# afronomy news

# Managing the European Corn Borer Using BT Corn

Transgenic plants produce insecticidal protein to provide better crop protection than insecticides.

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Several seed companies are now marketing Bt corn, one of the first commercially available products of biotechnology that has practical ramifications for U.S. and

Canadian corn farmers. Bt corn hybrids produce an insecticidal protein derived from the bacterium *Bacillus thuringiensis*, commonly called Bt. These Bt hybrids provide protection against *Ostrinia nubilalis*, the European corn borer (ECB), which is the most damaging insect pest of corn throughout the United States and Canada. Protection from Bt corn is usually far better than optimally timed

far better than optimally timed insecticides. Yield losses resulting from ECB damage and control costs exceed \$1 billion each year. This discussion provides an overview of Bt corn, an unprecedented technology for managing ECB, and examines how





to use this technology for long-term profitability.

Because Bt hybrids contain an exotic gene, they are commonly referred to as transgenic plants. The Bt gene in these plants produces a protein that kills ECB larvae. Most larvae die after ingesting only a few bites. Consequently, Bt corn provides high levels of yield protection even during heavy infestations of ECB. Plant geneticists created Bt corn by inserting selected exotic DNA (from Bt

(continued on page 2)

# **Bt Corn**

(Continued from page 1)

organism) into the corn plant's own DNA. DNA is the genetic material that controls expression of a plant's or animal's traits. Seed companies select elite hybrids for Bt transformation in order to retain important agronomic qualities for yield, harvestability and disease resistance. This genetic package is inserted into corn through a variety of plant transformation techniques.

Successful transformations, called "events," vary in the components of

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the genetic package and where this DNA is inserted into the corn DNA. The insertion site may affect Bt protein production and could affect other plant functions. Consequently, seed companies carefully scrutinize transformation events to ensure adequate production of Bt protein and no negative effects on agronomic traits. As of September 1997, the Environmental Protection Agency (EPA) has registered four unique transformation events for commercial use: 176 (Novartis Seeds and Mycogen Seeds), BT11 (Northrup King/Novartis Seeds), MON810 (Monsanto) and DBT418 (DEKALB Genetics Corp.). Event 176 is trademarked as "KnockOut" by Novartis and "NatureGard" by Mycogen. Both the BT11 and MON810 events are trademarked as "YieldGard" and the DBT418 event is trademarked as "Bt-Xtra."

Various seed 220 Grain Yield (Bu/A) 210 200 190 180 170 160 UNTREATED **TREATED** Insecticide Treatment bag. The

Figure 1: Grain yields for Bt and Non-Bt hybrid pairs and insecticide treatments at Yuma in 1997.

companies license each event, so when purchasing seed, note which trademark is present on the seed tag or

number of

events is likely to increase rapidly. Understanding these events and how they affect performance is critical to the wise selection of corn hybrids.

## Colorado Situation

Colorado com producers will find Bt corn hybrids widely available in 1998. We are interested to determine if use of Bt hybrids is a sound management scheme for Colorado's production environment. To do this, we compared the performance of many of the available Bt hybrids to non-Bt hybrids adapted to eastern Colorado during the 1997 season at two locations (Yuma and Wray). We also compared the ECB control efficacy of Bt corn hybrids to the current practice of properly scouted and timed insecticide applications. The results and conclusions in this report represent only a single crop year, in which ECB activity was greater than normal. They are intended to help decide whether to consider the Bt trait in the hybrid decision process, but keep in mind that corn borer control is only one of many performance traits to consider when selecting a corn hybrid.

## **Results - Pest Control**

The following remarks represent our observations from the twolocation study pertaining to pest control:

■ NON-BT

■ BT

First and second generation ECB were controlled both by insecticide and Bt hybrids. (continued on page 3)

# **Bt Corn**

(Continued from page 2)

Control patterns, as determined by position of ECB second generation larvae on the plant, were similar for both chemical and Bt hybrid control.

There was less stalk rot on Bt. hybrids compared to non-Bt hybrids at Yuma.

