2013 Meeting of WERA-60: MINUTES
Thursday, January 10, 2013
Conf. Rm 7, Marriott Rivercenter, San Antonio, TX

Attending: Katherine Stevenson (Chair), Mark Whalon (acting Secretary), Peter Ellsworth, Robert Nichols and Ryan Kurtz (Cotton, Incorporated), Andy Wyenandt, Bill McCLoskey, Lydia Brown

Call to Order: The meeting was called to order at 1:00pm by Katherine Stevenson (Chair)

WERA-60 Meeting Minutes: 2012 Minutes submitted and approved

WERA-60: Sponsored Programs, Meetings, Databases, and Newsletters:

1- **Insecticide Resistance Database** (pesticideresistance.com)

  a. 2012 = ~80,000 discreet searches lasting 10 minutes or longer annually

2- **Resistant Pest Management Newsletter** (Serialized Internationally since 1999)

  a. Vol. 22:1 8 Articles: insecticides and fungicides

3- **Fungicide Resistance Management Workshop**: Fungicide Resistance Development in North America for the 21st Century Workshop (August 3-4, 2012), Providence, Rhode Island

4- **WERA-60 Annual Meeting** (1/10/2013), held in conjunction with the 66th annual Belt Wide Cotton Production Conferences, San Antonio, TX.

Administrative Advisor Comments: by Tom Holtzer

WERA-60 Approved for Another 5 years: Western Agricultural Experiment Station Directors: NIMSS Petition for the Multistate Coordinating Committee, WERA060 Management of Pesticide Resistance: Approved Duration-Oct. 12, 2012 to Sept. 30, 2017.

2013 Meeting Presentations:

Bill McCloskey: Update on Glyphosate-resistant Palmer Amaranth in Arizona.

State Reports:

1- **Ian Heap:** Weed Resistance Database (weedscience.org) reported by Bill McCloskey and Bob Nichols

2- **Mark Whalon:** Reported upon the Arthropod Pesticide Resistance Database and the Pest Resistance Management Newsletter. Essentially, the database experienced over 60,000 visits in 2012 while the Newsletter was published biannually with the second edition published in December, 2012. A new Database revision process has been initiated and updated portions are already on-line.

3- **Peter Ellsworth:** Reported on the status of insecticide resistance in silverleaf whitefly (*Bemisia tabaci)* populations in Arizona, especially resistance to neonicotinoid insecticides. Imidacloprid has been used in leafy green vegetables, melons and cotton and has reduced populations on an area-wide basis from 1995-2004. However, from 2005 onward, whitefly populations started to increase. Acetamiprid showed some loss of susceptibility in assays in 2010; conservatively, 4 out of 16 populations were showed some tolerance. In

2011 several populations could survive 10x the recommended rate. In 2012 acetamiprid did not seem to suppress populations as well as the Neonicotinoids had performed in the early 2000's. There is some concern about the utility of neonicotinoid insecticide efficacy in the long term because of emerging resistance. Therefore, extension is supporting reducing reliance on the Neonicotinoids with intention of trying to reduce the frequency of resistant populations. Clothianidin, another neonicotinoid, use has increased in response to less effective control with acetamiprid. Pyriproxifen also has been used since 1996, but efficacy since 2005-6 has declined. To date no field failures have been reported. Pyriproxyfen represents about 10% of the insecticides used in the area and is restricted to one spray per season. In 2012 monitoring assays suggested significant reductions in Pyriproxyfen efficacy. There is also a correlation of detected Pyriproxyfen resistance, and the frequency of pyriproxifen use in the immediate proximity of the sample collection.

A lively discussion followed, addressing stewardship of pesticide efficacy with a focus on patent law, generic compounds, alternative management programs, and the interactions of industry, farmers, the green movement and U. S. policy maker's decisioning.

4- **Andy Wyenandt**: Fungicide Resistance in North America, 2nd Edition published. An overview of Fungicide Resistance Management Guidelines for Vegetable Crops Grown in the mid-Atlantic region-2013 published through resources from APS with a consortium of Andy Wyenandt, Nancy Gregory, Kathryne Everts, Steven Rideout and Beth Gugino. This is a very significant regional effort to communicate resistance management throughout the mid-Atlantic region.

5- **Katherine Stevenson**: Reported on gummy stem blight (GSB) of watermelon *Didymella bryoniae* in Georgia. Disease resistant cultivars are not available. Transmission via many different modes including transplants, overwintering on plant debris, etc. Management relies in part on pathogen-free seeds and transplants. Cultural practices like crop rotation, deep turning of crop debris, and irrigation management can help to manage the disease. However, fungicides are most important for control to date. Resistance to benomyl was first reported in 1994 and cross-resistance to thiophanate-methyl was documented. Registration of Azoxystrobin, a QoI fungicide, for use on watermelons in the late 1990s was followed by rapid and widespread development of resistance at high frequencies by 2004. With the exception of thiophanate-methyl, fungicide-resistant isolates have not been recovered from watermelon seed yet. An emergency exemption from USEPA for SDHI fungicide boscalid + pyraclostrobin (Pristine) in 2003 was followed by the first resistance detected in 2007. Tebuconazole (sterol inhibitors) was registered for use on watermelons in the late 2000s and remains very effective against GSB. Apparently only one base pair sequence change necessary to impart resistance to QoI and SDHI fungicides , therefore, a very volatile situation. Boscalid resistance was first detected in 2007 and widespread by 2010. Cross-resistance with penthiopyrad is commonplace, but not detected with fluopyram, both SDHIs. Successive research is isolating gene-specific resistance patterns (high resistance or very high resistance), and interestingly, some greater sensitivity in some instances associated with the resistance site. Therefore, a genotype-specific cross resistance pattern is emerging with this pathogen. A broad IPM program is recommended for controlling this disease and reducing risk of resistance development. In summary, resistance management is challenging because of rapid and widespread resistance to most classes of fungicides, and right now DMIs are most pressured because of their continued activity.

Discussion Topics:

1- **Funding for Pesticide Resistance Research:** Currently, USDA resistance management funding is very limited given the scale of the U. S. problems with resistance across both specialty and row crops nationally. Therefore, it is unlikely that current access to competitive grants and industry resources will address the scope and extent of resistance currently evolving in both row and specialty crops. In addition, given human population pressures and accelerating resistance occurring together with emerging resistance challenges in the genetically modified crop cultivars (GMOs); new attention is needed to bolster faltering efforts in pesticide discovery and resistance management. WERA-60 members believe that there is a crisis developing around adoption of resistance management practices in today's agriculture. Although the disciplines are committed, input from public institutions is much needed, and there is no convergence of mutual interest, plan of action, or leadership from NIFA for such a program. Research to establish new baselines and develop integrated resistance management standards could be incorporated into the USDA's budget.

