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SYMPOSIUM INTRODUCTION

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Comparing Conventional and Biotechnology-Based Pest Management

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ABSTRACT: Pest management has changed dramatically during the past 15 years by the introduction of transgenes into crops for the purpose of pest management. Transgenes for herbicide resistance or for production of one or more *Bt* toxins are the predominant pest management traits currently available. These two traits have been rapidly adopted where available because of their superior efficacy and simplification of pest management for the farmer. Furthermore, they have substantially reduced the use of environmentally and toxicologically suspect pesticides while reducing the carbon footprint of pest management as reduced tillage became more common, along with fewer trips across the field to spray pesticides. The most successful of these products have been glyphosate-resistant crops, which cover approximately 85% of all land occupied by transgenic crops. Over-reliance on glyphosate with continual use of these crops has resulted in the evolution of highly problematic glyphosate-resistant weeds. This situation has resulted in some farmers using weed management methods similar to those used with conventional crops. Evolution of resistance has not been a significant problem with *Bt* crops, perhaps because of a mandated resistance management strategy. Transgenic crops with multiple genes for resistance to different herbicides and resistance to additional insects will be available in the next few years. These products will offer opportunities for the kind of pest management diversity that is more sustainable than that provided by the first generation of transgenic crops.

KEYWORDS: biotechnology, *Bt*, glyphosate, herbicides, insecticides, pest management, transgenic crop

INTRODUCTION

Pest management has changed dramatically during the past 15 years by the introduction of transgenes into crops for the purpose of pest management. Transgenes for herbicide resistance or for production of one or more *Bt* toxins are the predominant pest management traits. The fact that almost 100% of the area planted with transgenic crops has one or both of these traits (herbicide resistance and *Bt* toxin-based insect resistance) is a little publicized fact. Almost all of the herbicide-resistant crops have one or more genes for glyphosate resistance. As of 2009, 14 million farmers in 25 countries were planting 134 million hectares in transgenic crops.[1] Approximately 75% of the soybean, 50% of the cotton and maize, and >25% of the canola grown worldwide are now transgenic varieties.[1] Herbicide resistance is available for all four of these major crops, and insect resistance is available in maize, cotton, and, more recently, soybean, often with both herbicide and insect resistance in the same variety. The rates of adoption of these crops have steadily increased since they were introduced, and the rate of increase has continued more recently mostly through more countries allowing the planting of these crops (e.g., Brazil and India).[1] In the case of transgenic (glyphosate-resistant) sugar beets, U.S. farmers went from 0 to almost 100% adoption within two years. The unprecedented adoption of these crops over the past 15 years is the most rapid adoption of a crop technology in the history of agriculture. Widespread adoption of these two pest management traits has revolutionized pest management for the crops in which they are marketed.

The question arises as to how pest management utilizing these biotechnology products differs from that without them. What has changed and what remains mostly the same? These are difficult questions to answer, as this technology has had indirect influences on pest management of even those not using it, so one cannot simply compare pretransgenic crop with transgenic crop pest management. Furthermore, even with this new technology, the playing field keeps shifting due to many factors. Still, the question has not been addressed in any formal way on a broad scale.

We (William P. Ridley, Keri Carstens, Nicholas Storer, and I) organized an Agrochemical Section symposium on this topic for the 239th national meeting of the American Chemical Society held in San Francisco, CA, in March 2010. In this symposium, we brought together experts on various aspects of this area to gain insight into some of the intriguing questions related to it. This paper is an introduction to some of the papers from the symposium found in a special section of this issue of the journal.[2−13] These papers cover many aspects of the symposium theme, but do not completely encompass this topic. This is partly because not every presentation from the symposium resulted in a paper and partly due to the lack of complete coverage by the 22 papers of the symposium.

In this introductory paper, the intention is not to discuss the following 12 papers from the symposium but to provide an overview of the broad topic in order to provide perspective for these papers.

