality, integrate data collected at varying frequencies (daily to weekly), and to code that uses open-source software. We refer to today's version as "Pestwatch 2.0", which currently houses the 2007 data and is running for 2008. The system also archives data, prior to 2007, from the 11 northeastern states (in "Pestwatch 1.0"); in the future we plan to merge the older data into the newer software. That would also enable us to incorporate data from other locations. PestWatch is now highly animated, and allows for quick comparisons of major flight activity throughout the eastern U.S.

Beyond direct IPM applications, PestWatch has also served as a valuable tool to couple with the "ZEA-MAP" effort to track pyrethroid resistance in *H. zea*, primarily for Midwestern U.S. locations (see: <http://www.vegedge.umn.edu/ZeaMap/zeamap.htm>). Pyrethroid resistance monitoring, underway since 2004, has included three complementary methods, including: a)-traditional, in-field Adult Vial Test (AVT), where live male moths are collected from pheromone-baited Hartstack traps, b)-field-collection of late instars, lab-rearing and AVTs on F1 or F2 moths, and c)-insecticide efficacy in multi-state, replicated field trials (e.g., Hutchison et al. 2007). We have primarily evaluated cypermethrin at previously developed discriminatory doses (5 or 10 ug active/vial) in the AVTs, as per historical use in the southern U.S. In 2007, we also evaluated lambda-cyhalothrin, a commonly used pyrethroid on many crops in the eastern U.S. To date, and since 2003, we have recorded significant levels of resistance, with survival in in-field cypermethrin AVTs ranging from 2-15%, with survival in the lab-based cypermethrin AVTs often >40% (via larval collections). Survival was much less in the cyhalothrin AVTs in 2007, usually <5%. Finally, percentage control in replicated sweet corn trials in Minnesota and Wisconsin, has typically averaged only 40-50% since 2003, using standard rates of all labeled pyrethroids (e.g., Hutchison et al. 2007).

On-line IPM and IRM Tools:

ZEA-MAP: home page for all CEW Tools:

<http://www.vegedge.umn.edu/ZeaMap/zeamap.htm>

PSU Web sites:

Full access to PestWatch pages, via the tabs: www.pestwatch.psu.edu

Data-entry screen (password needed): <http://www.pestwatch.psu.edu/cgi-bin/submitPestData.cgi>

NIU-CEW Daily Forecasts:

<http://agweather.niu.edu/IMRFForecast.html>

Katherine L. Stevenson
University of Georgia

FUNGICIDE RESISTANCE IN THE GUMMY STEM BLIGHT PATHOGEN OF WATERMELON

Gummy stem blight (GSB), caused by *Didymella bryoniae*, is one of the most destructive diseases of watermelons. Cultural practices can help to reduce disease, but preventive fungicide applications are by far the most effective option for management of GSB. Field trials conducted in 1999 on watermelons in Cordele, GA indicated reduced disease suppression of gummy stem blight with azoxystrobin (Quadris) compared to previously published results. Reduced disease suppression was also noted in several grower fields of both cucumber and watermelon. Isolates from several of these fields were later confirmed to be resistant to azoxystrobin. Results of more extensive surveys conducted in Georgia during 2001 and 2002 provided evidence of widespread resistance to azoxystrobin in populations of *D. bryoniae* in Georgia watermelon and cantaloupe fields.

Following the widespread development of resistance to azoxystrobin, watermelon growers relied primarily on the newest fungicide Pristine, a combination of pyraclostrobin (QoI) and boscalid (pyridine carboxamide). Until recently, Pristine was the most efficacious of any of the products tested against GSB. However, in 2007, control failures with Pristine were noted in a small number of grower fields and research plots in Georgia. Laboratory assays of 75 isolates of *D. bryoniae* from our stored culture collection that had not been exposed to boscalid were used to establish a baseline sensitivity distribution to boscalid. Sensitivity to boscalid was determined using a mycelial growth assay on fungicide-amended medium. The same assay was used to confirm resistance to boscalid in all five isolates of *D. bryoniae* collected in 2007 from the fields and research plots where Pristine failed to control GSB. All 75 of the previously unexposed baseline isolates of *D. bryoniae* were sensitive to boscalid. The range of EC_{50} values was quite narrow, ranging from 0.0153 $\mu\text{g/ml}$ to 0.1061 $\mu\text{g/ml}$, with a median of 0.0551. Relative growth on medium containing 3.0 $\mu\text{g/ml}$ was less than 10% for all 75 isolates. In contrast, all five isolates collected from watermelon fields in 2007 were highly resistant to boscalid. EC_{50} values for these five isolates were greater than 3.0 $\mu\text{g/ml}$, the highest concentration used in the in vitro assay. Relative growth on medium containing 3.0 $\mu\text{g/ml}$ was greater than 73% for all five isolates. Based on these results, a boscalid concentration of 3.0 $\mu\text{g/ml}$ was chosen as an appropriate discriminatory concentration for further sensitivity testing. Molecular analysis of these isolates conducted by BASF scientists in Germany showed that all five resistant isolates of *D. bryoniae* contained the same mutation in the SDH B gene.