# **Results - Agronomic Response**

The following represent our observations from the study pertaining to agronomic responses:

Grain moisture at harvest was higher in Bt hybrids than in non-Bt hybrids at both locations.

Lodging was reduced by insecticide treatments at both locations and by the use of Bt hybrids at Wray.

Bt hybrids yielded more than non-Bt hybrids at both locations (Figs. 1&2).

Insecticide-treated hybrids yielded more than untreated hybrids at Yuma. Much of this difference may have been due to Banks grass mite control.

The untreated Bt versions of the five isoline (identical hybrids except for presence of the Bt gene) comparisons out-yielded their untreated non-Bt counterparts by 18 bu/acre at Yuma (Fig. 1) and 22 bu/acre at Wray (Fig. 2). These yield differences were entirely due to ECB control.

## **General Observations**

Our general observations from the study are:

Superior non-Bt hybrids combined with properly scouted and timed insecticide applications may be a more economically sound pest management approach in areas with less consistent ECB activity.

Secondary pest management benefits, such as control of

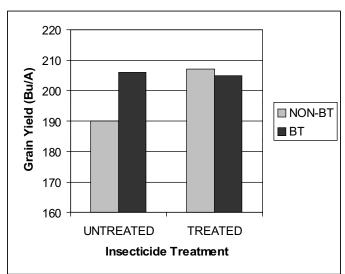


Figure 2: Grain yields for Bt and Non-Bt hybrid pairs and insecticide treatments at Wray in 1997.

western bean cutworm and western corn rootworm. associated with ECB treatments will not be realized on untreated Bt hybrids. Bt hybrids will have to be scouted for these pests and treated if necessary.

Untreated Bt hybrids may experience fewer insecticide-associated pest problems, particularly spider mite outbreaks, although this

was not observed in this year's study.

The use of Bt hybrids is a profitable and effective approach to ECB management in years of heavy corn borer activity and in areas with consistent year-to-year corn borer activity.

Consistent corn borer activity can, in part, be determined by historical records on light trap catches and soil type. Based on

> these factors, corn borer risk can be divided into three categories.

"Heavy soil," low corn borer risk based on light trap catch records. This category is represented at Bonny Dam, Burlington, and Kirk.

"Heavy soil," higher corn borer risk based on light trap catch records that

indicate a low-level, but continuous flight activity, represented by Clarkville, Yuma, and Holyoke.

"Light soil" consistently high ECB risk, represented by Eckerly, Wray, Wauneta.

# **Resistence Management**

One of the key management issues surrounding Bt corn is resistance management. It is possible that ECB larvae may become resistant to Bt corn. One principle of a

(continued on page 9)

# Using DNA Markers to Improve Wheat and Barley

Locating marker genes allow wheat and barley breeders to include desirable traits in new genetic lines more efficiently.

For many people, the word "biotechnology" conjures up images of creatures formed in the laboratory from a single cell such as "Dolly" the lamb, or the dinosaurs in the science fiction film "Jurassic Park". Most applications of biotechnology represent minor deviations from how things are presently done, but nonetheless have a dramatic impact on the efficiency and effectiveness of the process. One of these applications is the use of DNA markers to select for plants containing desired genes.

What are DNA markers and why can they be used for plant selection? DNA markers are pieces of DNA that lie near a specific gene of interest (e.g., gene for disease resistance) on the chromosome. Because of the proximity of a marker to a gene, the marker and the gene are usually inherited together in a population that is segregating for the trait. Plants containing a gene can therefore be identified by simply assaying for the presence of the associated DNA marker in the laboratory. The use of markers for selection offers several advantages over the use of morphology-based selection. Selection can be done at a very early stage of the plant, such as seedling stage or embryo stage, before the developmental stage when the trait is usually expressed. Because selection does not depend

on expression of the gene, selection for disease or insect resistance can be done without having to inoculate plants with a pathogen or infest with insects. This is particularly important in diseases where the inoculation method is time-consuming or expensive and is subject to many environmental effects.