IMPACTS OF HERBICIDE-RESISTANT CROPS

At the time of this writing, the only transgenic, herbicide-resistant crops being grown are those with glufosinate and

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glyphosate resistance, although bromoxynil-resistant crops were available for several years after the introduction of bromoxynil-resistant cotton in 1995.[14] Bromoxynil-resistant crops (cotton and canola) never had a large market share and were discontinued. Glufosinate-resistant crops, also first introduced in 1995, have shown more promise, but still have garnered only a relatively small portion of the transgenic crop market. Since glyphosate-resistant crops were commercially introduced in 1996, the combination of these crops with the herbicide glyphosate has grown to become the preferred weed management combination for farmers growing the crops for which this technology is available (maize, soybean, cotton, canola, sugar beets, and alfalfa) in the countries that have approved these crops for agricultural production.[1,14−16] Glyphosate-resistant crops cover approximately 85% of the land area devoted to transgenic crops.[1] Considering the dominant role of glyphosate-resistant crops, the rest of this section will focus on these crops.

Before transgenic crops, nonselective herbicides could not be used with crops unless the herbicide was sprayed before the crop was sown or emerged from the soil or was applied in a manner that did not allow the herbicide to reach the foliage of the crop (e.g., banded or shielded spraying). The only exception was the relatively limited use of imazapyr with nontransgenic, imidazolinone-resistant maize. With agronomic crops, nonselective herbicides such as glyphosate and glufosinate were rarely used for weed management after the crop emerged. The revolutionary aspect of weed management that came with glyphosate-resistant and, to a lesser extent, glufosinate-resistant crops was that a broad-spectrum, foliarly applied herbicide could be used for weed management to control virtually all weed species within the crop. Even with significantly increased costs for transgenic seeds, this technology simplified and generally lowered the costs associated with weed management. Farmers adopted this system extremely rapidly because of these factors, relying almost exclusively on glyphosate for weed management. The environmental benefits of this were generally positive, particularly because of the reduction in tillage, which is associated with soil loss and heavy fossil fuel use.[17] Reducing tillage also lowered weed management costs.

The technology has been so good that farmers have used it continuously, year after year.[18] Continual use of and reliance on a highly efficient pesticide is unsustainable for two reasons. The first is due to the fact that nature abhors a vacuum. The almost completely weed-free fields of glyphosate-treated, glyphosate-resistant crops provided a perfect habitat for weed species with a low level of natural resistance to glyphosate (tolerance). Before glyphosate-resistant crops, these species did not compete well for the agroecological niches then occupied by highly glyphosate-susceptible weed species or were well controlled by tillage. This change in problem weed species is termed “weed shifts” by most weed scientists.[19] Some of the weed shifts were to perennial species that thrive under no-till practices commonly used with glyphosate-resistant crops. This shift required farmers to occasionally return to tillage as a weed management tool, losing some of the environmental benefits of reduced tillage. On the basis of knowledge of weed population dynamics, weed shifts in these crops were predictable and, in most cases, relatively easily managed. In many situations, the new species could be managed with higher doses of glyphosate, as the level of tolerance of most of these shifted species was not high.

The other change was the evolution of glyphosate resistance in weeds, a topic that has received much publicity. The first substantiated case of an evolved glyphosate-resistant weed was that of *Lolium rigidum* reported in 1996,[20] the same year that glyphosate-resistant crops were introduced. The first few cases of evolved glyphosate-resistant weeds did not occur in glyphosate-resistant crops, but occurred in fields sprayed between crop plantings and in orchards. Shortly after the first case of an evolved glyphosate-resistant weed was reported, a paper was published, indicating that the likelihood of weeds evolving resistance to glyphosate at the site of action was remote.[21] The authors had a good rationale based on the need for mutations in at least two codons in order to have an adequately functional form of the target enzyme of glyphosate (5-enolpyruvylshikimate-3-phosphate synthase, EPSP synthase) that would be sufficiently resistant to glyphosate. However, the authors did not consider evolution of nontarget site resistance to glyphosate. Although the earliest cases of evolved glyphosate-resistant weeds did not occur in glyphosate-resistant crops, the widespread selection pressure of glyphosate in glyphosate-resistant crops led to most of the more recent examples of evolved glyphosate resistance. At present, 21 plant species have evolved resistance to glyphosate, most occurring in glyphosate-resistant crops.[20] The increase in species evolving resistance to glyphosate has been steady since 1996, and we can expect this to continue as glyphosate use becomes more widespread in existing and future glyphosate-resistant crops (Figure 1). The glyphosate resistance problem is acute for some farmers, forcing them to use other weed management options including glufosinate-resistant crops to obtain acceptable weed management.