2- **How to address low participation in WERA60?** It was suggested that perhaps we ought to divide along disciplinary lines and move ahead; however, given the historically low participation rates, this idea was quickly rejected. It was suggested that in the future, to be more effective and attract more participants to WERA60, we might meet in conjunction with different organizations, e.g., ESA, WSSA & APS, on a rotating schedule.

3- Why should WERA060 not serve as the focus for public institution to address resistance as a critical issue in pest management?

4- How could WERA060 attract other participants from commodities and industry?

5- **Is industry likely against the WERA mission?** Industry likely wants control, 17-year patent cycle, etc. (The focus of some companies may be on short term profitability linked to the 17-year patent life cycle. That is, when products patents are nearing expiration, companies see less value in them and do not try to extend their effective longevity. Such behavior is directly counter to the public interest which would be to maintain product efficacy at lower costs to growers.)

6- **The currently dire situation in weed management:** No new herbicide mechanisms of action (MOA) have been registered in the U. S. since 1993, and no herbicides with new MOAs are currently in development in the U. S. Resistance to one, and usually several, weed species is reported (www.weedscience.org) for the great majority of the MOAs that are principally used in crop agriculture worldwide. Evolution of wide-spread resistance to the herbicide, glyphosate, the dominant herbicide in U. S. agronomic crops, was of such concern to the U. S. pesticide regulatory community that USDA-APHIS, the agency that releases transgenic plants, and U. S. EPA, the agency that may authorize the use of herbicides upon them, formed a work group, and USDA-APHIS contracted with the Weed Science Society of America (WSSA) to prepare expert reports on (1.) herbicide resistance in plants and (2.) recommendations for management of weed resistance to herbicides. The work culminated in publication of two major articles (Norsworthy et al, 2012: Vencill et al., 2012) in a dedicated supplement to the journal Weed Science, formation of a Resistance Education Committee within WSSA (S-71), and a national symposium at George Washington University in Washington, D. C. that was jointly sponsored by the National Academy of Sciences and WSSA on address national concerns about the growth of herbicide resistance. Nine specific recommendations have officially been made by WSSA (Norsworthy et al., 2012). S-71 will meet on 2/4/13 at the annual meeting of WSSA in Baltimore and review progress on implementation of these recommendations.

Citations:

Norsworthy, J. K., S. Ward, D. Shaw, R. Llewellyn, R. L. Nichols, T. M. Webster, K. Bradley, G. Frisvold, S. Powles, N. Burgos, W. Witt, and M. Barrett. 2012. Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations. Weed Science Special Issue 60:31-62.

Vencill, W. K., R. L. Nichols, T. M. Webster, J. Soteres, C. Mallory-Smith, N. Burgos, W. G. Johnson, and M. D. Owen. 2012. Herbicide Resistance: Toward an Understanding of Resistance Development and the Impact of Herbicide-Resistant Crops. Weed Science Special Issue: 60:2-30.

Plans for 2014 Meeting and Beyond:

It was suggested that in the future, to be more effective, we might meet in conjunction with different organizations, e.g., ESA, WSSA & APS, on a rotating schedule (see discussion on participation above).

Next meeting (2014): in conjunction with WWSA meeting in Vancouver, BC, Feb 3-6, 2014

New Business:

Email List Updated

Elected Officer for 2014: Carol Mallory Smith—Chair Next Year (subject to her willingness to serve).

Adjourn: The meeting was adjourned at 6:10 pm by Katherine Stevenson (Chair)

2012 WERA60 Annual State Reports

Colorado 2012 Weed Research Update

Phil Westra & Dale Shaner, BSPM, CSU

Glyphosate Resistant Kochia. We have collected over 100 random samples of kochia from eastern Colorado where complaints of glyphosate resistant kochia have increased over the past 2 years. Kochia resistance threatens reduced till farming from the southern plains all the way into Canada.

In our greenhouse testing, some kochia plants survive up to 6 pounds of Roundup per acre. The photo below shows 2 kochia lines (2 and 4) totally killed by glyphosate while line 3 is unaffected.

Three random 2011 CO kochia accessions 4 WAT with .75 lb ae/A glyphosate: untreated con
in background; ACC 3 is 100% resistant – 27 plants. Greenhouse photo March, 2012.

Photo 1.

Molecular research has confirmed that glyphosate resistant kochia plants have many more copies of the EPSPS gene and that this makes them able to survive high rates of glyphosate (figure 1). This means that we will need to better develop other herbicides to help control kochia in various crops including Roundup Ready crops.

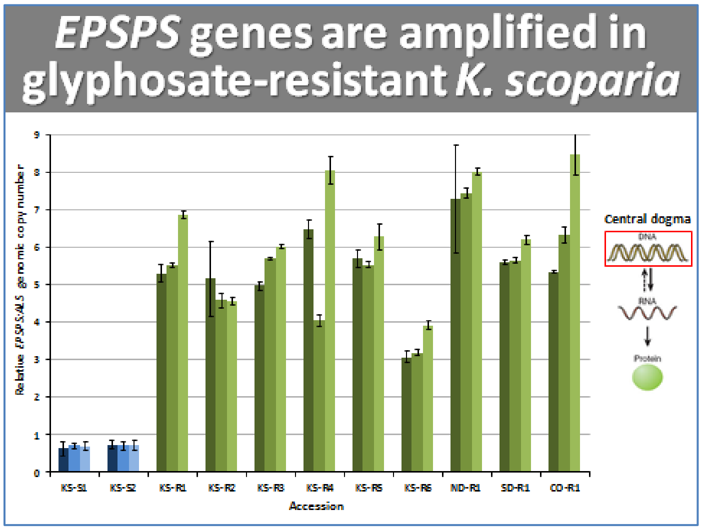
EPSPS genes are amplified in glyphosate-resistant *K. scoparia*

Figure 1.

Andrew Wiersma completed his MS on kochia in 2012, and is now doing kochia transcriptome analysis at Michigan State University.

Screening Feral Rye Response to Beyond Herbicide

The goal of this experiment was to identify resistant accessions of feral rye throughout the state of Colorado, and from this data perform an ALS assay to look at this as one of the possibilities for the mechanism of resistance. We used several teams of students in July 2012 to collect feral rye seed from 111 sites in eastern Colorado (figure 1). Each site was georeferenced and a minimum of 150 mls of seed was threshed from each accession. Seeds were planted in rows in greenhouse flats filled with commercial potting soil, and then watered and fertilized to promote good germination and growth.