In research programs at Colorado State University, DNA markers are being developed for genes controlling resistance to the Russian wheat aphid (RWA) in wheat and resistance to fusarium head blight or scab in barley. The RWA is the most significant pest problem in wheat in the United States. Since its introduction in the US in 1986, total US damages from the RWA are estimated to be \$475 million from 1987 to 1994 in the US. Colorado damages from 1986 to 1996 are estimated at \$112 million.(Peairs, personal communication). Damages were incurred both from yield losses and the cost of pesticide application. Many genes for resistance have been identified, however these are found in unadapted wheat cultivars or wild relatives of wheat which carry undesirable traits. Recently, three resistant wheat cultivars (Halt, Tam-107R, and Yumar) containing the Dn4 gene were released by Dr. Jim Quick at Colorado State

University. DNA markers for several RWA resistance genes are being developed to enable "pyramiding" or combination of two or more resistance genes in a single cultivar to provide a more durable type of resistance. At present, there is only one RWA biotype in the US although at least seven different biotypes have been identified worldwide. If new biotypes appear in the US, resistant cultivars containing the Dn4 gene could become ineffective against the aphid. Gene pyramiding could also be done using conventional selection methods, but the length of time to develop cultivars with two genes would be longer than using marker-assisted selection.

Scab is the most devastating disease that threatens barley production in the Midwest. Scab resistance is a more complex trait than resistance to RWA because it is controlled by many genes (a quantitative trait) and symptom expression is highly influenced by the environment. Environmental influence on quantitative gene expression makes breeding for quantitative traits difficult and inexact. DNA marker-assisted selection can vastly improve the efficiency of this process. Using markers, it is possible to determine not only how many genes control a trait, but also how much these genes

(continued on page 5)

organization of genes

in these species.

# **DNA Markers**

(Continued from page 4) contribute to the trait. Selection of a quantitative trait based on markers thus allows a breeder to identify those plants containing desired gene combinations. Thus far, we have identified four chromosome regions containing genes that contribute to scab resistance in barley.

The identification of markers linked to a gene also provides the basis for cloning of these genes by a process called positional cloning. This technique allows cloning of genes for which there are no known gene products, which is the case with most agronomic traits, including disease and insect resistance, yield, and tolerance to environmental stresses like drought and heat. Once genes responsible for these traits are cloned, they can be transferred into any cultivar in a matter of months and plants expressing the gene can be field tested. Furthermore, when a cloned gene is transferred to a cultivar by transformation, no undesirable genes are carried along with the desired gene, as often happens with conventional breeding. Positional cloning has been successfully applied for the cloning of genes for disease resistance in tomato and rice. Its application in wheat or barley has not been demonstrated and we are developing techniques to make gene cloning in these crops possible. Nora Lapitan

meet.



# Nora L. V. Lapitan is

an associate professor in the Department of Soil and Crop Sciences. Her research program involves the application of molecular techniques in wheat and barley improvement. Current projects in her laboratory are to develop DNA markers to tag genes for economically important traits, including resistance to Russian wheat aphid in wheat and resistance to fusarium head blight or scab in barley. Her group is working closely with wheat and barley breeders who will use the markers as selection tools in their breeding programs. Another goal of her program is to be able to clone RWA resistant genes for transfer into susceptible plants by transformation. Since gene cloning

in wheat and barley is not straightforward, research is being conducted to determine the

> Lapitan teaches a graduate level course entitled "Plant Molecular Genetics". Dr. Lapitan holds Ph.D. and M.S. degrees in Genetics from Kansas State University. Before coming to CSU, Nora was a postdoctoral associate at the Department of Plant Breeding and Biometry at Cornell University, where she conducted research on molecular mapping and genome organization in tomato.



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Editor

# Dissecting the Genetic Control of Complex Plant Traits

Evaluating experimental varieties with field and laboratory tests allows researchers to reveal the web of components for complicated inherited traits.