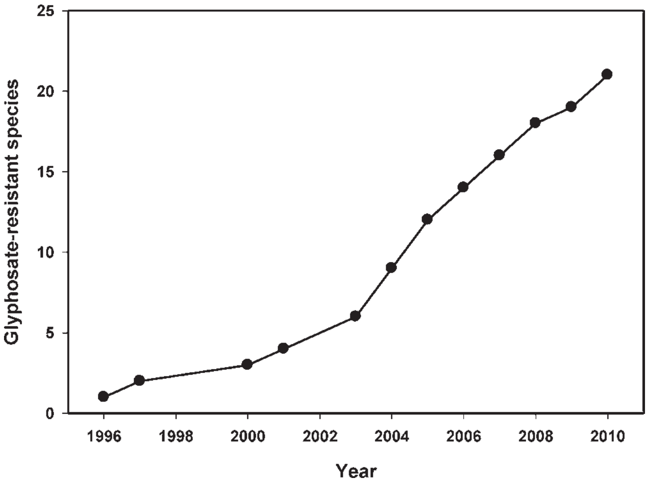

Figure 1. Increases in cases of evolved resistance to glyphosate worldwide. Data are from Heap.[20]

As predicted by Bradshaw et al.,[21] few of these species with evolved resistance have an altered EPSPS. The exact mechanism of resistance is unknown for most of the species, but in one case, gene amplification results in up to 100-fold more EPSPS in the plant, thus requiring substantially more glyphosate to block the aromatic amino acid pathway to which EPSPS contributes.[22] In another example, glyphosate is rapidly sequestered into vacuoles, perhaps by a mutant or an amplified ABC transporter.[23] Furthermore, there are cases of glyphosate resistance in which at least two mechanisms of resistance are utilized by the weed to enhance the level of resistance.[24] The recurring strong selection pressure of yearly use of glyphosate over broad expanses of glyphosate-resistant crops has resulted in weeds evolving unusual and unpredictable mechanisms of resistance to glyphosate.

Weed shifts and evolution of herbicide resistance are not new phenomena, as weeds evolved resistance to other herbicides before the advent of glyphosate-resistant crops.[20] For several herbicide classes, weeds are more predisposed to evolve resistance, as, unlike glyphosate, a one base pair change can provide a high level of site of action resistance. For example, there are several one base pair mutations in the gene encoding acetolactate synthase that cause a high level of resistance to herbicides that inhibit this enzyme.[25] These herbicides are generally used as part of a suite of herbicides, as each of the herbicides alone controls only a limited number of weed species. In most cases, the farmer had other herbicide choices when resistance evolved to one of these herbicides, but the introduction of glyphosate-resistant crops made glyphosate the most dominant and valuable herbicide in history,[15] largely because they allowed farmers to effectively and economically control virtually all weeds with one herbicide. Now, due to overuse of this important weed management tool, the utility of glyphosate is being jeopardized by the evolution of resistance. Farmers with severe infestations of glyphosate-resistant weeds have returned to the more complex weed management strategies used before the advent of glyphosate-resistant crops.[5] These strategies involve the use of several herbicides on a crop, as well as cultural methods such as tillage. This situation has also improved the market for glufosinate-resistant crops.

As discussed by Tranel et al.,[3] the alternative herbicide options for some glyphosate-resistant weed species have become limited by the fact that they are also resistant to other herbicide classes by separate mechanisms. The biotechnology industry is developing new herbicide-resistant crops that will provide additional tools for weed management.[5,16,26] However, all of the herbicide-resistant crops that are being actively developed are resistant to herbicides with modes of action that have been used for decades. In most cases, there are already weed populations that are resistant to these herbicides. To avoid even greater problems of weeds evolving resistance to multiple modes of action (see, e.g., ref 3), herbicides with new mechanisms of action are badly needed.

