Accomplishments by Participant and State

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DEVELOPMENT OF RESISTANCE MANAGEMENT TECHNIQUES FOR CORN INSECT PESTS IN NEBRASKA

Resistance management of pest insect species represents a serious challenge to corn growers that utilize both transgenic corn varieties as well as conventional pesticides. Research associated with this project is intended to provide information for design of effective and long-term pest management solutions.

Individual European corn borer that have been phenotyped for resistance to Bt toxins have been sent to collaborators at the USDA ARS Corn Insect and Crop Genetics Research Lab who are currently developing linkage maps using AFLP markers to identify quantitative trait loci for mapping resistance genes. Additionally, we have been collaborating with researchers at the University of Valencia, Spain to clone putative receptors that have previously been identified as being involved with resistance.

Monitoring programs for the European corn borer have continued in 2006. Routine monitoring of European corn borer populations at diagnostic concentrations have indicated that this target pest species of transgenic maize remains susceptible to Cry1Ab and Cry1F toxins. Laboratory selections for resistance to both toxins have resulted in significant levels of resistance. Additional strains from field populations have been identified and are currently being characterized with regard to inheritance, fitness, and biochemical basis of resistance. Additionally, we have initiated experiments to document the ability of these strains to utilize transgenic plant tissues. Preliminary results indicate that although survival is significantly reduced, it appears that development of the resistant strains on transgenic plants is possible. The resistance to Cry1Ab is associated with reduced binding to a cadherin-like protein from the midgut brush border membrane. However, resistance to Cry1F remains uncertain, because assays to measure toxin binding and midgut proteolosis have not indicated and differences between resistant and susceptible strains. The genes for putative toxin receptors been cloned and sequenced and expression studies indicate that this protein is involved with both binding and toxicity. Susceptibility assessment of corn rootworm larvae to the Cry3Bb toxin has continued with representative field populations. Additional studies have been initiated to measure baseline susceptibility to Cry 34/35 which has recently been registered for corn rootworm control and the neonicotinoid insecticide chlothianidin which is the active ingredient used as a seed treatment of rootworm management. We are currently developing RNA interference assays to assess gene function and identify potential target sites for novel control strategies.

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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 probably because OPs (especially chlorpyrifos) continue to be used for citricola scale. Citricola scale is not susceptible to pyriproxyfen. During 2006 we documented low levels of pyriproxyfen resistance in several California red scale populations, using a fruit dip bioassay. We also documented chlorpyrifos resistance in a large number of citricola scale populations using a leaf dip bioassay. 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 resistance in citricola scale is a very serious situation.

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A DECADE OF SUCCESSFUL MANAGEMENT OF RESISTANCE TO INSECTICIDES IN ARIZONA

Two arthropod pests have been the major focus of efforts to manage resistance to insecticides in the Southwestern US. They are the pink bollworm, *Pectinophora gossypiella*, and the sweetpotato whitefly, *Bemisia tabaci*. Pink bollworm is a severe pest of cotton and is the major pest targeted by transgenic Bt cotton in our area. Sweetpotato whitefly is a severe pest of many crops produced in the southwestern including cotton, melons and vegetables. It has also become an increasingly common problem in glasshouse production systems.

Attempts to control whiteflies and pink bollworm with conventional broad-spectrum insecticides have had devastating results in many desert agro-ecosystems. Severely reduced natural enemy populations have been associated with resurgences of these pests, outbreaks of secondary pests, and rapid evolution of pest resistance. Under such conditions, these pests have developed resistance to essentially all insecticides to which it has been repeatedly exposed. Such was the case in 1995, when whitefly numbers reached crisis proportions in Arizona cotton despite application of 6 to 15 insecticide treatments per acre.

In consultation with researchers in Israel and the United Kingdom, emergency alternatives for whitefly control were formulated and implemented in 1996 that replaced broad-spectrum insecticides during the early season with once-per-season use of the insect growth regulators, pyriproxyfen and buprofezin. Concomitant registration of Bt cotton significantly reduced treatments of conventional insecticides for lepidopteran pests. Additionally, neonicotinoid insecticides provided exceptional whitefly suppression in the other major whitefly hosts, melons and winter vegetables. The end result has been over a decade of unprecedented low insecticide use in cotton, and equally unprecedented effectiveness of biological control in cotton fields.

