SCS-2015-03

Guide for Trait Selection in Corn

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Introduction

Transgenic traits are widely available in corn hybrids today. Use of hybrids containing transgenic traits has increased dramatically in Texas and the U.S. over the past twelve years (Figure 1). As of 2012, transgenic varieties comprise 85% of all Texas corn acreage planted, just below the U.S. average of 88%. With the options available today and the cost associated with each technology, it is important to understand the terminology and biology of transgenic traits. The following summary of transgenic corn traits will be divided into three sections: insect resistance, herbicide tolerance, and drought tolerance.

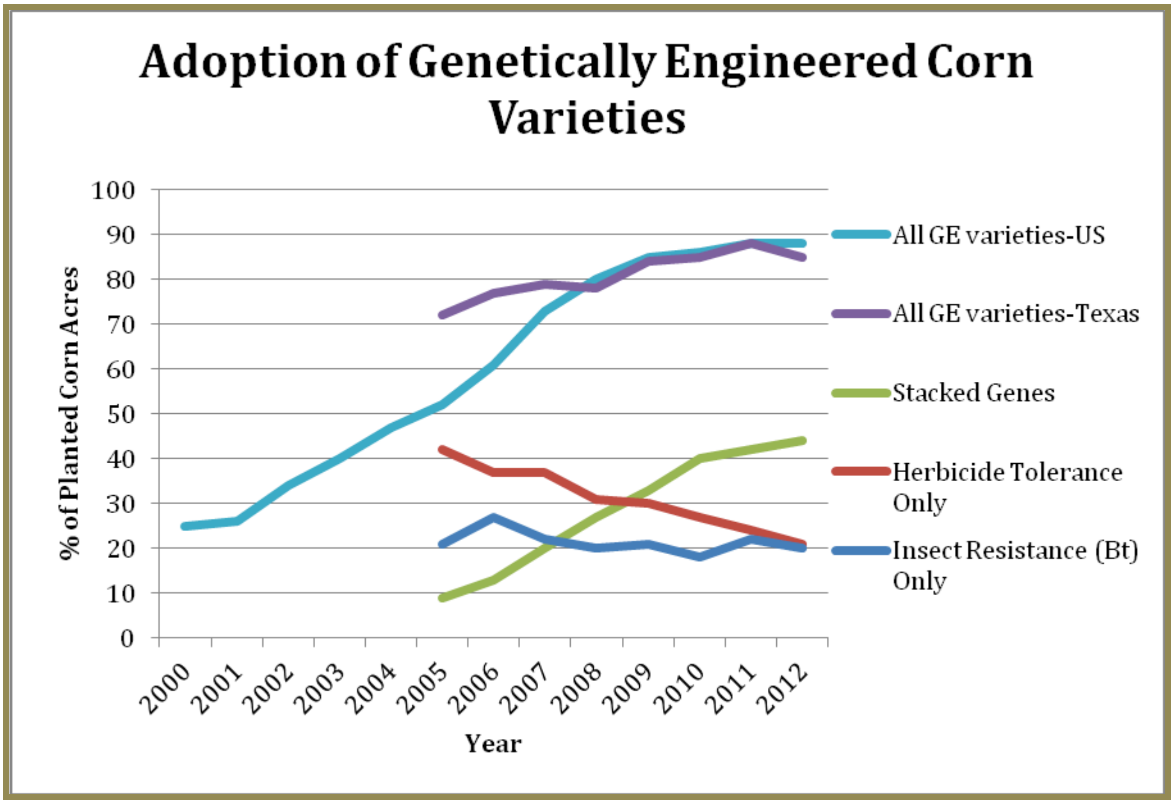

Figure 1. Percent of planted acres using genetically engineered corn varieties for the U.S. and Texas (adapted from: Adoption of genetically engineered crops in the US. 2012. USDA-ERS).

Plant Incorporated Protectants for Insect Control

Modern insect-protected transgenic corn contains combinations of several protectants all of which are derived from various subspecies of the bacterium *Bacillus thuringiensis*. This is why we use the term Bt corn. However, newer technology that does not rely on Bt is on the horizon.

The plants contain genes that code for crystalline proteins (Bt proteins) present in the natural Bt bacteria. When certain insects ingest these proteins, they break down into smaller subunits, some of which bind on the wall of the insect gut. This binding eventually causes a small hole to form in the gut wall. Natural bacteria present in the gut then move into the body of the insect resulting in death from bacterial septicemia. It is the case that there is a wide range of susceptibility to Bt toxins among broad insect groups (such as caterpillars, beetles, flies etc.) and among even closely related insects within a small group (such as caterpillars). For example, the Bt proteins active against corn rootworm have absolutely no affect on caterpillars. Similarly, the tobacco budworm is very much affected by Bt toxins, but the corn earworm (cotton bollworm), which is in a closely related genus, is far less affected by the same Bt proteins. The acidity of the insect gut is important in that, and the wrong acidity prevents the crystal subunits from forming. Additionally, the insects must have the right type of receptors on the gut wall in order for the protein subunits to bind, and many insect species lack the appropriate receptors for the Bt toxins they encounter when feeding on Bt plants.