As discussed by Gerwick,[26] there have been no major new modes of action for herbicides introduced in about 20 years. The widespread use of glyphosate with glyphosate-resistant crops led to a lack of incentive for the development of new herbicides. Furthermore, relatively few of the recent patents for new herbicides appear to be for compounds with new modes of action compared to insecticides and fungicides.[26] To paraphrase Gerwick, whereas the costs of introducing a new pesticide went up considerably (from ca. \$150 million in 1995 to more than \$256 million in 2005−2008), raising the bar, passing over the bar became less rewarding due to glyphosate-resistant crops.

■ IMPACTS OF *Bt* CROPS

Bacillus thuringiensis (*Bt*) is an aerobic, Gram-positive, endospore-forming bacillus; its spores and crystal proteins were first commercialized in France in the late 1930s as a biopesticide spray.[27] The insecticidal activity of *Bt* commercially comes from proteinaceous endotoxins (Cry proteins) included in crystals formed during sporulation, although “vegetative insecticidal proteins” (Vips) from before sporulation have also been developed. The term commonly used for *B. thuringiensis* toxins is also *Bt*. A large variety of >100 Cry toxins expressed from single genes are specific for certain orders of insect pests (Lepidoptera, larvae of butterflies and moths; or Coleoptera, beetle larvae). Schnepf and Whiteley[28] first cloned and characterized the genes coding for crystal proteins, and *Bt* was first introduced into tobacco plants in 1987.[29] More effective insect-protected plants were developed using synthetic genes modeled on those from *Bt* but designed to be more compatible with plant expression.[30] Currently, the *Bt* proteins targeted for the control of Lepidoptera include wild type or modified Cry1Ab, Cry1Ac, Cry1A.105, Cry1F, Cry2Ab2, and Vip3A, whereas wild type or modified Cry3A, Cry3Bb1, Cry34Ab1, and Cry35Ab1 target Coleoptera.[31] Cry34Ab1 and Cry35Ab1 must be expressed together to form the binary toxin necessary to kill the target insect.[32] A vast majority of the cropland devoted to approved, insect-protected crops is planted in *Bt* cotton and maize, but other *Bt* products for controlling insects in potato, tomato, and recently soybean have also been developed.

Extensive testing of *Bt* crops has been conducted and has demonstrated the safety of these products to humans, animals, and the environment.[33,34] Mammalian toxicology and digestive fate studies conducted on the Cry proteins in currently approved *Bt* crops have confirmed that these proteins are nontoxic to humans and pose no significant concern for allergenicity. The food and feed derived from *Bt* crops have been shown to be as nutritious and safe as the food and feed derived from conventional crops with a demonstrated history of safe use.[11,35−38] Deleterious effects on nontarget organisms under field conditions have either not been detected or were not significant.[39,40] The Cry proteins produced in *Bt* crops have been shown to rapidly degrade when crop residue is incorporated into the soil.[41] The human and environmental safety of *Bt* crops is further supported by the long history of safe use for *Bt* microbial pesticides around the world.[42]

Wherever maize is grown, it can be infected with mycotoxigenic fungi that produce toxic secondary metabolites known as mycotoxins. Dietary exposure to mycotoxins can cause a variety of adverse health effects in farm animals and humans. Using maize manually infected with European corn borer (*Ostrinia nubilalis*), Munkvold and colleagues[43] showed that *Fusarium* ear rot levels and the resulting levels of fumonisin mycotoxin were dramatically reduced in *Bt*-protected maize containing Cry1Ab compared to non-*Bt* maize. Because the Cry1Ab protein virtually eliminated corn borer-induced tissue damage in maize, the fungal spores were less able to germinate and reproduce. Fumonisins, highly toxic mycotoxins from *Fusarium* spp., were monitored in maize grain collected from *Bt* hybrids grown in the United States in 2000−2002.[44] Over the three years of field trials with natural infestation by European corn borer, there were 126/210 comparisons when fumonisin levels in the grain from control hybrids exceeded the U.S. FDA guidance of 2 ppm for human food. Grain from *Bt* hybrids was at or below 2 ppm for fumonisins for 58 of the 126 comparisons, indicating that *Bt* maize can increase the percentage of grain that would be suitable for use in food and feed. In addition to positive impacts on health, the reductions in mycotoxins seen in *Bt* maize have had beneficial economic impacts.[45]