Following the initial detection of boscalid resistance in *D. bryoniae* and establishment of the baseline sensitivity distribution, additional single-lesion isolates of *D. bryoniae* were collected from GSB-infected watermelons in Georgia and northern Florida and tested for sensitivity using an in vitro mycelial growth assay on a single discriminatory concentration of 3.0 $\mu\text{g/ml}$ boscalid in PDA and a non-amended PDA control. Isolates that were able to grow on PDA containing a discriminatory concentration of boscalid (3.0

µg/ml) were considered to be resistant and those that failed to grow on the same concentration were considered sensitive to boscalid. Of the 21 isolates collected from watermelon fields in six different locations, only 8 isolates were sensitive to boscalid. These sensitive isolates were found at only two of the six locations. All isolates collected from the other four locations were resistant to boscalid. Interestingly, the field in Quincy, FL had never been planted to watermelon prior to 2007 and had no history of carboxamide use. And yet all four isolates of *D. bryoniae* collected from this field were resistant to boscalid.

Evidence collected to date suggests that boscalid-resistant isolates are common in some locations, but in other locations, the pathogen population is still predominantly sensitive. Based on our results to date, it appears that fungicide-resistant isolates may be introduced into the field as inoculum from an outside source (seed, transplants, or airborne ascospores), rather than arising from selection pressure in the form of fungicide exposure in the field. However, this evidence is based on a relatively small number of isolates and sampling locations. The sources of inoculum and fungicide resistance and the consequences for management of GSB epidemics are currently under investigation.

Beth Grafton-Cardwell
University of California Riverside

My research team monitors for insecticide resistance in California red scale and citricola scale, the leading scale pests infesting San Joaquin Valley California citrus. Organophosphate and carbamate resistance was documented using an esterase enzyme assay in a large number of populations of California red scale in the early 1990s. We continue to monitor a subset of these populations and even though organophosphate and carbamate use has been replaced by pyriproxyfen for California red scale, OP and carbamate resistance has not declined significantly. This is most likely because OPs (especially chlorpyrifos) continue to be used for citricola scale. Citricola scale is not susceptible to pyriproxyfen. During 2005-07 we documented low levels of pyriproxyfen resistance in 12% of 50 California red scale populations tested, using a fruit dip bioassay. In these bioassays, scales showed more than 10% survival of 10 ppm pyriproxyfen. These laboratory bioassays have not been correlated with field problems with California red scale control, suggesting that resistance is in the early stages. During 2006-07 we documented chlorpyrifos resistance in a large number of citricola scale populations using a 5-day leaf dip bioassay. We found that 40% of the 41 populations tested showed 20-68% survival of a discriminating concentration of 178 ppm chlorpyrifos. In the field, this level of resistance translates to single year control of citricola scale versus 3-5 years of control in the absence of resistance. In citrus, citrus thrips, which produces 6-8 generations per year, developed resistance to OPs and carbamates in the 1980s; California red scale, which has 4-5 generations per year developed resistance in the 1990s; and now citricola scale which has only one generation per year has developed resistance in the 2000s. Alternative insecticides for citricola scale control are only suppressive and biological control is ineffective in this region. Thus organophosphate resistance in citricola scale is a very serious situation.