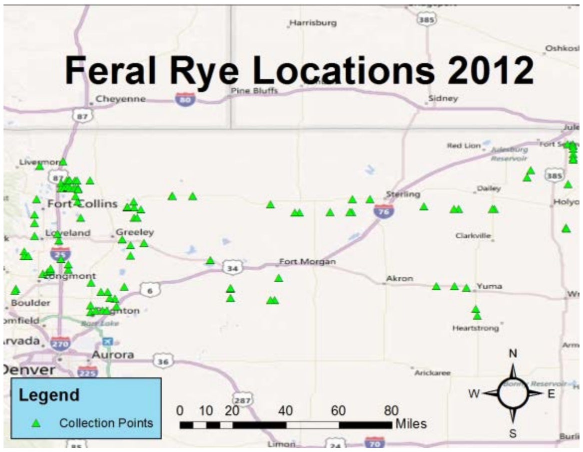

Figure 1

Once the plants reached a height of close to 4 inches, we applied Beyond herbicide at 3 oz/acre, 6 oz/acre, and 12 oz/acre (photo 1). The highest labeled rate in the field for control of feral rye with imazamox is 6 oz/acre. After spraying and allowing them to regrow for about a week, we cut all of the plants an inch from the soil, and the resistant plants quickly showed re-growth. After allowing them adequate time for regrowth, about 1 month, we sprayed all of the treated flats with the 6 oz/acre rate. We did this in order to surely eliminate any susceptible biotypes that survived the initial treatment.

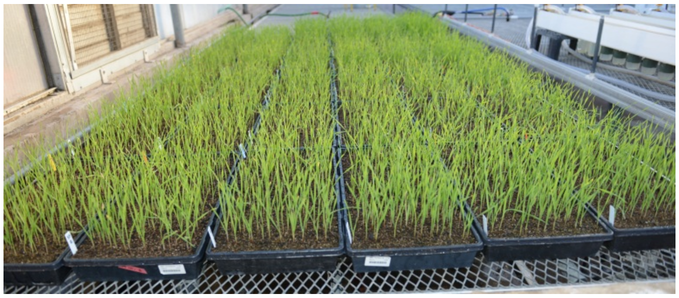

Photo 1.

After identifying possible resistant accessions, we performed an ALS assay to determine the inhibitory effect of imazamox on the resistant plants. Several feral rye accessions survived 12 oz/acre of imazamox, suggesting they would not be controlled by a field rate of the herbicide. From the assay we graphed optical density vs. herbicide concentration to see the amount of inhibition for a single susceptible accession (F1), and three resistant accessions (F 33, 21, and 13) (figure 2).

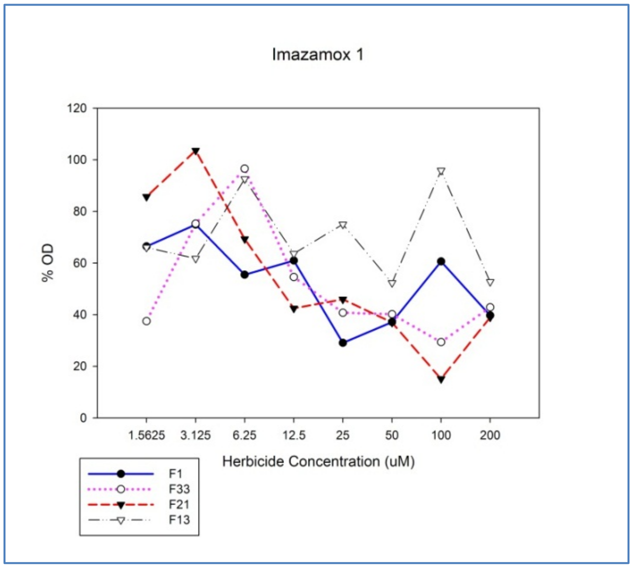

Figure 1.

To date, this research with a large number of feral rye accessions from eastern Colorado shows a wide range of responses to a given rate of Beyond herbicide. Some are controlled by 3 oz/acre of Beyond, while others are not controlled by 12 oz/acre. Ongoing research will attempt to further characterize the reasons for these differences.

Derek Sebastian, MS student working on the feral rye study.

Gene Flow from Wheat to Jointed Goatgrass

Craig Beil is finishing a MS program using Genomic In Situ Hybridization to paint each genome of wheat and jointed goatgrass a different fluorescent color. Although this is very difficult and tedious research, it will help us characterize the amount of DNA that is exchanged in gene flow between wheat and jointed goatgrass.

Glyphosate Induced Rapid Necrosis in Giant Ragweed

Christopher Van Horn, PhD student working on giant ragweed.

We have assembled a collection of 20 accessions of giant ragweed from the US and Canada with three phenotypes exhibiting differential response to lethal rates of glyphosate; glyphosate susceptible, glyphosate resistant with a slow rate normal survival response, and glyphosate resistant exhibiting rapid mature whole leaf necrosis within 24 hours following treatment. It appears that glyphosate triggers very rapid cell death at the whole leaf level, thereby excluding glyphosate in treated leaves that die and drop off the plant. Immature leaves and meristem tissues do not exhibit this response. The response requires light, and is seen in accessions from multiple sites that are distant from each other. Molecular research shows no implicated mutation in the EPSPS gene from resistant plants, and there is no indication of overexpression of EPSPS protein.

Stability of EPSPS gene amplification is Palmer amaranth across clonal generations

Darci Giacomini PhD student working on EPSPS gene amplification stability in Palmer amaranth

The stability and influence of increase EPSPS gene copy number in Palmer amaranth is being studied at the molecular level to determine the genetic mobile element responsible for the large increase in copy number seed in glyphosate resistant plants. Since some progeny with high copy number eventually revert back to a susceptible response to glyphosate, the possibility of gene silencing is being evaluated. This project contains a large bioinformatics component for sequence data analysis.