Cotton is attacked by a complex of insects; however, on a worldwide basis, the main pests are the diverse set of Lepidoptera that feed on the cotton buds or bolls. Cotton receives the most insecticide use of any crop worldwide.[46] The National Center for Food and Agricultural Policy (NCFAP) conducted an analysis of the influence of *Bt* cotton on insecticide-use patterns in the United States and concluded that the average application rates declined from 0.41 kg/ha in 1995, the year before the commercial introduction of *Bt* cotton, to 0.13 kg/ha in 2000.[47] A recent report from a 2-year farm-scale evaluation in Arizona concluded that the use of transgenic cotton producing Cry1Ac in large commercial cotton fields reduced broad-spectrum insecticide use and increased yields at fixed insecticide levels.[48] On the basis of a survey conducted in northern China in 1999, farmers who did not use *Bt* varieties sprayed pesticides on average 20 times per season, whereas the *Bt* cotton users sprayed on average only 6.6 times per year. The quantity of formulated pesticide on *Bt* varieties also fell substantially. Farmers using *Bt* varieties applied 11.8 kg/ha, <20% of the quantity used by non-*Bt* cotton farmers (60.7 kg/ha).[49] India is now the country with the largest *Bt* cotton area (8.4 million hectares in 2009).[50] Data collected in India for three growing seasons between 2002 and 2006 indicated an average 41% decrease in insecticide use, a 37% increase in yield, and an 89% increase in profit among the smallholder-dominated cotton production systems.[51] Analysis of the population dynamics of cotton bollworm (*Helicoverpa armigera*) from 1992 to 2007 in China indicated a marked decrease in regional outbreaks of this pest in multiple crops was associated with the planting of *Bt* cotton.[52] The data from six provinces in northern China suggest that *Bt* cotton not only controls cotton bollworm but also may reduce the presence of the insect pest on other host crops (maize, peanuts, soybeans, and vegetables), thereby decreasing the need for insecticide sprays in general. Control of cotton bollworm and reduced use of insecticide with *Bt* cotton could be responsible for the appearance and subsequent spread of nontarget insect pests (e.g., mirid bug) in some situations at an agro-landscape level.[53] Nevertheless, the widespread adoption of *Bt* cotton over the past 15 years suggests that farmers are satisfied with the technology from an economic point of view.

The reduction in insecticide use associated with *Bt* maize is more difficult to assess because a majority of growers do not use insecticide to control the primary pest, the European corn borer (*O. nubilalis*).[46] A recent paper by Hutchison and colleagues[54] highlighted the area-wide suppression of European corn borer throughout the Midwestern U.S. Corn Belt. Cumulative benefits over 14 years of *Bt* maize use were estimated to be $3.2 billion for maize growers in Illinois, Minnesota, and Wisconsin with more than $2.4 billion of this total accruing to non-*Bt* maize growers. Soil-applied insecticides and seed treatments have been used to control corn rootworm (*Diabrotica* spp.) in maize, and *Bt* maize containing Cry3Bb1 has been shown to control this pest.[55] However, few data are available to determine the reduction in insecticide usage associated with the adoption of this below-ground *Bt* pest control.