This differential activity by Bt proteins on different, even closely related, insect species explains why some Bt toxins work better on some pests than on others. A good illustration of this phenomenon is that fall armyworm is relatively less affected by the Bt toxin Cry1Ab than by the Bt toxin Cry1F. However, our stalk borers, southwestern corn borer and European corn borer are extremely susceptible to both toxins, so much so that we have driven down the size of their populations just by planting Bt corn. The new vegetative insecticidal toxin Vip3a is very effective on fall armyworm and corn earworm, but has little effect on stalk borers, which are unable to survive doses of Cry1Ab and Cry1F. This is why Vip3a is always sold as a combination toxin (pyramid) with at least one other toxin that will control stalk borers.

Figure 2. Corn hybrids without Bt traits for caterpillar control (foreground) and with Bt traits for caterpillar control (background).

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

To understand modern insect-protected Bt hybrids, it is important to know the meaning of two terms, "stacked traits" and "pyramid traits". Stacked traits have been around a long time. Stacking is where toxins that act against totally different groups of insects are put together in a single hybrid. A good example of this is Pioneer's Optimum® AcreMax®1 technology that has one toxin to kill caterpillar pests (with Cry1F) and a different toxin to kill corn rootworm larvae (with Cry34/35Ab1). These are "stacked" together to target two very different types of insect threats. In this particular 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 insect pests. Similarly, herbicide tolerance (glyphosate and glufosinate) is also added to Optimum® AcreMax®1, which makes this a three-way stack of traits.

Pyramids are where two or more types of Bt toxins that act on the same group of pests are combined in a plant. The simplest example on the market today is Pioneer's Optimum®

Intrasect® that has only two toxins, Cry1F and Cry1Ab, both of which are targeted at caterpillars. This type of corn has no toxins for corn rootworm but is pyramided for toxins to protect against caterpillars.

Stacked pyramids are currently the highest evolution of Bt technology and are both stacked for toxins active against very 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) and is therefore a pyramid toxin plant for caterpillars. Additionally, it has two toxins directed specifically at corn rootworm larvae (Cry3Bb1 and Cry34/35Ab1) so it is pyramided against rootworms as well. Technically speaking, Cry34/35Ab1 is a binary toxin, but 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.

It can be difficult to keep up with all of the new types of Bt corn and determine which might be more effective at insect control based on the number of pyramids and stacks it contains. The best place to go for this information is the "Handy Bt Trait Table", a publication jointly produced by the University of Wisconsin and Michigan State University. This publication is updated twice per year and can be found in the list here: http://labs.russell.wisc.edu/cullenlab/extension/extension-publications/. The Handy Bt Trait Table lists the toxins in all registered Bt corn and breaks them down into which pest species are controlled by toxin or group of toxins. Refuge requirements for each type of corn are listed in the publication but these do not apply to Texas except for the 20 counties at the northern tip of the Panhandle.

Table 1. Currently registered Bt toxins active against insects.

Target Pests	Toxin
Lepidoptera (caterpillars)	Cry1Ab, Cry1F, Vip3a, Cry1A.105, Cry2Ab2
Corn rootworm	mCry3a, eCry3.1Ab, Cry3Bb1, Cry34/35Ab1

It can be very difficult to keep up with the proper stewardship practices required for the many types of Bt corn on the market today. As a response to that problem, the seed companies have partnered with the National Corn Growers Association to make it easy to know the stewardship practices for any type of Bt corn grown anywhere in the country. There is a web-based tool here: http://www.ncga.com/for-farmers/issue-briefs-ipm/irm-refuge-calculator, and downloadable apps for iPhone and Android devices are available on this page as well.

Insect Resistance Management

The best way to preserve the benefits of Bt traits in corn is to develop and implement a resistance management plan. A corn refuge is a key component of Insect Resistance Management (IRM). The primary purpose of a refuge is to maintain a population of insect pests that are not exposed to Bt proteins. This lack of exposure allows susceptible insects emerging from the refuge to mate with resistant insects that may emerge from the Bt crop. Susceptibility to Bt technology would then be passed on to their offspring, helping to preserve the effectiveness of Bt technologies.