Management of resistance in the Southwestern USA is focused intensively on sustaining effective, selective insecticides. This includes statewide detection and isolation of resistance in cotton, vegetables, melons and glasshouses, and collaborative research to characterize critical toxicological, genetic and ecological parameters of resistance in laboratory and field experiments.

The contributions to resistance management of major research programs based at the University of Arizona are detailed below.

Bruce Tabashnik
University of Arizona

DNA SCREENING REVEALS PINK BOLLWORM RESISTANCE TO BT COTTON REMAINS RARE AFTER A DECADE OF EXPOSURE

Transgenic crops producing toxins from the bacterium *Bacillus thuringiensis* (Bt) kill insect pests and can reduce reliance on insecticide sprays. Although Bt cotton and Bt corn covered 25 million ha worldwide in 2005, their success could be cut short by evolution of pest resistance. Monitoring the early phases of pest resistance to Bt crops is crucial, but has been extremely difficult because bioassays usually cannot detect heterozygotes harboring one allele for resistance. We monitored resistance to Bt cotton with DNA-based screening, which detects single resistance alleles in heterozygotes. We used polymerase chain reaction primers that specifically amplify three mutant alleles of a cadherin gene linked with resistance to Bt cotton in pink bollworm, *Pectinophora gossypiella* (Saunders), a major pest. We screened DNA of 5,571 insects derived from 59 cotton fields in Arizona, California, and Texas during 2001 to 2005. No resistance alleles were detected despite a decade of exposure to Bt cotton. In conjunction with data from bioassays and field efficacy tests, the results reported here contradict predictions of rapid pest resistance to Bt crops.

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FUNGICIDE RESISTANCE IN CUCURBIT POWDERY MILDEW

Activities pertaining to fungicide resistance in cucurbit powdery mildew conducted in 2006 in New York were evaluating fungicides at-risk for resistance, monitoring of resistance in production fields, determining baseline sensitivity for new fungicides, and requesting emergency exemption from registration (FIFRA Section 18) for a new fungicide. 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 squash and melon. Effective control necessitates products able to move to the lower leaf surface, where this disease develops best. Unfortunately they are prone to resistance development because of their single-site mode of action.

The annual conventional fungicide evaluation experiment in 2006 included individual products and fungicide programs. The QoI fungicide Cabrio was ineffective, which was not surprising considering that resistance to this fungicide group is qualitative and resistant strains have been detected since 2003 through monitoring in NY. The DMI fungicide Nova (at 5 oz/A, the highest label rate) also did not control powdery mildew likely due to resistance. Another DMI, Procure (at 6 oz/A, the intermediate rate which is recommended by the manufacturer) provided poor control (34% on lower leaf surfaces). These products were among the most effective in the 2005 experiment. A gradual change in efficacy due to resistance was expected because DMI resistance is quantitative. It was first documented in the US in 1990 following control failure with an inherently less active DMI fungicide, Bayleton. A new fungicide, Quintec, provided excellent control (88%). When Procure was applied in alternation with Quintec starting with Procure, powdery mildew was not controlled as effectively (64%) as when the alternation started with Quintec (77%), which was as effective as Quintec alone. This later observation further documents the difficulty of detecting resistance when a fungicide program is used, as in commercial production fields

Fungicide resistance was monitored in commercial production fields. Early in powdery mildew development in spring cucurbit crops resistance to 2 chemical groups (QoIs and MBCs) and moderate resistance to a third (DMIs) were common. Since QoI and MBC resistance is qualitative, fungicides in these groups would not suppress resistant strains. Growers were provided this information with guidelines for managing powdery mildew in main season crops.

Baseline sensitivity data was obtained for a new powdery mildew fungicide, boscalid, which will serve as a benchmark for assessing if the pathogen is developing resistance in the future.

Growers in NY were able to effectively control powdery mildew in 2006 because EPA granted a FIFRA Section 18 for Quintec. Results of research described above on fungicide efficacy and resistance monitoring provided justification for another request for 2007.