2012 WERA60 Annual Report -- Georgia

Submitted by:
Katherine L. Stevenson
Department of Plant Pathology
University of Georgia

Accomplishment: A two-day international workshop on "Fungicide Resistance Development in North America for the 21st Century" was held in August 2012 in Providence RI, in conjunction with the Annual Meeting of the American Phytopathological Society (APS). The meeting was organized and sponsored by the APS Pathogen Resistance Committee, three members of which (Katherine Stevenson, Meg McGrath, and Andy Wyendandt) are also active participants in WERA60. The workshop featured presentations from invited speakers from the major fungicide manufacturers and scientists working in the area of fungicide resistance in the major crops in North America, including tree fruits and nuts, vegetables, soybeans, corn, peanuts, sugar beets, turfgrass, ornamentals, and rice. This was the first workshop of its kind since 1987. The second edition of a book titled "Fungicide Resistance in North America" based on the proceedings from the workshop is currently in preparation, edited by WERA60 participants Katherine Stevenson, Meg McGrath, and Andy Wyendandt.
Impact: This workshop held in conjunction with the APS meeting provided a forum to highlight the current state of fungicide resistance in major North American crops and an opportunity to share information, discuss management strategies and focus attention on current fungicide resistance issues and attract interest and participation from a wider audience.

Accomplishment: Research was conducted on resistance of the fungal pathogen *Didymella bryoniae*, which causes gummy stem blight (GSB) of watermelon, to the quinone outside inhibitor (QoI) fungicide azoxystrobin. Azoxystrobin resistance in *D. byroniae* was first confirmed in 2001 and was found at high frequencies in Georgia watermelon fields by 2002. Azoxystrobin sensitivity in *D. bryoniae* (and many other fungal pathogens) is usually determined using conidial germination assays. However, mycelial growth assays are less labor intensive (do not require microscopic examination) and thus are more suitable for screening large numbers of fungal isolates. Experiments were conducted to compare the sensitivity of conidial germination and mycelial growth of *Didymella bryoniae* to azoxystrobin (AZO) to evaluate the potential utility of mycelial growth assays for detecting azoxystrobin-resistant isolates of *D. bryoniae*. Results provided evidence that mycelial growth assays on medium amended with 1.0 µg azoxystrobin/ml could be used to effectively discriminate resistant and sensitive isolates. Isolates with relative growth values ≥0.90 are AZO-R and isolates with relative growth values ≤0.70 are AZO-S.
Impact: A simple mycelial growth assay using a discriminatory concentration of 1.0 µg/m azoxystrobin serves as an efficient tool for monitoring the frequency of azoxystrobin resistance in populations of the gummy stem blight pathogen and evaluating various fungicide programs for GSB management that include azoxystrobin (or other QoI fungicides).

Accomplishment: Resistance of the cucurbit gummy stem blight pathogen, *Didymella bryoniae*, to succinate-dehydrogenase-inhibiting (SDHI) fungicides boscalid and penthiopyrad was recently reported in the southern U.S. However, cross-resistance to the SDHI fungicide fluopyram has not yet been reported in this pathogen and isolates resistant to boscalid and penthiopyrad were confirmed as sensitive to fluopyram based on in vitro mycelial growth assays. In this study, baseline sensitivity to fluopyram was established using 98 isolates of *D. bryoniae* with no previous exposure to SDHI fungicides, using an *in vitro* mycelial growth assay on two different types of medium amended with fluopyram. The baseline will be used as a basis for establishing discriminatory concentrations for further

resistance monitoring. Although yeast bactone acetate (YBA) medium has been recommended for SDHI sensitivity assays, based on our results, the mycelial growth assay using fungicide-amended potato dextrose agar medium was more reliable than YBA and produced more consistent results for determining sensitivity of this pathogen to fluopyram.
Impact: The baseline sensitivity of *D. bryoniae* to fluopyram will serve as a basis for establishing discriminatory concentrations for further resistance monitoring in this pathogen.

LIST OF RELEVANT PUBLICATIONS

Avenot, H., Thomas, A., Gitaitis, R. D., Langston, D. B. Jr., and Stevenson, K. L. 2012. Molecular characterization of boscalid- and penthiopyrad-resistant isolates of *Didymella bryoniae* and assessment of their sensitivity to fluopyram. Pest Management Science 68:645–651.

Stevenson, K. L., Keinath, A. P., Thomas, A., Langston, D. B. Jr., Roberts, P. D., Hochmuth, R. C., and Thornton, A. C. 2012. Boscalid insensitivity documented in Didymella bryoniae isolated from watermelon in Florida and North Carolina. Plant Health Progress doi:10.1094/PHP-2012-0518-01-BR.

Thomas, A., Langston, D. B. Jr., and Stevenson, K. L. 2012. Baseline sensitivity and cross-resistance within succinate-dehydrogenase-inhibiting fungicides and demethylation-inhibiting fungicides in *Didymella bryoniae*. Plant Dis. 96:979-984.

Thomas, A., Langston, D. B. Jr., Sanders, H. F., and Stevenson, K. L. 2012. Relationship between fungicide sensitivity and control of gummy stem blight of watermelon under field conditions. Plant Disease (in press).

Report of the MSU Arthropod Pesticide Resistance Database and Resistance Pest Management Newsletter (WERA 60 “Management of Pesticide Resistance”) 2012

Mark E. Whalon, David Mota-Sanchez, Brittany Harrison and Rebeca Gutierrez. Department of Entomology. Michigan State University, East Lansing, MI48864

MSU Arthropod Pesticide Resistance Database

1) Accomplishments. The occurrence of pesticide resistance frequently leads to the increased use, overuse, and even misuse of pesticides that pose a risk to the environment, phytosanitation, market access, global trade, and public health. It can also result in serious economic loss and social disruption. The economic impact of pesticide resistance in the US has been estimated at $1.4 billion to over $4 billion annually (Pimentel et al 1991, 1993). Arthropods have been evolving for millions of years to defeat natural toxins. Since the first written report of insecticide resistance was published in 1914 by Melander, 597 species, 354 compounds, and 11,403 cases of pesticide resistance have been counted (Figure 1), most of which have been recorded over the last 60 years of intensive pesticide use. Most of the cases were found in agricultural, forest and ornamental plants (65.9%). Another 30.6% occurred in medical, veterinary and urban pests. Only 3.1% of the cases reported described the development of resistance in natural enemies such as predators and parasitoids, 0.4% in other species such as pollinators, and non-target insects. Conventional insecticides (organochlorines, organophosphates, carbamates and pyrethroids) make up about 85.2% of the total resistance cases. We have observed that there is an increase in the number of resistance cases in groups of compounds with novel chemistries and modes of action such as insect growth regulators, avermectins, neonicotinoids, IGRs, bacterial agents (Bts) and spynosins, among others.

In addition, the Insecticide Resistance Action Committee (IRAC) has reported resistance grouped by *insecticide mode of action*. These reports are hosted in our MSU arthropod pesticide resistance database at: http://www.pesticideresistance.org/irac/1/. The IRAC database content reflects the current working knowledge of a wide range of experts from industry, academia, and state and local cooperative extension, with IRAC making the ultimate decision on rankings of resistance status. IRAC makes no claim of completeness or accuracy because situations can change quickly due to many factors.