There are different types of refuge strategies for different types of Bt corn, and these often vary depending on where the corn is grown. Texas is split in to two zones. The 20 counties at the top 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 some toxins similar to those found in Bt corn. All corn in Texas grown south of the 20 counties in the northern Panhandle has a 20% or 50% mandated refuge depending on whether the corn has single toxin or pyramid toxins and is active against corn rootworms or caterpillar pests or both. Regardless of the complexities involved in the sentence above (and there are many), all of the refuge in the counties south of the 20 counties in the northern Panhandle (the cotton zone) must be a “structured refuge”. This means that the refuge corn must be in its own rows, either a 4-row (minimum) or wider strip or a block planting in part of the field or in an adjacent field (with restrictions depending on the Bt traits). Seed blend refuges, sometimes referred to as “refuge in the bag” are where the non-Bt seeds are blended with the Bt seeds at the right ratio to give the minimum refuge stated in the stewardship agreement. There are some seed blend refuges permitted in the cotton zone, but these must have an additional block refuge planted as well. Agrisure Viptera® 3220 E-Z Refuge™ is one example of this seed blend + structured refuge scenario.

Seed companies must report “compliance data” to the EPA each year. Compliance is the number of growers who are following the stewardship guidelines and the number who are not. EPA has noted a slip in compliance in recent years, especially in the south (cotton zone) and has turned up the heat on companies to increase compliance. The seed companies know that a lack of compliance means it is more likely that resistance will develop to their Bt toxins and will eventually lead to the demise of the technology. Taken together, this is why corn growers can expect visits from seed company representatives to insure they are in compliance with the stewardship agreement(s). The greatest concern is if resistance develops, the EPA can force the removal of certain Bt technologies from the market. This has already happened in Puerto Rico. Of course, the other concern is that resistance means Bt hybrids stop working and growers once again lose money to the pests that have developed resistance. Growers in the Midwest are now paying the technology fee for rootworm Bt corn (with the highest levels of seed treatment available) in addition to purchasing soil applied insecticides to put on top of it, as well as paying again to spray adult rootworm beetles in the summer, all the while losing yield to corn rootworm. Resistance means vastly increased costs and lower farm profits.

Seed companies have begun to cross-license Bt toxins from each other in order to build multi-toxin pyramids for caterpillar pests and corn rootworm. There are two very good reasons for this. It will improve efficacy 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. As a general statement, better insect control results from more toxins in the pyramid, although this can vary somewhat on the particular toxins being used.

Pyramids are critical in IRM because we do have insects that have the genes to survive some of the individual toxins in our hybrids if they encounter these toxins one at a time. For example, fall armyworm is resistant to Cry1F in Puerto Rico and parts of the southern USA, and continuing to use Cry1F (only) corn will make a larger and larger percentage of the population resistant each year. The answer is to add a second or even a third toxin so that the insects with genes to live through Cry1F will most probably not have the genes to live through the second and third toxin. This effectively removes the Cry1F resistant insects from the population and resistance to Cry1F does not continue to develop. The toxins currently on the market for caterpillar pests are Cry1F, Cry1Ab, Cry1A.105, Cry2Ab2 and Vip3a (a vegetative insecticidal protein). With pyramid Bt corn, it is now possible to avoid most significant economic loss to direct feeding by caterpillars. Don't expect the pyramid Bt corn to be damage-free, but know that the damage is less than would be the case with non-Bt corn.

Corn rootworm can be a significant pest anywhere in Texas (except in the Coastal Bend and south Texas where the southern corn rootworm is common) and the best defense against corn rootworm is crop rotation. However, many growers cannot rotate out of corn and they grow corn year after year in the same field. Laboratory studies have shown that corn rootworm can develop resistance to any of our current rootworm Bt toxins in as little as four years of continuous use. Western corn rootworm has become resistant to Cry3Bb1 in parts of the Midwest, and the first confirmed cases of resistance were in fields that were in continuous corn planted to Cry3Bb1 for four or more years. Unlike our caterpillar toxins that are relatively toxic to their target pests, corn rootworm toxins are relatively less toxic overall and we already know that the natural populations of corn rootworms have the genes to survive our current toxins; Cry3Bb1, Cry34/35Ab1, mCry3A and eCry3.1Ab. There is some cross resistance between Cry3Bb1 and mCry3A so insects that become resistant to one of these toxins will have partial resistance to the other.

They key to preventing resistance to corn rootworm toxins is to practice crop rotation where possible and rotate corn rootworm toxins where crop rotation is not possible. Never plant the same rootworm technology in the same field for more than three years, and fewer years than three is better. This might mean buying seed from a different company, but it is vital that this three-year limit be observed if resistance is going to be delayed on a particular farm. Corn rootworm adults tend to stay in the same field where they fed on roots as larvae, and most corn rootworm resistance can be tracked right back to a specific field where toxins were not rotated.

Herbicide Tolerance

Similar to Bt traits, several herbicide tolerance traits are available in corn hybrids 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 must be inserted in the plant using a genetic package. The package includes the gene, promoter and other necessary components for proper expression. Herbicide traits differ greatly in how they provide tolerance to various herbicides.