Scott Nissen
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RECENT ACCOMPLISHMENTS IN HERBICIDE RESISTANCE WEED RESEARCH AT COLORADO STATE UNIVERSITY

Herbicide resistance research at CSU has focused on three major issues affecting weed management in Colorado. These areas include 1) dicamba resistance kochia (*Kochia scoparia*), 2) the pollen mediated flow of genes that confer herbicide resistance between wheat varieties and between wheat and jointed goatgrass (*Aegilops cylinderica*), and 3) issues related to the continuous use of glyphosate-tolerant crop technology.

Dicamba Resistant Kochia: Experiments were conducted 1) to determine ethylene production by dicamba resistant and susceptible kochia following dicamba applications 2) to establish the relationship between ethylene production and herbicide symptoms, and 3) to evaluate the response of dicamba resistant kochia to fluroxypyr. Dicamba resistant kochia treated with 280 g ha^{-1} dicamba produced significantly less ethylene than susceptible kochia 24 HAT. Ethylene production increased faster in susceptible than resistant kochia as dicamba rate increased. Dicamba resistant and susceptible kochia treated with fluroxypyr produced similar amounts of ethylene as herbicide rate increased. GR_{50} values for susceptible and resistant kochia treated with dicamba were 45 and 1331 g ha^{-1}, respectively. GR_{50} values for susceptible and resistant kochia treated with fluroxypyr were 10 and 34 g ha^{-1}, respectively. The ethylene inhibitor AOA inhibited ethylene production but did not reduce dicamba symptoms.

Pollen Mediated Gene Flow: The potential introduction of wheat (*Triticum aestivum* L.) cultivars with transgenic traits has generated increased interest in pollen-mediated gene flow (PMGF). The objectives of this study were to estimate wheat PMGF between commercial fields across multiple years and locations, and to compare field-scale estimates to smaller experimental plots. The study was conducted in 2003, 2004 and 2005 in eastern Colorado. Fifty-six commercial field locations planted to one or more of 18 imidazolinone-susceptible (IS) winter wheat cultivars were sampled at distances of 0.23 to 61 m from a bordering imidazolinone-resistant (IR) cultivar. At least one sample from all 56 commercial fields and from all evaluated cultivars had detectable PMGF. The highest observed PMGF was 5.3% at 0.23 m. The farthest distance at which PMGF was detected was 61 m and the highest PMGF at that distance was 0.25%. No PMGF was detected in 27% of samples. Higher levels and greater distances of PMGF were detected in commercial fields than in experimental plots. Based on estimates from a generalized linear mixed model with a random location effect, the distance required to ensure 95% confidence that 95% of locations would have PMGF less than 0.9% is 41.1 m for the earliest heading cultivars and 0.7 m for intermediate heading cultivars. These confidence limits should represent the highest levels of PMGF expected to occur in winter wheat and will be useful for wheat biotechnology regulation.

Gene flow between jointed goatgrass and winter wheat is a concern because transfer of herbicide resistance genes from imidazolinone-resistant (IR) winter wheat varieties to jointed goatgrass could restrict weed management options for this serious weed of Colorado winter wheat cropping systems. Project objectives were (1) to investigate the frequency of interspecific hybridization between IR wheat and jointed goatgrass in eastern Colorado, and (2) determine the gene action of the IR acetolactate synthase (ALS) allele in IR wheat by jointed goatgrass and IR wheat by imidazolinone-susceptible (IS) wheat backgrounds.

Jointed goatgrass was sampled side-by-side with IR wheat and at distances up to 53 m away in both experimental plots and at commercial field study sites in 2003, 2004, and 2005. A greenhouse screening method was used to identify IR hybrids in collected jointed goatgrass seed. The average percent hybridization across sites and years when IR wheat and jointed goatgrass were grown side-by-side was 0.1% and the maximum was 1.6%. The greatest distance over which hybridization was documented was 16 m. The mutant ALS allele was found to be partially dominant in a jointed goatgrass by IR wheat cross and additive in an IS wheat by IR wheat cross. The hybridization rate between jointed goatgrass and partial dominance of the IR wheat ALS allele will both influence trait introgression into jointed goatgrass.