The primary threat to the continued success of *Bt* crops is the evolution of resistance by insect pests. The refuge strategy has been the chief approach used worldwide to delay evolved pest resistance to *Bt* crops.[56] This strategy, which has been required in the United States and other countries, is based on the theory that most of the rare, resistant target insects surviving on *Bt* crops will mate with abundant susceptible pests from nearby refuges of host plants without *Bt* toxins.[57] If inheritance of resistance is recessive, the hybrid progeny from matings will die on *Bt* crops, substantially slowing the evolution of resistance. The refuge strategy works best if the dose of toxin ingested by insects on *Bt* plants is high enough to kill all or nearly all of the hybrid progeny.[57,58] Although first-generation *Bt* crops produced a single *Bt* toxin, second-generation crops produce two distinct *Bt* toxins that are active against the same pest (http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm). This approach has been called a "pyramid" and is expected to delay pest resistance most effectively when selection of resistance to one of the toxins does not cause cross-resistance to the other toxin.[59] The refuge requirements for pyramids are smaller than those for single events. The introduction of *Bt* seed mixed with nontransgenic seed in the same product bag ("refuge in the bag") is simplifying compliance with refuge management requirements. Alternative natural hosts have played a role in delaying evolution of resistance insects to *Bt* crops through providing additional refugia.[60,61] Better utilization of this resource may further enhance future integrated resistance management.

The major pests targeted by *Bt* crops have been monitored for the evolution of resistance, which is defined as a heritable decrease in a population's susceptibility to a toxin.[56] Susceptibility is usually measured by sampling insects from a field population and determining how their progeny respond to the toxin in laboratory bioassays. Such bioassays document field-evolved resistance if one or more populations with a history of exposure to the toxin in the field are less susceptible than conspecific field populations or laboratory strains that have less exposure. Laboratory documentation of resistance does not always indicate that there will be control problems in the field.[62] After more than a decade since the initial commercialization of *Bt* crops, most target pest populations remain susceptible. Tabashnik and colleagues[56,63] have reported field-evolved resistance in some populations of three noctuid moth species: *Spodoptera frugiperda* to Cry1F in *Bt* maize in Puerto Rico, *Busseola fusca* to Cry1Ab in *Bt* maize in South Africa, and *Helicoverpa zea* to Cry1Ac and Cry2Ab in *Bt* cotton in the United States. However, there is controversy about the significance of reported resistance in the field.[64] The factors delaying resistance including recessive inheritance of resistance, sufficient refuges on non-*Bt* host plants, and two-toxin *Bt* crops deployed separately from one-toxin *Bt* crops are consistent with the observed field outcomes and highlight the importance of continued monitoring to enhance the durability of insect-protected crops.

THE FUTURE

Transgenic crops have clearly revolutionized pest management in agronomic agriculture. After more than 15 years of safe use, virtually all transgenes for pest management revolve around glyphosate-resistant crops for weeds and *Bt* crops for insects. These two traits have been rapidly adopted where they are available because of their superior efficacy and simplification of pest management for the farmer. These benefits come with increased profitability for farmers in developed and developing countries. Furthermore, they have substantially decreased the use of environmentally and toxicologically suspect pesticides as well as shrunk the carbon footprint of pest management due to reduced tillage and fewer trips across the field to spray pesticides.

In the case of glyphosate-resistant crops, these benefits are being jeopardized because of the evolution of glyphosate-resistant weeds. Farmers with this problem are having to return to weed management methods similar to those used with conventional crops. Crops with transgenes for resistance to multiple herbicides that will soon be introduced will be useful in mitigating the glyphosate-resistant weed problem. However, these new products are not likely to return weed management to the nearly ideal state that it was with glyphosate-resistant crops before evolution of widespread glyphosate resistance.

Bt crops are not encountering widespread evolved insect resistance, perhaps because of the mandated resistance management strategy (refugia) discussed above. This apparent success should be an indicator that required strategies for fighting evolution of herbicide resistance would be beneficial to farmers, the agricultural product industry, and the public. Prevention and mitigation strategies are well understood, but there has been little will to implement them. This may change if the severity of the glyphosate-resistant weed problem intensifies.

In the next few years, the number of biotechnology-based pest management options will increase dramatically. We hope that these products will help to provide the basis for diversity in pest management that will be sustainable and environmentally sound.

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