Many plant traits important to crop production, such as yield potential, stress tolerance, or end-use quality, are governed by quantitative inheritance. That is, they are controlled by many genes acting together to produce the desired plant type. Until recently, it has been difficult to focus on the individual genes (known as quantitative trait loci or QTLs) that contribute to a quantitative trait. However, the advent of DNA molecular markers and the chromosome maps derived from them has enabled researchers to "dissect" trait inheritance into its component parts as never before. Locating the QTLs in specific chromosome regions, estimating the size of their effects, and determining their type of gene action are initial steps in manipulating the genes in breeding programs to produce superior varieties.

Two traits we have chosen to investigate with QTL methodology are heat tolerance in wheat and Fusarium wilt resistance in dry beans, characteristics important to Colorado growers, according to CSU plant breeders Jim Quick and Mark Brick. The scheme for conducting QTL analysis is shown in the accompanying diagram. Typically, parents having large differences for the trait of interest,

e.g., a heat tolerant and a heat susceptible wheat line, are crossed together to create a segregating population of 150 to 200 families. Each family in the population is evaluated both for the agronomic trait of interest and for DNA markers. To evaluate heat tolerance we will use a combination of field measurements (such as yield in heat-stressed locations) and laboratory analyses (such as membrane thermostability, a characteristic which is correlated with field performance under hot conditions).

At the same time, we will determine DNA marker patterns for each family. These markers are landmarks along the plant's chromosomes that indicate which parent's DNA was inherited in a particular chromosome region; normally each chromosome will be a mosaic of regions inherited from both parents. When 75 to 100 markers have been scored across the whole population, linkage maps showing the order and distance between markers can be constructed for each chromosome, as shown in the diagram.

The final step in QTL analysis is to combine the trait data with the DNA marker data and run a series of statistical analyses. For each DNA marker, the families are divided into three classes, those having the parent A pattern, the parent B pattern, or the AB pattern from both parents. We then ask the question, Do mean values of the measured trait differ among the three classes? If the classes do differ significantly, we conclude that a OTL influencing the trait is located in the vicinity of the marker. The graph at the bottom of the diagram represents a typical QTL analysis output and indicates the probability that a OTL exists along the chromosome. The peak of the curve corresponds to the most likely position of the OTL, in this case between markers 3 and 4.

How would a plant breeder use this information? The most direct way is to conduct marker-assisted selection, in which lines in a breeding program are screened for the appropriate DNA bands at markers flanking the QTL. For example, if the desired QTL effect is shown to be from parent A, then lines having the parent A pattern at the adjacent markers are selected. The attractiveness of conducting marker-assisted selection will depend on the cost and logistical difficulty of measuring the trait per se relative to evaluating marker patterns. With regard to heat tolerance in wheat, the use of

# QTL ANALYSIS

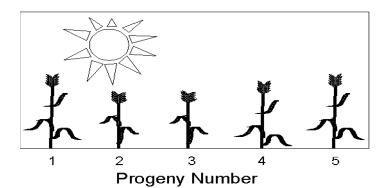
Cross parent lines A x B.

From the cross, develop population of 150-250 progeny families. Evaluate each progeny for agronomic traits and molecular markers.



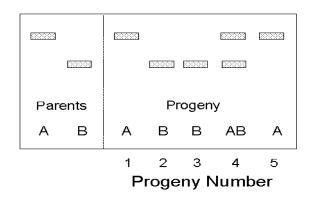
# Field Evaluation

Evaluate traits of interest in the field, in this case yield under heat stress. Typically, multiple traits are evaluated in several environments.



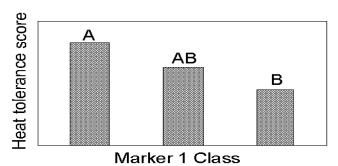
# Lab Evaluation

Use gel electrophoresis to determine size differences of specific DNA fragments (markers). Score each progeny as having the Parent A or Parent B pattern or both. Repeat for 75-150 markers.

