Transgenic traits give growers valuable tools to manage weeds, insects, and plant stresses. These traits are widely available, with new traits being developed every year. With so many trait options and the costs associated with each technology, corn growers can select hybrids and manage crop production more effectively if they understand the biology and terminology that applies to developing transgenic traits.

Transgenic plants have genes from another species inserted into their genetic makeup. These genes can come from another plant or from another organism, such as a bacterium. The inserted genes modify the plant to improve traits such as resistance to disease, pests, herbicides, and stress or to increase crop yield and quality.

The use of hybrids with transgenic traits has increased dramatically in the past 12 years (Fig. 1). As of 2015, transgenic varieties comprise 89 percent of all Texas corn acreage, just below the U.S. average of 92 percent.

**Plant-Incorporated Protectants for Insect Control**

Modern insect-protected transgenic corn contains combinations of protectants derived from various subspecies of the bacterium *Bacillus thuringiensis* (Bt). However, newer technology that does not rely on Bt may soon be available.

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1Assistant Professor and Extension Agronomist, 2Professor and Extension Entomologist, 3Professor and Extension Weed Specialist, 4Associate Professor and Extension Agronomist; all from the Texas A&M University System

**Figure 1.** Percent of planted acres using genetically engineered corn varieties for the United States and Texas (adapted from *Adoption of Genetically Engineered Crops in the US*. 2012. USDA-ERS).
The plants contain genes that specify the genetic code for crystalline proteins (Bt proteins) present in the natural Bt bacteria. When certain insects eat them, these proteins break down into smaller subunits that can bind to the wall of the insect gut, eventually causing a small hole. Bacteria in the gut then move into the body and the insect dies from bacterial septicemia.

The acidity of the insect gut is also important; the wrong acidity level prevents the crystal subunits from forming. The insects must also have the right type of receptors on the gut wall for the protein subunits to bind, and many insect species lack the appropriate receptors for the Bt toxins they ingest from Bt plants.

Susceptibility to Bt toxins varies widely among broad insect groups such as caterpillars, beetles, and flies, and also among closely related insects within a small group such as caterpillars (Fig. 2). For example, the Bt proteins active against corn rootworm have no effect on caterpillars. Bt toxins greatly affect the tobacco budworm, but the same Bt proteins have much less impact on the corn earworm (cotton bollworm), which is in a closely related genus.

These varying effects explain why some Bt toxins work better on some pests than others. A good illustration is that the Bt toxin Cry1Ab affects the fall armyworm less than the Bt toxin Cry1F does. However, stalk borers, the southwestern corn borer, and the European corn borer are so susceptible to both toxins that planting Bt corn has reduced their populations (Fig. 3).

A new class of insecticidal protein, known as vegetative insecticidal toxin (Vip3a) is effective on fall armyworms and corn earworms, but has little impact on stalk borers, which cannot survive doses of Cry1Ab and Cry1F. This is why Vip3a is sold as a combination toxin (pyramid) with at least one other toxin that controls stalk borers.

Modern Bt hybrids now contain multiple toxins for insect control (Table 1), both for the broader spectrum of control they provide and to delay the development of resistance to the toxins in the suites (trait packages).

Table 1. Currently registered Bt toxins active against insects.

<table>
<thead>
<tr>
<th>Target pests</th>
<th>Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepidoptera (caterpillars)</td>
<td>Cry1Ab, Cry1F, Vip3a, Cry1A.105, Cry2Ab2</td>
</tr>
<tr>
<td>Corn rootworm</td>
<td>mCry3a, eCry3.1Ab, Cry3Bb1, Cry34/35Ab1</td>
</tr>
</tbody>
</table>

Figure 2. Bt traits and insect resistance. Corn hybrids without Bt traits for caterpillar control (in foreground) have damaged leaves, and plants with Bt traits for caterpillar control (in background) are tall and green with healthy leaves.

Figure 3. Corn borer damage to a corn stalk. This corn variety is not a Bt hybrid and does not have protection against corn borers. This crop would require timely scouting and insecticide application to control the pest.
Two important terms related to insect-protected Bt hybrids are stacked traits and pyramid traits. With stacked traits, toxins that act against different groups of insects are combined in a single hybrid. For example, Pioneer’s Optimum AcreMax 1 technology has one toxin to kill caterpillar pests (Cry1F) and a different toxin to kill corn rootworm larvae (Cry34/35Ab1). These are “stacked” together to target two different types of insect threats.