2) Impacts. Our database is visited frequently; recording about 58,000 page views to our web site (www.pesticideresistance.org) per year, and is perhaps one of the most complete databases in resistance of organisms to xenobiotics. It is our intention that this effort in reporting arthropod pesticide resistance should contribute to the design of better alternatives for resistance pest management; and in the end contribute to the world’s effort to reduce hunger, and improve human and animal health and food security.

3) Publications:

Whalon, M.E., Mota-Sanchez, D. and Robert M. Hollingworth. 2013. Arthropod Pesticide Resistance Database 2013. On-line at: www.pesticideresistance.org

This effort in reporting resistance was supported through a partnership between the Insecticide Resistance Action Committee (IRAC), USDA/CSREES/IPM, WERA 60, USDA and Michigan State University.

Resistant Pest Management (RPM) Newsletter

Accomplishments and impacts.

The Resistant Pest Management (RPM) Newsletter was developed to spread knowledge of resistance around the world. The goal of the RPM Newsletter is to inform researchers, industry workers, pesticide policy and field personnel worldwide of ongoing changes and advances in pesticide resistance management, provide an archival resource to national and international policy leaders, and enhance communication of ideas among resistance managers worldwide. Since its 1989 inception, the Newsletter has published over 680 articles, including 17 articles in 2012. The Bi-annual publication has over 1,150 electronic subscribers (mostly in government, industry and academia), and hard copies are now part of 60 libraries serial listings worldwide. Example countries with serial listings include the United States, Germany, Italy, the United Kingdom, India, Japan, Taiwan, Egypt, Kenya, Costa Rica, Australia, Malaysia, Pakistan and New Zealand.

Publications:

Resistant Pest Management Newsletter. 2012. A Biannual Newsletter of the Center for Integrated Plant Systems (CIPS) in Cooperation with the Insecticide Resistance Action Committee (IRAC) and the Western Regional Coordinating Committee (WRCC-60) Vol. 22, No. 1 (Fall 2012). On-line at: **http://whalonlab.msu.edu/Newsletter/pdf/22_1.pdf**

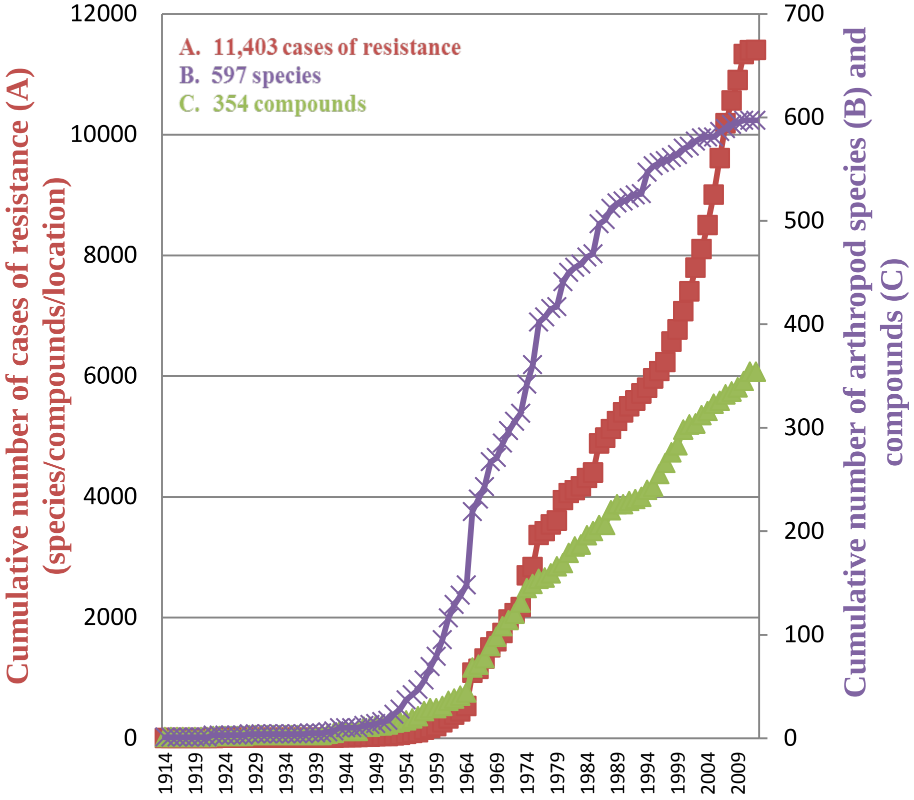

Fig 1. Evolution of arthropod insecticide resistance from 1910 to 2012. (species, compounds and total number of case)

Source:

Whalon, M.E., Mota-Sanchez, D. and Robert M. Hollingworth. 2013. Arthropod Pesticide Resistance Database 2013. On-line at: www.pesticideresistance.org

WERA60 2012 Annual Report

PROJECT TITLE: Physiological and Ecological Evaluation of Metabolism-Based Herbicide Resistance in *Avena fatua* (Wild Oat)

PROJECT COORDINATOR: William E. Dyer, Ph.D.
Organization: Montana State University
Address: Department of Plant Science and Plant Pathology
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E-mail address: wdyer@montana.edu
Fax No.: (406) 994-1848

Overview: Significant progress was made on all objectives. Competitive funding in the amounts of $500,000 (USDA/NIFA) and $50,000 (Environmental Protection Agency Strategic Agricultural Initiative Program) was obtained to conduct this research.

Accomplishments:

Objective 1. Characterize the molecular regulation of multiple herbicide resistance in the *A. fatua* HRm biotype.

The MHR wild oat biotypes MHR3 and MHR4 were derived from seeds collected in 2006 from two wild oat populations not controlled by 60 g a.i. ha^{-1} pinoxaden in two production fields separated by approximately 8 km in Teton County, Montana, USA. Herbicide susceptible biotype HS1 was derived from seeds of untreated plants in an adjacent field, and a second susceptible biotype HS2 is the nondormant inbred SH430 line used in seed dormancy research. Dose response experiments showed that the MHR biotypes are resistant to nine herbicides from five mechanism of action families, including three acetyl-CoA carboxylase (ACCase) inhibitors, three acetolactate synthase (ALS) inhibitors, the carbamothioate herbicide triallate, the membrane disruptor paraquat, and the growth inhibitor difenzoquat. Pre-treatment with the cytochrome P450 inhibitor malathion indicates that P450-mediated enhanced metabolism rates may be associated with resistance to flucarbazone (both MHR biotypes), imazamethabenz (MHR4), difenzoquat (MHR4), and pinoxaden (MHR3), but not for tralkoxydim, fenoxaprop-P, or triallate.