Peter C. Ellsworth
University of Arizona

Resistance management is a critical aspect of all Integrated Pest Management (IPM) programs and activities at the University of Arizona. Our cross-commodity IPM program focuses on developing, delivering, and evaluating adoption of cross-commodity IPM recommendations aimed toward sustaining efficacy of key chemistries while providing near-term management options for key pests.

Several interrelated extension efforts comprise our cross-commodity IPM program. The Arizona Pest Management Center (APMC) and the Arid Southwest IPM Network (ASIPMN) provide organizational structure for these efforts in Arizona and the surrounding low desert regions, linking researchers and extension personnel with stakeholders and federal agencies. Through our Cross-commodity Research and Outreach Program (CROP), we engage stakeholders in the development, implementation, and evaluation of IPM guidelines that span multiple crops in our system and incorporate resistance management goals. Through the Crop Pest Losses program, we work with clientele to quantify crop losses, economic impact and pesticide use in lettuce, melons, alfalfa and cotton. Since 2005, the APMC has focused effort on the development of quantitative IPM assessment techniques that account for the spatial components of insect ecology and cross-commodity management issues. Many of our efforts have centered on a Western Regional IPM (WRIPM) Competitive grant to spatially define and analyze adoption of cross-commodity IPM guidelines for whitefly control, a major component of which is resistance management.

In 2007, we completed development of a statewide pesticide use reporting (PUR) database, which is being merged with GIS field map data to facilitate analysis of cross-commodity IPM guidelines adoption. Along with the Crop Pest Losses program, the PUR database provides rich data for identifying clientele needs, responding to federal pesticide information requests and quantifying clientele pest management and resistance management behaviors. We are proceeding with the analysis of guidelines adoption in 2008, including PCA interviews to document user rationale behind adoption and non-adoption of cross-commodity guidelines in various communities and circumstances. This qualitative analysis will help us further refine the guidelines.

The CROP & Crop Pest Losses programs include growers, PCAs, industry representatives, extension personnel and other stakeholders, and inform IPM and resistance management efforts across crops in Arizona. In addition to this direct stakeholder input, we maintain a number of important partnerships in the Cross-commodity IPM program. The PUR database was developed in partnership with the Arizona Department of Agriculture (ADA) and USDA's National Agricultural Statistics Service (AZ-NASS). The ADA and the Arizona Crop Protection Association (AzCPA) are also currently partnering with us on a complete revision of education materials for PCA licensing in Arizona. Another major cooperator is the Arizona Cotton Research and Protection Council (ACRPC), which has provided accurate and updated field maps needed for the spatially-explicit analysis of guidelines adoption.

Final analysis of cross-commodity IPM guidelines adoption will be conducted in 2008. Preliminary analyses conducted to date show significant, but less than complete, adoption of the guidelines, suggesting some impact of intensive extension education efforts in 2003-2005 (see <http://ag.arizona.edu/crops/presentations/06Bemisia%20X-IPMvF4lo.pdf> and http://ag.arizona.edu/crops/presentations/2006/Palumbo_ESA2006_IRAC.pdf). While data support that some portion of clientele is considering the guidelines in decision-making processes, we do not yet have a clear understanding of motivations for their behaviors. The significant time and resources we have invested in developing both the PUR database and the spatially-explicit data for the WRIPM project will soon pay dividends in terms of quantifying clientele pest management behaviors. In particular, for the first time, we will be able to assess when and why pest managers observe resistance management guidelines and when and why they do not. The results of this qualitative and quantitative exercise will lead us to the development of new, updated and modified guidelines for the next generation of whitefly control chemistry.

Data now available through Crop Pest Losses and the PUR database will help us quantify behaviors and plan research and educational outreach. New insecticide registrations since the guidelines were developed have made many of the same classes of insecticides available across multiple crops, including melons, leafy vegetables and cotton. Insect management behaviors of clientele across these crops will greatly impact the potential for development of insecticide resistance and field failures for some of our key control tools. Maintenance of the viability of important selective chemistries (such as neonicotinoids) through cross-commodity IPM will focus many of our activities. We continue to engage clientele on cross-commodity and resistance management concerns.