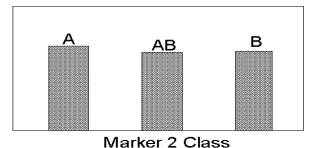




# Analyze combined field and lab data



Class differences indicate a QTL is linked to this marker.



l values for all classes ind

Equal values for all classes indicate that no QTL is linked to this marker.

(continued from page 6) markers may be effective because of the unpredictable occurrence of heat stress in the field and the resulting difficulty of evaluating heat tolerance directly.

In the longer term, QTL analysis

results can provide insights into the biological basis of trait expression. When combined with other information on gene location and function in wheat and related crops, QTL data on heat tolerance might suggest that the trait is associated

with a heat-stable enzyme, a more efficient cooling mechanism, or an anatomical adaptation of the plant. This information, in turn, should lead to strategies for further genetic manipulation of the trait. *Patrick Byrne* 

# Biotechnology Impacts Weed Management Issues in Corn Production

# Herbicide resistant corn helps farmers combat common weeds in Colorado's third-ranked cash crop.

If you are wondering how biotechnology is going to impact crop production on your farm, look no further than your own corn fields where Poast Protected, Liberty Link, Roundup Ready, or IMI corn could be a part of your corn production system in 1998. All of these technologies offer new and exciting use of herbicides. Advanced breeding, tissue culture, and biotechnology techniques have been used to develop herbicide resistant corn varieties for these herbicides.

## **Background**

Corn producers in the Central Great Plains have benefitted from the many genetic advances that improved corn hybrids over the past 50 years. Although corn originated in a more tropical climate, it has been genetically altered to provide favorable yields under irrigated and dryland conditions in Colorado and other western states. Grain corn sustains commercial cattle and hog feeding operations of Colorado and surrounding states. Dryland corn is becoming an important rotational crop used to break the life cycle of winter annual weeds such as jointed goatgrass or downy brome. In Colorado, corn usually ranks in the top 3 cash crops on an annual basis.

#### Weed Effects in Corn

Weeds cause several damaging effects in corn production. Weeds can impact the severity of corn

disease problems or harbor insects which attack corn. The most obvious damage caused by weeds is reduced corn yields due to competition for light, water, or nutrients. It is estimated that weeds annually reduce corn yields by an average of 10% in the United States. This translates into more than \$1 billion annually lost corn due to weeds.

# **Post-emergence Corn Herbicide Technologies**

For the past 35 years, the backbone of most corn weed control packages consisted of a soil applied pre-plant-incorporated or pre-emergence herbicide such as Lasso, Dual, Atrazine, or Eradicane. Environmental concerns and economics have slowly driven corn growers to shift to use of more post-emergence herbicides. While products such as 2,4-D, dicamba, atrazine, bladex, accent, beacon, permit, and exceed have been used post-emergence in corn, they rarely have formed the foundation of good weed management in corn.

All this has changed with the advent of new post-emergence herbicide technologies from several companies. Roundup-Ready corn, Liberty Link corn, IMI tolerant corn, and Poast Protected corn are just some of the new technologies available to corn growers for 1998. These new herbicide-resistant corn technologies offer growers

powerful tools to control troublesome weeds while maintaining environmental quality and minimizing the cost of weed control in corn.

For troublesome grass weeds such as wild-proso millet, sandbur, shattercane, foxtail and barnyard grass, Poast-protected corn offers excellent grass weed control. Because numerous weed species normally are present in corn fields, other herbicides must be added to Poast for complete weed control. IMI-tolerant corn provides broad spectrum control of many annual grass and broadleaf weeds, but will not provide control of the IMI herbicide resistant weeds. Lightning will provide soil residual activity on many important weeds, however, crop rotation restrictions may be a concern in some areas. Liberty Link corn will allow for broad spectrum control of many common Colorado weeds particularly if growing conditions are ideal. Under stress conditions, however, or situations of multiple weed flushes, either higher Liberty rates or tank mixes with residual herbicides will be required for commercial weed control.