DNA sequencing of the ACCase CT Domain and an ALS domain known to contain single nucleotide polymorphisms conferring herbicide resistance did not reveal any mutations associated with resistance in either MHR biotype.

Polymerase chain reaction (PCR) using primers based on the *O. sativa CYP81A6* sequence generated five unique but highly homologous (96-99% nucleotide identity) clones which shared 92-93% and 80-81% amino acid identity with the *L. rigidum CYP81B1* and the *O. sativa CYP81A6* cDNAs, respectively.

In Northern hybridizations, the labelled 212-bp subfragment from *AfCYP81L* hybridized to a 1550-base band in RNA from HS and MHR biotypes. *AfCYP81L* mRNA levels were unchanged in HS1 and HS2 plants, but levels were consistently higher in MHR plants and increased in MHR3 plants after herbicide treatment. Thus, in both experiments using independent seedlots, *AfCYP81L* expression was constitutively higher in the MHR4 biotypes than the HS biotypes, and mRNA levels increased after herbicide treatment of MHR plants. Further, levels of *AfCYP81L* mRNA were induced to much higher levels than previously observed when HS1 and MHR4 plants were pre-treated with malathion before imazamethabenz treatment. Significantly, elevation of *AfCYP81L* mRNA levels in both HS and MHR plants indicates that malathion induces *CYP* gene expression in wild oat plants regardless of herbicide resistance phenotype. To our knowledge, this is the first report of malathion-induced *CYP* gene expression in any plant species.

The qualitative differences between HS and MHR *AfCYP81L* expression levels we observed in Northern analyses were subsequently confirmed and quantified in qPCR assays.

Objective 2. Evaluate biological and environmental stressors determining fitness costs associated with enhanced herbicide metabolism in the *A. fatua* HRm biotype.

We investigated two MHR wild oat (*Avena fatua* L.) populations from Montana, USA and hypothesized that they would exhibit fitness costs compared to two herbicide susceptible (HS) populations. This was accomplished in greenhouse studies, where we assessed differences between MHR and HS populations in seed germination, plant growth, and reproduction.

We did not detect differences in seed germination rates across the four wild oat populations, and there were slight trends but no significant differences in relative growth rates at low or high nitrogen levels. Similarly we did not detect consistent differences in the ratio of root to shoot resource allocation among wild oat MHR and HS populations. Overall, our results do not indicate a consistent fitness cost, and thus generally do not strongly support expectations emerging from the resource-based allocation theory that MHR population should be less fit than HS populations.

Greenhouse competition studies between wild oat and wheat plants did not reveal signficant differences in competitive ability of MHR or HS wild oats in the presence of wheat under several levels of nitrogen stress. As expected, wild oat biomass declined with increasing wheat biomass, although this relationship was minimal in the no nitrogen treatments where both wild oat and wheat biomasses were quite low compared to in the 50 and 100 kg N ha-1 treatments. The similar pattern observed with respect to wild oat RGR can also be attributed to the low wheat biomass in the unfertilized pots compared to fertilized ones. More importantly, there were no differences in the response of the HS and MRH populations to wheat competition or nitrogen stress, suggesting that there were no growth-related fitness costs for herbicide resistance.

Objective 3. Develop and deliver an extension program to educate producers and land managers about preventing and managing enhanced-metabolism herbicide resistance.

Between 2010 and 2013, a total of 24 extension presentations on herbicide resistance prevention and management were delivered across Montana. These presentations directly reached an estimated audience of 928 attendants. Additionally, herbicide resistance was discussed at the 2011 Crops and Weeds Field Day in front of 65 farmers and agricultural professionals. We plan to present the results at the 2013 Crops and Weeds Field Day.

Impacts: These studies provide initial characterization of the MHR phenotype in wild oats. Resistance to multiple herbicides may be due in part to enhanced cytochrome P450-based metabolism, and we report the first example of a cytochrome P450 mRNA with elevated constitutive and inducible (by malathion and herbicide treatment) expression in any MHR biotype of any species.

Our greenhouse studies do not support the presence of fitness costs associated with the MHR phenotype in wild oats, which is in conflict with the resource allocation theory.

Outputs:

Extension Bulletins

Menalled, F. Weed management lessons from a dry and hot summer. Produced 8/21/2012. MSU IPM Bulletin.

Menalled, F. Spring cropland weeds IPM, news and update. Produced 03/11/11. MSU IPM Bulletin.

Publications and Presentations in Professional Meetings

Lehnhoff, E., B. K. Keith, W. E. Dyer, R. K. Peterson, and F. D. Menalled. *In press*. Characterization of multiple herbicide resistance in wild oat (*Avena fatua*) and its impacts on physiology, germinability, and seed production. Agronomy Journal.

Lehnhoff, E. A., B. Keith, W. Dyer, and F.D. Menalled. *In Press*. Does multiple herbicide resistance modify crop-weed competitive interactions? Impact of biotic and abiotic stresses on multiple herbicide resistant wild oat (*Avena fatua*) in competition with wheat (*Triticum aestivum*). PLOS One.

Keith, B., E. Lenhoff, E. Burns, F. Menalled, and W Dyer. *In review*. Elevated constitutive and inducible expression of a Cytochrome P450 mRNA in multiple herbicide resistant wild oat (*Avena fatua* L.).

Mayes, E, Z. Miller, and F. Menalled. 2013. Is there a cost of herbicide resistance?: Effects of environmental and biological stressors on fitness of herbicide susceptible and multiple herbicide resistant *Avena fatua* L. (wild oat) biotypes. Western Society of Weed Science Meeting. March 11-14, 2013. San Diego, CA.

Lehnhoff, E., F. Menalled, B. Keith, and W. Dyer. 2012. Fitness costs of multiple herbicide resistant wild oat. Western Society of Weed 65th Annual Meeting. March 12-15, 2012. Reno, Nevada

Miles, G., E. Kalinina, and W.E. Dyer. 2011. Investigation of multiple herbicide resistance in *Avena fatua* L. Montana State University Undergraduate Scholars Conference, March, 2011.

Boyd, M., B. Keith, and W.E. Dyer. 2012. Are glutathione S-transferases involved in multiple herbicide resistance in *Avena fatua*? Montana State University Undergraduate Scholars Conference, March, 2012.

Davis, E.S., W.E. Dyer, and F. Menalled. 2013. Herbicide Resistant Wild Oat Occurrence in Diverse Cropping Systems: Two Case Studies. Proc. West. Soc. Weed Sci. 66:nnn.