Issues related to glyphosate tolerant crop technology: Glyphosate resistance has recently been reported in Palmer amaranth (*Amaranthus palmeri*) populations from glyphosate-tolerant cotton cropping systems in Georgia. Seeds were obtained from scientists in Georgia and screened with an in-vivo shikimate accumulation assay. Using a range of glyphosate concentrations from 100 to 2,000 µM, susceptible plant leaf discs accumulated shikimate in 100 µM glyphosate while resistant plant leaf discs accumulated detectable shikimate only in 2,000 µM glyphosate. Candidate glyphosate resistance mechanisms under investigation include mutations in EPSPS and over-expression of EPSPS. Gene sequences have been obtained for 1,056 base pairs of EPSPS from resistant and susceptible plants. These results have been compared using current bioinformatics protocols to determine whether any detected mutations may be significant. Semi-quantitative PCR has been used to determine whether EPSPS is over-expressed in resistant plants. Preliminary results indicate that resistant plants may have higher EPSPS expression than susceptible plants. The exact mechanism of glyphosate resistance in Palmer amaranth has not yet been determined.

A long-term, multi-state project to examine the effects of continuous glyphosate use on possible weed shifts and the selection of glyphosate resistant weeds is another research emphasis at CSU. This research project compares continuous glyphosate use, alternating glyphosate and conventional programs, and conventional programs. Crop rotations are being examined as a sub-plot effect. After 8 years at four different sites, continuous glyphosate use has consistently provided the greatest yields and has significantly reduced the weed seed bank. No glyphosate resistant weeds have been identified; however, there has been a shift toward weeds that are traditionally harder to control with glyphosate, i.e., wild buckwheat (*Polygonum convolvulus*) and lambsquarters (*Chenopodium album*).

Mark Whalon
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The Arthropod Pesticide Resistant Database (**APRD**) **system--** Resistance is a widespread phenomenon where arthropod populations evolve the genetic ability to escape the lethal effects of normally fatal concentrations of one or more pesticides. Therefore, resistance is a significant and on-going issue for trade, homeland security, animal and human health protection and agricultural production and profitability. The occurrence of pesticide resistance frequently leads to the increased use, overuse and even misuse of pesticides that may pose a risk to the environment, phytosanitation, market access, global trade and public health. In 1989 I initiated the Global Arthropod Pesticide Resistance Database (APRD) at Michigan State University, which has progressively grown into the world's premier web-based, pesticide-policy influencing tool in the US, the EU and most of the rest of the world (www.pesticideresistance.org). Both Drs. Robert M. Hollingworth and David Mota-Sanchez joined this critical effort in 1992 and 1996 respectively. In December 2006 workers at MSU including Mr. Lee Duynslager (technical management) and Dr. Qiang Xue (software development) effectively transferred the old Access database to a "user-interactive" HTML system. Thus the database now features remote access and a peer-reviewed (publication), case-submission system. With these user friendly adaptations, the APRD is interactively accessible via the www and receives more than 1500 daily visits from arthropod resistance workers from around the globe.

This effort was supported through a partnership between the Insecticide Resistance Action Committee (IRAC), USDA/CSREES/IPM, USDA and Michigan State University. As of April 17^{th} 2007 the current global number of Insecticide and Miticide resistant cases as well as the number of resistant species is 7,558 and 553, respectively. The new www APRD hosted by MSU registered over 540,000 visits in 2006 lasting longer than 10 minutes each.

The APRD now has the capability for remote case submissions from anywhere in the world. It also features an electronic-based peer-reviewed process for publication of resistance cases. Thus the website now affords directed searching, extracting and analyzing resistance information across an array of factors including various time horizons and modes of pesticide action. Each case submitted is peer reviewed with an editorial body made up of volunteer arthropod resistance scientists and industry workers from all over the world. Each reviewed and accepted case is assigned an accession number and a case submission title equivalent to a peer reviewed publication.

The APRD www now provides routine reports detailing the number of resistant cases by species, pesticides, mode of action, use and severity. Historically, all resistance case reports were extracted from peer reviewed literature and we will continue to maintain a search process of over 43 different referred journals globally so long as resources permit. However, we now anticipate that hundreds, perhaps thousands, of cases from around the world will be peer-reviewed and published electronically into the www via the APRD annually.