William Moar
Auburn University

PRODUCTION, CHARACTERIZATION, AND MONITORING OF BT CRY1AC RESISTANCE IN BOLLWORM, *HELICOVERPA ZEA*

Laboratory-selected *Bt*-resistant colonies are important tools for elucidating *Bt* resistance mechanisms and helping to determine appropriate resistance management strategies for *Bt* crops. Two laboratory populations of *Helicoverpa zea* resistant to *Bt* Cry1Ac, were established by selection with either Cry1Ac activated toxin (AR) or MVP II (MR) from an unselected parent strain (SC). Stable and high level resistance was achieved in AR but not in MR. AR was only partially cross-resistant to MVP II. AR was highly cross-resistant to Cry1Ab toxin but not other toxins tested. Toxin binding assays showed no significant differences, indicating that resistance was not linked to a reduction in binding.

In response to selection, heritability values for AR increased in generations 4 to 7 and decreased in generations 11 to 19. While rearing on Cry1Ac treated diet, AR had significantly increased pupal mortality, a male-biased sex ratio, and lower mating success compared to SC. AR males had significantly more mating costs compared to females.

AR had significantly higher fitness costs in involving larval mortality, weight, and period; pupal weight, period, and mortality compared to SC. Cry1Ac-resistance was not stable in AR in the absence of selection.

In laboratory experiments with field-cultivated *Bt* and non-*Bt* cotton squares AR significantly outperformed SC. However, AR could not complete larval development on *Bt* cotton. Additionally, a significantly lower percentage of AR larvae reached pupation on non-*Bt* compared with SC resistance.

Monitoring of Bt Cry1Ac resistance in field collected *H. zea* and tobacco budworm, *Heliothis virescens* continued for 2007. No change from baseline susceptibility was observed.

Michael E. Scharf
University of Florida

DEVELOPMENT OF RESISTANCE MONITORING AND MANAGEMENT PROGRAMS FOR URBAN, LANDSCAPE AND VETERINARY INSECT PESTS IN FLORIDA

Arthropod pest management in Florida poses unique challenges because of the state's large population and because of its level of urban development, prominent tourism industry, and significant agricultural holdings at the urban-agricultural interface. The demand for pest-free environments and commodities and for effective vector management has led to resistance in many of the state's pest species. Florida pest species with significant resistance concerns include cockroaches and mosquitoes (urban environment), houseflies (urban-agriculture interface), and chinch bugs and thrips (turf and landscape environments). In 2007, research continued or was initiated on resistance in each of these pest species.

M.E. Scharf (Insect Toxicology): In urban environments research projects include development of cockroach resistance monitoring and management programs for indoxacarb gel baits, and identification of negative cross-resistance factors in Diptera. Cockroach resistance to gel baits is a unique problem because of the co-evolution of behavioral and physiological resistance mechanisms. Contact and feeding assays are being developed for cockroach resistance monitoring. In mosquitoes, negative cross resistance (NCR) is being considered as a possible strategy for identifying rotation strategies for new chemistries, and for understanding how to integrate new and older insecticidal materials into existing management programs. Both mosquitoes and resistant *Drosophila* strains (as a model) are being used in NCR research.

P.E. Kaufman (Veterinary Entomology): At the urban-agriculture interface, a project funded by the Florida dairy industry is being conducted on housefly resistance management. This project is addressing resistance management proactively by selecting for resistance using new chemistries, including neonicotinoids. This work is elucidating rates of resistance evolution and cross-resistance profiles after insecticide selection.

E.A. Buss (Turf Entomology): In turf and landscape, chinch bug insecticide resistance continues to be a significant problem across Florida. In 2007, chinch bug research focused on developing laboratory rearing methods, comparison of resistance bioassays, characterization of insecticide susceptibility profiles across geographic areas, and product efficacy testing.

L.S. Osborne (Landscape Entomology): Research continued in 2007 on control and resistance management in chili thrips (*Scirtothrips dorsalis*). This invasive pest, which is capable of surviving on dozens of landscape ornamentals, is heavily targeted with insecticides. It is becoming apparent that long-term chili thrips pest management will require effective resistance management practices to preserve susceptibility.