In this case, Cry1F has no effect on corn rootworm and Cry34/35Ab1 has no effect on caterpillars, but the combination of the two provides protection against both pests. Adding herbicide tolerance (glyphosate and glufosinate) to Optimum AcreMax 1 makes this a three-way stack of traits.

Pyramid traits combine two or more types of Bt toxins that act on the same group of pests. An example is Pioneer’s Optimum Intrasect that has only two toxins, Cry1F and Cry1Ab, both of which target caterpillars. This type of corn has no toxins for corn rootworm but is pyramided for toxins to protect against caterpillars.

Currently, stacked pyramids are the most advanced Bt technology. They are stacked for toxins active against different types of insects and pyramided for two or more toxins active against a particular type of insect or pest group.

A good example is Genuity SmartStax. It contains three different toxins targeted at caterpillars (Cry1F, Cry1A.105, and Cry2Ab2), making it a pyramid toxin plant for caterpillars. Because it also has two toxins directed at corn rootworm larvae (Cry3Bb1 and Cry34/35Ab1), it is pyramided against rootworms as well. Even though Cry34/35Ab1 is a binary toxin (made up of two parts: a toxin and a membrane-altering component that makes it easier for the toxin to enter the cells), both components are always present, so it acts as a single toxin. Genuity SmartStax also has genes for tolerance to two different types of herbicides, which makes these hybrids stacked pyramids.

Keeping up with new types of Bt corn and determining how effectively they control insects based on the number of pyramids and stacks they contain is a challenge. The Handy Bt Trait Table lists the toxins in all registered Bt corn and indicates which toxin or group of toxins control which pest species. It also lists the refuge requirements for each type of corn.

The IRM Refuge Calculator, developed by the National Corn Growers Association and seed companies, is a web-based tool that lists stewardship practices for any type of Bt corn grown in the U.S. It is also available as a downloadable app for iPhone or Android devices.

**Insect-Resistance Management**

Developing an Insect Resistance Management (IRM) plan is the best way to preserve the benefits of Bt traits in corn. A key component of this plan is to plant a non-Bt variety in a corn refuge area to maintain a population of insect pests that are not exposed to Bt proteins. When susceptible insects from the refuge mate with resistant insects from the Bt crop, they pass on the susceptibility to Bt technology to their offspring, helping preserve the effectiveness of Bt technologies.

There are varying refuge strategies for different types of Bt corn, depending on where the corn grows. Refuge requirements in Texas are split into two zones. The 20 counties in the northern part of the Panhandle follow the refuge requirements for the Corn Belt. Counties south of Amarillo have larger refuges because of the dominance of Bt cotton, which contains toxins similar to those in Bt corn.

Corn grown south of the 20 counties in the northern Panhandle has a 20 percent or 50 percent mandated refuge, depending on whether the corn has single or pyramid toxins and is active against caterpillar pests, corn rootworms, or both. The refuge in these counties (the cotton zone) must be a “structured refuge”—the refuge corn must be in its own rows in either a 4-row (minimum) or wider strip, or in a block planting in part of the field or in an adjacent field (with restrictions depending on the Bt traits).

In seed-blend refuges, sometimes referred to as “refuge in the bag,” the non-Bt seeds are blended with Bt seeds in a ratio that meets the minimum refuge stated in the stewardship agreement (Fig. 4). Some seed-blend refuges are permitted in the cotton
zone, but these must have an additional block refuge planted as well. Agrisure Viptera 3220 E-Z Refuge is an example of this seed-blend-plus-structured-refuge scenario.

Seed companies must report compliance data to the United States Environmental Protection Agency (EPA) each year, noting the number of growers who do and do not follow the stewardship guidelines. To increase compliance, seed company representatives confirm that corn growers have planted refuge areas that fulfill stewardship agreements.

Lack of compliance makes it more likely that resistance to Bt toxins will develop and can lead to the demise of the technology. The greatest concern if resistance develops is that the EPA can force the removal of certain Bt technologies from the market. This has already happened in Puerto Rico.