Our short term goal for the APRD has been to reduce the reporting, analysis and publication burdens of resistance reporting in such a way that routine analysis tables, figures, reports and attributions will be generated and posted monthly for pesticide policy workers globally. Our long term goal is to develop and publish on the www a spatial/temporal explicit geographical mapping system reflecting historical and current case summaries at greater spatial detail until sufficient precision is available to facilitate the development of effective resistance policy in geographically relevant management units. In addition, user remote APRD literature and case searches will be accessible from anywhere at anytime on the www.

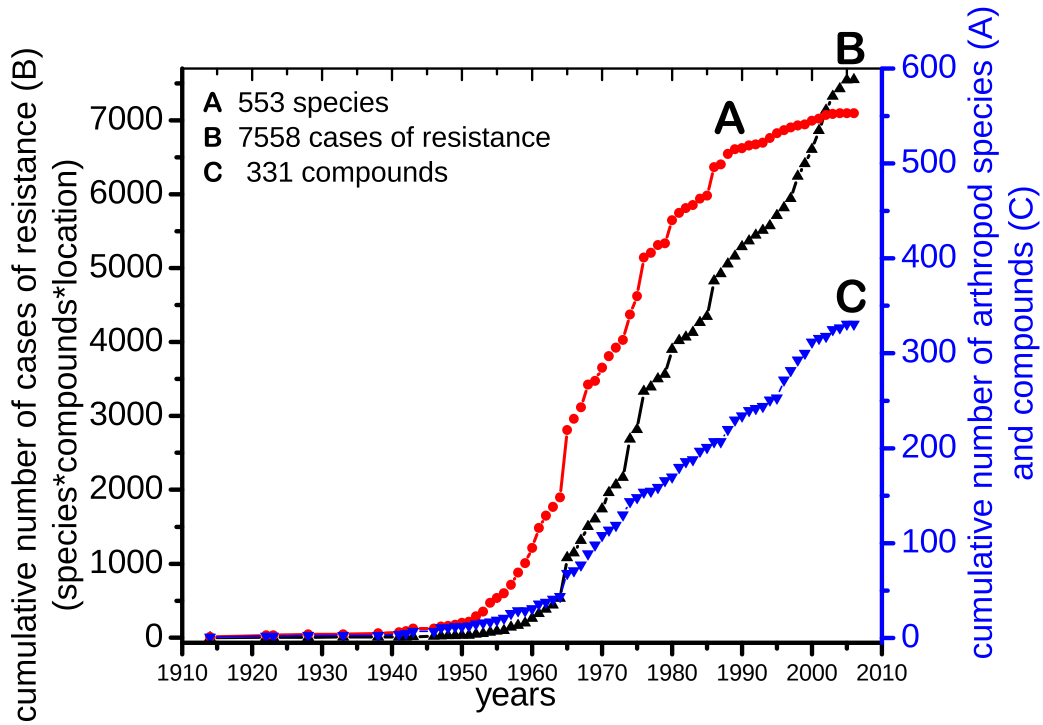

Fig 1. A century of evolution of arthropod insecticide resistance

Given the APRD's global reach and continued development, new products will be trialed and, in time, become regular contributions to resistance policy and management. Our strategy is a deliberate, peer reviewed, partner participation process of new product development and introduction. For instance, in December of 2006 we began presenting for the first time, the number of arthropod resistance cases reported in the APRD by the Insecticide Resistance Action Committee's *International Mode of Action Classification System* and these data have been well received by APRD users.

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 bureaucrats 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 550 articles, including 36 articles in 2006. The Bi-annual publication has 1,091 electronic subscribers (mostly in government, industry and academia), and hard copies are now part of 58 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, and New Zealand. The newsletter can be viewed online at http://whalonlab.msu.edu/rpm/index.html and has received 12,076 visitors since May 2006.

In part, as a result of MSU's APRD and RPM Newsletter, GREEEN has helped to pioneer and perpetuate the WERA-60, an international-in-scope, USDA/CSREES project that addresses pesticide resistance and resistance management policy in the Upper

Midwest, US, EU and the world. MSU's efforts are a key note of this important process, and we have developed these communication tools into a strong cooperative process with several other Land Grant research groups nationally and other cooperators internationally are using to derive up-to-the-minute resistance information via the www. MSU's APRD program recently sponsored a resistance management workshop in conjunction with the 4th IPM Global Symposium in Indianapolis, IN, 2006.