Margaret Tuttle McGrath
Cornell University

FUNGICIDE RESISTANCE IN CUCURBIT POWDERY MILDEW

Activities pertaining to fungicide resistance in cucurbit powdery mildew conducted in 2007 in New York were monitoring of resistance in production fields, evaluating fungicides at-risk for resistance, and determining baseline sensitivity for new fungicides. Fungicides are an important tool for managing cucurbit powdery mildew to avoid losses in quantity and/or fruit quality. This is the most common disease of cucurbit crops, which include pumpkin, squash and melon. Effective control necessitates products able to move to the lower leaf surface, where this disease develops best. Unfortunately these mobile products are prone to resistance development because of their single-site mode of action, and the pathogen has demonstrated its ability to develop resistance several times with different fungicide classes. There are 8 mobile fungicides labeled for cucurbit powdery mildew in the US. Because of resistance, current recommendations often include only 3 of these: Procure, Pristine, and Quintec. Quintec is only labeled for use on melons.

A seedling bioassay was used to examine sensitivity to fungicides of the powdery mildew fungus in pathogen populations in commercial fields during the growing season. Additionally, pathogen isolates were collected and tested in the laboratory with a leaf disk assay to determine the sensitivity of individuals. The bioassay was conducted in spring squash crops in LI, NY, and also in PA. Powdery mildew starts to develop in these crops before later-planted main-season crops such as pumpkin and melon. Thus results from the bioassay are especially useful for guiding fungicide recommendations in main season crops. Resistance to MBC fungicides (FRAC code 1) and to QoI fungicides (code 11) was detected in cucurbit powdery mildew populations at the start of disease development. Since resistance to these groups is qualitative, these fungicides would have been ineffective. These results confirmed the expectation that QoI resistance is common, thereby supporting the recommendation to not use fungicides, and the results documented that MBC resistance persists under at most very limited use of this chemistry. Resistance is known to be quantitative to DMI fungicides (code 3) and suspected to be quantitative

to boscalid (code 7). Strains of the pathogen were detected able to tolerate as high as 120 ppm of the DMI myclobutanil and 200 ppm boscalid. In contrast, 5 ppm was the highest concentration tolerated of quinoxyfen (code 13), and very few isolates were able to grow on leaf tissue treated with this dose.

These findings combined with results from a fungicide evaluation conducted with these fungicides using field-grown pumpkin plants yielded valuable information for elucidating the relationship between the pathogen's sensitivity to these fungicides and their efficacy, in particular the concentration tolerable in an assay that corresponds to control failure with the fungicide due to resistance. In the fungicide evaluation, boscalid was not as effective as quinoxyfen (73% versus 87% control) while the DMI (code 3) fungicide tested (triflumizole) provided an intermediate level of control (84%). Degree of control with all 3 fungicide classes was considered good. Shifts toward greater insensitivity to these fungicides was documented in the treated plots. In commercial crops of Halloween (decorative) pumpkin, however, inadequate control was achieved with fungicide programs that included a DMI fungicide and a product containing boscalid. Quinoxyfen is not registered yet for this use.

The leaf disk assay was used to examine sensitivity to new fungicides and compare to sensitivity to registered fungicides. It was anticipated that the powdery mildew fungus would be found to be more sensitive to new DMI fungicides; surprisingly, it was found that this pathogen is less sensitive to one of these than to the DMIs currently in use. The pathogen was found to be highly sensitive to two new chemistries in development, and moderately sensitive to another new fungicide (similar sensitivity to quinoxyfen).

For another important disease of cucurbit crops, downy mildew, poor to ineffective control as a result of resistance was documented for QoI fungicides (code 11) and Ridomil fungicides (code 4).

Jeffrey G. Scott
Cornell University

CHARACTERIZATION OF SPINOSAD RESISTANCE IN HOUSE FLIES

Spinosad is a new and highly promising insecticide, derived from the bacteria *Saccharopolyspora spinosa*, with efficacy against a wide range of insects. The mechanism of action of spinosad appears to be unique, with a primary site of attack being the nicotinic acetylcholine receptor and a secondary site of attack being GABA receptors. Nicotinic acetylcholine receptors (nAChR) belong to the Cys-loop superfamily of ligand-gated ion channels that include γ -aminobutyric acid (GABA)-gated channels, glycine receptors, glutamate-gated Cl^- channels and 5-hydroxytryptamine type 3 receptors. Neural nAChRs are composed of five subunits, with a minimum of 2 α s. Receptors consisting of only α subunits are known in vertebrates, but not in invertebrates. Each subunit possesses a large N-terminal extracellular domain that includes the acetylcholine (ACh) binding site and four transmembrane domains (M1-4) with M2 contributing most of the amino acids that line the ion channel. The relatively small number of nAChR