To understand how glyphosate tolerance works, it is necessary to understand how glyphosate affects susceptible plants. The enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is needed by plants to produce aromatic amino acids. Glyphosate binds to EPSPS, inhibiting the formation of amino acids needed for growth and development by the plant. The plant eventually dies as supplies of certain amino acids are exhausted. Glyphosate tolerance is provided by means of a "backup enzyme" that was derived from multi-site mutations of EPSPS in maize (mEPSPS) or from *Agrobacterium* sp. strain CP4 EPSPS. The backup enzymes mEPSPS (event GA21) and CP4 EPSPS (event NK603) have a modified shape that prevents glyphosate from binding to it. This enables the modified enzyme to continue to catalyze amino acid synthesis in the presence of glyphosate, allowing the plant to grow normally. Both genes utilize promoters to allow expression in all plant cells. In addition, the coding sequence for chloroplast transit peptide (CTP2) is included with the gene. This helps direct expression of modified EPSPS to the chloroplast of plant cells, which is the site of the EPSPS pathway and glyphosate site of action.

Tolerance to glufosinate is accomplished very differently than tolerance to glyphosate. Glufosinate application results in death of susceptible plants by binding to the enzyme glutamine synthetase, which is needed to detoxify ammonium in plant cells. When the glutamine synthetase enzyme is not available following glufosinate application, death of the plant occurs within hours of application due to hyper-accumulation of ammonium in cells. Rather than using a modified enzyme to enable amino acid synthesis, glufosinate tolerant plants produce an enzyme that detoxifies glufosinate molecules. The enzyme involved in detoxification of glufosinate is called phosphinothricin acetyltransferase (PAT). The PAT gene was derived from the soil bacteria *Streptomyces viridochromogenes*. As with glyphosate tolerance, the PAT gene is expressed in all plant cells. Glufosinate tolerance was originally utilized as a selectable marker in breeding programs but is also expected to aid in the management of glyphosate-resistant weeds.

Herbicide Resistance Management

The first step in preserving transgenic herbicide tolerance traits involves the implementation of comprehensive weed control program for resistance management. The use of different herbicide chemistries to rotate the mode of action against target weeds is critical to any resistance management program. Over-use and reliance on single herbicides has resulted in the development of several herbicide-resistant weeds in Texas. Similar to Bt resistance, repeated exposure to only one herbicide will select for resistant types within a population of weed species over time. Palmer amaranth and waterhemp pigweeds have shown resistance to glyphosate in Texas for over 10 years.

There are several practices that should be used to manage the development of herbicide resistance. Always include residual herbicides, with different modes of action, into the program starting from preplant burndown through layby. This can be accomplished by applying herbicides as tank-mixes or through sequential application of contrasting herbicides. Another practice to manage herbicide resistance is crop rotation. Rotate conventional, Roundup Ready®, and Liberty Link® corn hybrids when possible. Rotation to alternative crops can create opportunities for using different herbicide chemistries. Only apply the full, recommended labeled rates and make applications when weeds are small to ensure applications are effective. Although the reduced costs associated with conservation tillage are a major advantage, mechanical weed control may be very helpful when difficult to control weeds are found. Ultimately, a strong focus on minimizing seed production by in-season weed escapes and post-harvest recruits is imperative for minimizing the risk of herbicide resistance evolution. It may mean that growers need to spend some additional money and effort to keep their fields free of troublesome species such as the 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 tolerance in corn hybrids is a relatively new area of focus for marketing transgenic traits. As of 2014, only one company (Monsanto) was marketing a drought tolerant transgenic trait, with multiple companies now sub-licensing the trait and incorporating it into various hybrids. Other companies have hybrids marketed as drought resistant but the traits were developed through selection of native trait genes (non-transgenic). Transgenic drought tolerant hybrids from other companies are expected in the near future. The transgenic drought tolerant hybrids offered by Monsanto (event code: Mon87460) were developed to reduce yield loss under water-limited environments. Drought tolerance is conferred from the expression of the cold shock protein B (CspB) derived from the bacteria *Bacillus subtilis*. CspB serves as a molecular chaperone, preserving RNA stability and translation. This results in the preservation of normal plant functions under stressful conditions, such as water stress. Initial field trials have demonstrated that drought tolerant transgenic hybrids do have better grain set under water limiting environments resulting in increased yields.

Summary

Many transgenic traits are available today and new traits are being developed every year. Understanding the biology and terminology used in trait development and management is essential for growers making decisions about hybrid selection and transgenic traits associated with various commercial hybrids. Transgenic traits provide growers valuable tools to manage weeds, insects, and plant stresses. However, these traits must be deployed appropriately to maximize the useful longevity of these tools.

Produced by the Department of Soil and Crop Sciences
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