#### **Issues In Corn Production**

Roundup Ready corn offers broad spectrum control of annual grass and broadleaf weeds as well as excellent activity on key perennial

# Weed Management

(continued from page 8)

weeds. Since Roundup is a systemic herbicide, it translocates in weeds to kill growing points both above and below ground. Two applications of Roundup in a growing season will normally provide control of most Colorado weeds.

Where heavy weed pressure is anticipated, it may be desirable to use a pre-emergence herbicide to reduce weed pressure or ensure a relatively clean field at the time of planting Roundup Ready corn. Since Roundup has no soil activity, it will only control emerged, growing weeds. Use of a residual herbicide will allow more time for corn crop competition to help control weeds or may provide help in controlling later germinating weeds.

## **Transgenic or New Corn Varieties**

Corn growers soon will have access to Roundup Ready Corn, Liberty Link Corn, IMI Corn, Sethoxydim Resistant Corn. Regardless of how these varieties were developed, they offer powerful new corn weed management options with herbicides which previously would have killed the corn. In the future, we will see corn hybrids with additional genetic traits stacked with herbicide resistant traits. Improved abilities to grow no-till corn will protect the environment and allow dryland corn producers to maximize moisture capture by the corn crop. Broad spectrum control of annual and perennial weeds will allow for fewer annual herbicide applications and reduce the need for excessive tillage. Fitting these transgenic corn hybrids into production fields with varying degrees of weediness will be a challenge for both researchers and growers in the future.

#### **Conclusion**

As corn producers move to a new millennium, advances in science and technology will increasingly impact the crop production systems we thought we knew so well. New methods of pest management, protection of the environment, and delivery of higher economic returns to the grower will help ensure that future generations will have the ability to feed the world's population with production from the fields of Colorado. Phil Westra

# Bt Corn

(Continued from page 3) resistance management plan is the use of refuges. A refuge is any non-Bt host of ECB such as non-Bt corn, and some other non-corn hosts such as potatoes, forage sorghum, millet, and some native weeds. The purpose of the refuge is to provide a source of ECB, not exposed to Bt corn or Bt insecticides that could mate with potential resistant moths emerging from nearby Bt corn. The actual amount and location of refuges is still in question. Discuss resistance management strategy prior to purchasing Bt seed corn.

The following management strategies are quoted from the publication, Bt Corn & European Corn Borer: Long-Term Success Through Resistance Management. North Central Regional ESA Publication 602: Plant non-Bt corn refuge(s) to protect 20-30% of the ECB larval populations from

exposure to the Bt Cry proteins. Plant non-Bt corn a similar time and in close proximity to Bt corn. In corn production areas, where corn is the primary refuge, at least 20 to 30% of the corn acreage should be non-Bt corn. Where spraying of non-Bt corn is anticipated, increase the refuge size to 40%.

Transgenic crops, such as Bt corn, are at the forefront of a revolution in pest management. The concept of managing insects by a simple seed choice is a powerful tool. As with any new technology, Bt corn brings mixed feelings: excitement of using new technology, desire to know more about it, apprehension about its wise use, and uncertainty about its value. In the next few years, much will be learned about how to use this powerful new tool wisely. Hopefully this new approach toward ECB management will not falter because of development of resistance by the ECB to Bt corn. By working together, producers, seed companies, scientists and regulators can better ensure the longevity of Bt corn. For additional information on this topic refer to Ostlie et al (1997). (Shanahan, Peairs and, Pilcher) John Shanahan

References: Ostlie, K.R., W.D. Hutchison, and R.L. Hellmich. 1997. Bt corn and European corn borer. NCR Publication 602. University of Minnesota Extension Service, St. Paul, MN.

Corn borer photographs taken from NCR Publication 602. University of Minnesota Extension Service, St. Paul, MN.

# web sites

http://www.foodbiotech.org/ news.cfmFood Biotechnology Communication Network homepage http://www.nbiap.vt.edu/ centers.html Biotechnology Centers Involved in Agriculture/Environment homepage

http://ificinfo.health.org/ index14.htm Food Biotechnology

http://www.vmsrf.com/links.htm Biotechnology Links

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