Another concern is that resistance means Bt hybrids stop working and growers face increased costs and lower farm profits. For example, growers in the Midwest pay a technology fee for rootworm Bt corn with the highest levels of seed treatment available, purchase additional soil-applied insecticides, and pay to spray adult rootworm beetles in the summer, all while losing yield because of the corn rootworm.

Figure 4. Seed-blend refuge. Different color dyes are sometimes used for refuge and transgenic seed. The purple seeds in this example are refuge seed. This product is known as “refuge in the bag.”

Seed companies cross-license Bt toxins to build multitoxin pyramids for caterpillar pests and corn rootworms and to improve their effectiveness against target pests and IRM. Improved efficacy is easy to understand; two toxins are better than one, and three toxins are better than one and possibly two. Generally, having more toxins in the pyramid results in better insect control.

Pyramids are critical in IRM because some insects can survive individual toxins in hybrids if they encounter these toxins one at a time. For example, the fall armyworm is resistant to Cry1F in Puerto Rico and parts of the southern U.S. Continuing to use Cry1F (only) corn each year increases the percentage of the resistant population. The answer is to add a second or even a third toxin so that the insects with genes that can survive Cry1F will probably not have the genes to survive through the second and third toxin. With the Cry1F-resistant insects removed from the population, resistance does not continue to develop.

The toxins currently on the market for caterpillar pests are Cry1Ab, Cry1F, Vip3a (a vegetative insecticidal protein), Cry1A.105, and Cry2Ab2 (Table 1). With pyramid Bt corn, it is possible to avoid most significant economic losses caused by direct-feeding caterpillars. Although the pyramid Bt corn will not be damage-free, the damage is less than with non-Bt corn.

The corn rootworm can be a significant pest in Texas (except in the Coastal Bend and in south Texas where the southern corn rootworm is common), and crop rotation is the best defense against it. However, because many growers cannot rotate out of corn, they grow it year after year in the same field. Laboratory studies show that corn rootworm can develop resistance to any of the current rootworm Bt toxins in as little as 4 years of continuous use.

In parts of the Midwest, where the western corn rootworm has become resistant to Cry3Bb1, the first confirmed cases of resistance were in fields planted in corn to Cry3Bb1 for 4 or more continuous years. Unlike caterpillar toxins that are relatively toxic to their target pests, corn rootworm toxins are less toxic overall and natural populations of corn.
rootworms have the genes to survive the toxins Cry3Bb1, Cry34/35Ab1, mCry3A, and eCry3.1Ab. There is some cross-resistance between Cry3Bb1 and mCry3A, and insects that become resistant to one of these toxins will have partial resistance to the other.

Crop rotation is the key to preventing resistance to corn rootworm toxins. If crop rotation is not an option, rotate the corn rootworm toxins. Do not plant the same rootworm technology in the same field for more than 3 years (less than 3 years is even better). This might mean buying seed from a different company, but the 3-year limit is vital for delaying resistance on a particular farm. Corn rootworm adults tend to stay in the same field where they feed on roots as larvae, and most corn rootworm resistance can be traced back to a specific field where toxins were not rotated.

**Herbicide Tolerance**

Similar to Bt traits, some corn hybrids have herbicide-tolerance traits such as glyphosate (Roundup Ready) and glufosinate (Liberty Link) tolerance. New traits, such as 2,4-D, dicamba, and FOP (aryloxyfenoxypropionate) herbicide tolerance could be available in the near future.

Like all transgenic traits, genes that provide tolerance are inserted in the plant using a genetic package that includes the gene and other components necessary for proper gene expression or function.

Herbicide traits differ greatly in how they provide tolerance to various herbicides. For example, understanding how glyphosate affects susceptible plants helps explain how glyphosate tolerance works. Plants need the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) to produce amino acids for plant growth and development. Glyphosate binds to EPSPS, preventing the development of these amino acids, and without them, the plant dies.