Rangasamy, M. and B.D. Siegfried. 2012. Validation of RNA interference in western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) adults. Pest Manag. Sci. 68: 587-591.

BACKGROUND: RNA interference (RNAi) is commonly used in insect functional genomics studies and usually involves direct injection of double-stranded RNA (dsRNA). Only a few studies have involved exposure to dsRNAs through feeding. For western corn rootworm (Diabrotica virgifera virgifera) larvae, ingestion of dsRNA designed from the housekeeping gene, vacuolar ATPase (vATPase) triggers RNAi causing growth inhibition and mortality; however, the effect of dsRNA feeding on adults has not been examined. In this research, WCR adults were fed with vATPase-dsRNA-treated artificial diet containing a cucurbitacin bait, which is a proven feeding stimulant for chrysomelid beetles of the subtribe Diabroticina to which rootworms belong.

RESULTS: Real-time PCR confirmed suppression of vATPase expression and western blot analysis indicated reduced signal of a protein that cross-reacted with a vATPase polyclonal antiserum in WCR adults exposed to artificial diet treated with dsRNA and cucurbitacin bait. Continuous feeding on cucurbitacin and dsRNA-treated artificial diet resulted in more than 95% adult mortality within 2 weeks while mortality in control treatments never exceeded 20%.

CONCLUSIONS: This research clearly demonstrates the effect of RNAi on WCR adults that have been exposed to dsRNA by feeding and establishes a tool to screen dsRNAs of potential target genes in adults. This technique may serve as an alternative to target screening of larvae which are difficult to maintain on artificial diets.

Chen, H., H. Wang, and B.D. Siegfried. 2012. Genetic differentiation of western corn rootworm populations (Coleoptera: Chrysomelidae) with resistance to insecticides. Ann. Entomol. Soc. Am. 105: 232-240.

As the single most important pest of field corn, Zea mays L., throughout most of the Corn Belt, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), has undergone repeated selection for resistance to a variety of insecticides that persist widely among Nebraska populations. In this study, we used 11 microsatellite markers to genotype two populations with high levels of resistance to methyl-parathion and aldrin (Polk and

Stromsburg), two populations with low and intermediate levels of resistance (Mead and Clay Center) from Nebraska, and one population from outside the Corn Belt (Safford, AZ). The genetic diversity measured by observed heterozygosity (H_0) was reduced 15–32% in the highly resistant populations compared with the more susceptible populations in Nebraska. Significant genetic differentiation was detected between the resistant and susceptible populations (Polk and Stromsburg versus Mead and Clay Center) in Nebraska (FST = 0.016) and between all the populations from Nebraska and Arizona (FST = 0.059). The average observed heterozygosities in the populations were positively correlated with insecticide susceptibility based on mortality at diagnostic concentrations of aldrin and methyl-parathion, respectively. These results indicate that the insecticide selection from exposure to aldrin and methyl-parathion may be a contributing factor in shaping the genetic structure of western corn rootworm populations in Nebraska. Factors including isolation by distance and a Wolbachia-induced breeding barrier may have contributed to differentiation of rootworm populations from Nebraska and Arizona.

Coates, B.S., A. Alves, H. Wang, K. Walden, B. W. French, N.J. Miller, C.A. Abel, H.M. Robertson, T.W. Sappington, and B.D. Siegfried. 2012. Distributions of genes and repetitive elements in *Diabrotica virgifera virgifera*: prelude to assembling a large, repetitive genome. J. Biomed. Biotechnol. doi:10.1155/2012/604076.

Abstract: Feeding damage caused by the western corn rootworm, Diabrotica virgifera virgifera, is destructive to corn plants in North America and Europe where control remains challenging due to evolution of resistance to chemical and transgenic toxins. A BAC library, DvvBAC1, containing 109,486 clones with 104 ± 34.5 kb inserts was created, which has an ~4.56X genome coverage based upon a 2.58 Gb (2.80 pg) flow cytometry-estimated haploid genome size. Paired end sequencing of 1037 BAC inserts produced 1.17 Mb of data (~0.05% genome coverage) and indicated ~9.4 and 16.0% of reads encode, respectively, endogenous genes and transposable elements (TEs). Sequencing genes within BAC full inserts demonstrated that TE densities are high within intergenic and intron regions and contribute to the increased gene size. Comparison of homologous genome regions cloned within different BAC clones indicated that TE movement may cause haplotype variation within the inbred strain. The data presented here indicate that the D. virgifera virgifera genome is large in size and contains a high proportion of repetitive sequence. These BAC sequencing methods that are applicable for characterization of genomes prior to sequencing may likely be valuable resources for genome annotation as well as scaffolding.

Siegfried, B.D. and R.L. Hellmich. 2012. Understanding successful resistance management: The European corn borer and Bt corn in the United States. Special Issue of GM Crops and Foods 3: 184-193.

Abstract: The European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) has been a major pest of corn and other crops in North America since its accidental introduction nearly a hundred years ago. Wide adoption of transgenic corn hybrids that express toxins from Bacillus thuringiensis, referred to as Bt corn, has suppressed corn borer populations and reduced the pest status of this insect in parts of the Corn Belt. Continued suppression of this pest, however, will depend on managing potential resistance to Bt corn, currently through the high-dose refuge (HDR) strategy. In this review, we describe what has been learned with regard to *O. nubilalis* resistance to Bt toxins either through laboratory selection experiments or isolation of resistance from field populations. We also describe the essential components of the HDR strategy as they relate to *O. nubilalis* biology and ecology. Additionally, recent developments in insect resistance management (IRM) specific to *O. nubilalis* that may affect the continued sustainability of this technology are considered.

WERA-060 2012 Annual Report for New Jersey

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Fungicide resistance management in vegetable crop production continues to be a major focus in New Jersey as well as the rest of the mid-Atlantic region (PA, DE, MD, and VA). The 7th edition of the Fungicide Resistance Management Guidelines for Vegetable Crop Production in the mid-Atlantic Region was published in 2012. Since 2007, over 15,000 of these guides have been distributed to growers, extension agents and specialists, crop consultants, and industry representatives throughout the region representing to our best estimates between 75,000 to 100,000 A of commercial vegetable production.