subunits in insects is compensated for by diversification due to alternative exon use and RNA editing. Spinosad resistance has been selected for and characterized in several insect species. Resistance is monofactorial and cannot be overcome by insecticide synergists. Resistance is also recessive which makes detection of heterozygous resistant individuals by bioassay problematic. Spinosad resistance in the house fly maps to chromosome 1 and three nAChR subunit genes ($\alpha 5$, $\alpha 6$, and $\beta 3$) are predicted to exist on chromosome 1 based on *Drosophila/Musca* homology maps. However, cloning and sequencing of *Mda5*, *Mda6*, and *Md β 3* from susceptible and spinosad resistant strains of house fly found no differences that could be associated with resistance.

William E. Dyer
Montana State University

Herbicide resistance research at MSU focuses on two projects: 1) dicamba resistance in kochia (*Kochia scoparia*) and 2) multiple herbicide resistance in wild oat (*Avena fatua*).

Dicamba-resistant biotypes of kochia were examined to determine the mechanism of resistance. Since dicamba is an auxinic herbicide, resistance was unexpected by some researchers. Initial research ruled out reduced uptake and translocation as potential mechanisms of resistance. Subsequent research compared differential gene expression patterns in dicamba-resistant and susceptible kochia biotypes shortly after dicamba treatment. We have identified a number of genes differentially expressed in each biotype using differential display and suppressive subtraction hybridization. In particular, we are interested in clones with significant sequence similarity to: 1) the spliceosome U2 auxiliary factor-small subunit, 2) a 4Fe-4S ferredoxin, iron-sulfur binding protein, 3) a conserved domain (DUF862) that is part of the Permuted Papain fold Peptidases, and 4) a Fe(II)/ α -ketoglutarate-dependent hydroxylase subfamily of the mononuclear nonheme iron(II) dioxygenase enzymes. This last enzyme is capable of carrying out a known dicamba detoxifying reaction and thus may be responsible for dicamba metabolism and thus the resistance phenotype.

Wild oats resistant to four different herbicide families appeared in farmers' fields in 2006. Specifically, these biotypes are resistant to members of the Group 1 ACCase inhibitors, Group 2 ALS inhibitors, Group 8 lipid synthesis inhibitors, and an unclassified herbicide. Reversal of the resistance phenotype was achieved by greenhouse spray treatments with malathion, a known cytochrome P450 inhibitor. This result indicates that resistance may be due to induction of metabolic enzymes. Current work is identifying other herbicides in the resistance spectrum and focusing on potential cyt P450 isozyme candidates for expression analysis.

Tracy Sterling
New Mexico State University

EDUCATION COLLABORATION: COLORADO, MONTANA, AND NEW MEXICO

Activities during the Year: Web-based Weed Science educational materials for multiple type learners have been developed in collaboration with University of Nebraska's Plant and Soil eLibrary (<http://www.wsweedscience.org/Lessons/lessons.asp>). Several of these lessons have been published in the peer-reviewed, on-line journal, *Journal of Natural Resources and Life Science Education* (JNRLSE).

Using these materials, Bill Dyer, Scott Nissen, and Tracy Sterling have offered a shared, graduate-level Herbicide Physiology course (PSPP 546 Herbicide Physiology) via Distance Education from Montana State University in Fall 2007 (<http://btc.montana.edu/courses/asp/descrip3.aspx?TheID=104>). Major topics include an in-depth overview of Herbicide Mode of Action and Resistance. Eight students from across the U.S. (AZ, CO, IA, MO, MT, OR) enrolled with two dropping in 2007 because of time constraints. Students came from multiple backgrounds – those seeking M.Sc. and Ph.D. degrees as well as several from industry and consulting businesses, and one professor; this diversity really added to the quality of the discussions and insights shared. Student reviews were very favorable, emphasizing knowledge gained, clarity of expectations, and in-depth coverage of topics. This 14-week, 3-credit course will be offered every Fall semester via WebCT through the Burns Technology Center at Montana State University for ca. \$700 tuition.