A “backup enzyme” from EPSPS mutations in maize (mEPSPS) or from *Agrobacterium* sp. strain (CP4 EPSPS) provides glyphosate tolerance. Because these backup enzymes (mEPSPS (event GA21) and CP4 EPSPS (event NK603)) have a modified shape that glyphosate cannot bind to, the plant produces amino acid in the presence of glyphosate, allowing the plant to grow normally.

Glufosinate tolerance works very differently. Glufosinate kills susceptible plants by binding to the enzyme glutamine synthetase, which plants need to detoxify ammonium in plant cells. After glufosinate application, this enzyme is not available and the plant dies within hours because ammonium hyper-accumulates in the plant cells.

Unlike glyphosate-tolerant plants that use a modified enzyme to enable the plant to produce amino acids, glufosinate-tolerant plants produce an enzyme, phosphinothricin acetyltransferase (PAT) that detoxifies glufosinate molecules. Glufosinate tolerance was originally used as a selectable marker in breeding programs. Like glyphosate tolerance, the PAT gene is expressed in all plant cells, which provides an additional tool to manage glyphosate-resistant weeds.

**Herbicide-Resistance Management**

A comprehensive weed control program to manage resistance is the first step in preserving transgenic herbicide-tolerance traits. Using different herbicide chemistries to rotate the mode of action (the way a chemical affects the plant at a cellular level) against target weeds is critical to any resistance-management program.

Several herbicide-resistant weeds have developed in Texas because of over-use and reliance on single herbicides. Similar to Bt resistance, repeated exposure to one herbicide encourages the development of resistant types within a weed population. In Texas, Palmer amaranth and waterhemp pigweeds have shown resistance to glyphosate for more than 10 years (Fig. 5).

To manage herbicide resistance, include residual herbicides with different modes of action into the herbicide program, starting from preplant burn-down through layby. Apply the herbicides as tank mixes or through a sequential application of contrasting herbicides.

Crop rotation is another way to manage herbicide resistance. When possible, rotate conventional,
Roundup Ready, and Liberty Link corn hybrids to use different herbicide chemistries. Apply the full, recommended labeled rates when the weeds are small. Although the reduced costs of conservation tillage are a major advantage, difficult-to-control weeds may require mechanical weed control.

Minimizing seed production by in-season weed escapes (weeds not controlled by in-season herbicide application) and post-harvest recruits (weeds that emerge and produce seed post-harvest during fallow periods) reduces the development of herbicide resistance.

Growers may need to spend more money and effort to keep their fields free of troublesome species such as pigweeds. However, the short-term efforts are well worth it, considering the long-term benefits in cost savings and profits. When it comes to resistance management, whether for insects or for weeds, being proactive is critical because reactive measures can be costly and damaging.

**Drought Tolerance**

Drought stress is common with dryland corn production in Texas (Fig. 6) and drought tolerance traits could provide some benefits to producers. Drought tolerance in corn hybrids is a relatively new area for marketing transgenic traits. As of 2014, Monsanto was the only company marketing a drought-tolerant transgenic trait. Now, several companies sublicense the trait and incorporate it into various hybrids. Other companies market some hybrids as drought resistant, but the traits were developed through selecting native trait genes (nontransgenic). Other companies will probably offer transgenic drought-tolerant hybrids in the near future.

Monsanto developed transgenic, drought-tolerant hybrids (event code: Mon87460) to reduce yield loss in water-limited environments. The cold shock protein B (CspB), from the bacteria *Bacillus subtilis*, promotes drought tolerance because it preserves normal plant functions under stressful conditions such as water stress. Field trials show that drought-tolerant transgenic hybrids have increased yields because of their better grain set under drought conditions.
Summary

Growers have more transgenic corn hybrids to choose from than ever before, with more trait options available each year. While these traits offer resistance to insects, herbicides, disease, and drought, growers must manage these hybrids appropriately and adhere to stewardship agreements and other requirements to preserve these benefits and the technology that produces them.

Resources

Handy Bt Trait Table  
Produced by the University of Wisconsin and Michigan State University. Updated twice a year, this publication lists the toxins in registered Bt corn and the pests they control.

IRM Refuge Calculator  
http://www.ncga.com/irm-calculator  
Easy reference for stewardship practices for Bt corn grown in the United States. Available as a web-based tool or as downloadable apps for iPhone and Android devices.

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