Publications: none

WERA-060 2012 Annual Report for New York

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New York

FUNGICIDE RESISTANCE IN CUCURBIT POWDERY MILDEW

Activities pertaining to fungicide resistance in cucurbit powdery mildew being conducted in New York are 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. Only 3 of the 5 fungicide chemical groups labeled for cucurbit powdery mildew in the US currently are recommended: FRAC Codes 3, 7, and 13. Resistance to FRAC Code 1 and 11 fungicides has been shown to be generally common through previous research conducted in NY. Spores of this pathogen (*Podosphaera xanthii*) can be wind dispersed long distances enabling widespread dispersal of resistant strains.

Sensitivity to fungicides was examined for 55 pathogen isolates collected at the end of the 2011 growing season from commercial and research cucurbit fields. A leaf disk bioassay was used. Resistance to QoI fungicides (FRAC code 11) was detected in 79% of the isolates tested (not all isolates were tested with this fungicide). Resistance to this fungicide chemistry is qualitative, thus pathogen isolates are either sensitive or resistant, and fungicides are ineffective against resistant isolates. There is a fungicide (Pristine) with a FRAC code 11 active ingredient that has continued to be recommended because it contains another active ingredient (FRAC code 7). Applying Pristine could select for pathogen strains resistant to FRAC code 11 fungicides, thereby maintaining this resistance in the pathogen population. Resistance to most fungicide chemistry is quantitative, including active ingredients in Pristine, Procure, Rally, and Quintec. With this type of resistance, pathogen isolates exhibit a range in sensitivity. Several concentrations are used in assays to characterize sensitivity. Ability to grow on leaf disks with a high concentration (500 ppm) of boscalid, an active ingredient in Pristine, was detected in only 6% of the pathogen isolates tested. This concentration is in the range of what would be in the spray tank when Pristine is applied at labeled rates, therefore isolates tolerating 500 ppm are likely fully resistant to this fungicide, which means they would not be controlled by Pristine. Each of the three isolates were obtained from a different farm. In contrast, 43% of the isolates collected in 2010 from similar locations were resistant. With myclobutanil, the active ingredient in Rally, a DMI (FRAC code 3) fungicide, 4% of isolates tolerated 80 ppm, 33% tolerated 40 ppm, while 16% were sensitive to 10 ppm. The concentration in the spray tank would be 150 ppm for Rally applied at the lowest label rate (2.5 oz/A) and 50 gpa. With quinoxyfen, the active ingredient in Quintec (FRAC code 13) fungicide, 4% of isolates tolerated 80 ppm, 24% tolerated 40 ppm, while 22% were sensitive to 10 ppm. The concentration in the spray tank would be 141 ppm for Quintec applied at the lowest label rate (4 fl oz/A) and 50 gpa. One of the two isolates able to tolerate 80 ppm quinoxyfen was also resistant to boscalid Sensitivity to Topsin M was examined for some isolates to determine if the pathogen is maintaining resistance to this old fungicide. It was found that resistance continues to be common to this fungicide group (MBC; FRAC code 1); however, there were fewer resistant isolates in 2011 (50%) than in previous years when most isolates were resistant (97% in 2010).

Efficacy of fungicides at-risk for resistance was assessed in a replicated experiment conducted in 2012 with products applied individually to pumpkins using a tractor-sprayer. These results in comparison with those from experiments in previous years provides some indication when the pathogen might be developing resistance. Among currently registered fungicides, Pristine (FRAC Code 7 and 11) applied at its highest label rate was ineffective. In previous years at this location, pathogen isolates resistant to both components of this fungicide have been detected, and the fungicide has exhibited variable performance in previous evaluations. Powdery mildew also was not effectively controlled by Fontelis (FRAC 7), a chemically-related fungicide registered in 2012. Procure (FRAC 3) applied at its highest label rate was effective through 28 August on both leaf surfaces. Quintec (FRAC 13) was highly effective through the last assessment on 6 Sep when the other registered fungicides were no longer effective.

A seedling bioassay was conducted in research fields early in the 2012 season to obtain estimates of the proportion of the pathogen population able to tolerate fungicides at the concentrations applied to the seedlings. Strains of the pathogen were detected able to tolerate 500 ppm boscalid (active ingredient in Pristine), 120 ppm myclobutanil (Rally) and 10 ppm quinoxyfen (Quintec). Ability to tolerate 500 ppm boscalid is of concern because this concentration is in the range of what would be in the spray tank when Pristine is applied. Resistance is common to the other active ingredient in Pristine, which is in FRAC code 11. Therefore Pristine would not be expected to be able to control these strains. On average, a lower proportion of the pathogen populations were able to tolerate 10 ppm quinoxyfen than 500 ppm boscalid. Therefore Quintec was expected to be the most effective fungicide in 2012. Quintec was very effective while Pristine was ineffective in the fungicide efficacy experiment.

2012 Publications

McGrath, M. T., and Hunsberger, L. K. 2012. Efficacy of fungicides for managing cucurbit powdery mildew and pathogen sensitivity to fungicides, 2011. Plant Disease Management Reports 6:V080.

WERA-060 2012 Annual Report for New York

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1) Accomplishments

We investigated the population genetics of target site resistance to pyrethroid insecticides in house flies and Colorado potato beetles. We found that for both species there were multiple evolutionary origins of kdr (Vssc mutation L1014F). In addition, house flies have super-kdr and kdr-his alleles. These alleles also have multiple evolutionary origins, although super-kdr appears to evolve only in flies that already have the kdr mutation.

2) Impacts

Pyrethroid insecticides continue to be widely used, but the evolution of resistance is reducing their effectiveness in many locations (against many species). Our finding of multiple evolutionary origins for resistance mutations in Vssc indicate resistance management must focus on a relatively localized resistance management plan, as immigration is not necessary for this type of resistance to appear in a population.

3) Publications relevant to this project, since the last report.

Rinkevich, F. D., Hedtke, S. M., Leichter, C. A., Harris, S. A., Su, C., Brady. S. G., Taskin, V., Qiu, X. and Scott, J. G. 2012. Multiple origins of kdr-type resistance in the house fly, Musca domestica. PLOS One 7:e52761.

Rinkevich, F. D., Su, C., Lazo, T. A., Hawthorne, D. J., Tingey, W. M., Naimov, S. and Scott, J. G. 2012. Multiple evolutionary origins of knockdown resistance (kdr) in pyrethroid-resistant Colorado potato beetle, Leptinotarsa decemlineata. Pestic. Biochem Physiol. 104: 192-200.

Wang, Q., Li, M., Pan, J., Di, M., Liu, Q., Meng, F., Scott, J. G. and Qui, X. 2012. Diversity and frequencies of genetic mutations involved in insecticide resistance in field populations of the house fly (Musca domestica L.) from China. Pestic. Biochem. Physiol. 102: 153-159.