ABSTRACT

Title of Thesis: *Bt* GENETICS EFFECT ON CORN HYBRID PERFORMANCE: A COMPARISON OF TWO NEAR ISOLINE CORN HYBRIDS

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Most corn (*Zea mays* L.) hybrids planted in the U.S. are the result of genetic modification that gives them a *Bt* gene or genes obtained from the bacterium, *Bacillus thuringiensis* (Berliner) (*Bt*), that express insecticidal proteins and enables these hybrids to be resistant to several insects. European corn borer (ECB) (*Ostrinia nubilalis*, Hübner) is the main *Lepidopteran* pest targeted by the *Bt* corn technology. All *Bt* events used in current corn hybrids provide 100% control of ECB. This has led to widespread use of *Bt* hybrids and has resulted in a drastic decrease in the ECB population. This raises the question whether it is still economically feasible to plant *Bt* hybrids that have higher seed costs in environments where the ECB pest level is low. The objectives of this study were: 1) compare the yield and agronomic performance of a pair of corn near-isoline hybrids with and without the *Bt* traits; and 2) evaluate the agronomic and economic optimums for yield and nitrogen (N) rate for each near-isoline hybrids. A two-year study at three University of Maryland research farms in 2013-2014 examined each hybrid type for stalk damage due to ECB, yield

performance, the optimum N rate for maximizing yield, and the economic returns the two hybrids provided. This study found minimal ECB stalk damage and no consistent agronomic or economic yield difference between the *Bt* and non-*Bt* hybrids. Neither hybrid type was determined to have a consistent nitrogen use efficiency (NUE) advantage. The results of this study indicate that producers should not have concerns over hybrid type choice, now that there is significant regional suppression of ECB below economic levels.

Bt GENETICS EFFECT ON CORN HYBRID PERFORMANCE: A COMPARISON OF TWO NEAR ISOLINE CORN HYBRIDS

by

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Chapter 1: Literature Review

Benefits of *Bt* Corn

The European Corn Borer (ECB) has been a major *Lepidopteran* pest for corn production in the Mid-Atlantic region. In its larval stage, it feeds on corn tissue and often will bore into the corn stalk, which can result in lodging that often reduces yield (Bode and Calvin, 1990). From research conducted in Pennsylvania, Bode and Calvin (1990) found that a yield reduction of up to 6% occurred for every larva present in a corn plant. During the 80's and early 90's, corn producers who wanted to limit their yield losses caused by ECB, relied on labor-intensive field scouting to determine the level of ECB infection followed by costly and time-sensitive insecticide applications for control if scouting determined it necessary.

In 1996, an alternative approach for managing ECB as well as several other major *Lepidopteran* corn pests became available to producers. The new approach used corn hybrids that had been genetically modified to express insecticidal endotoxins (Cry proteins) derived from a common soil bacterium, *Bacillus thuringiensis* (*Bt)*. More specifically, scientists using DNA recombinant techniques were able to modify the genome of corn by inserting specific *Bt* genes, allowing the plant to express the production of *Bt* Cry proteins throughout its tissues (Gianessi and Carpenter, 1999; Witkowski et al., 2016). Corn producers quickly adopted the technology because it offered superior protection against insect pests without the need for insecticide applications.

Bt corn hybrids provide almost 100% protection against ECB damage because the Cry proteins are highly costly toxic to the pest (Kocourek and Stara, 2012; Burkness et al., 2002). Dillehay et al. (2004) conducted research in environments where ECB infestation regularly occurred. They found that *Bt* corn hybrids provided superior protection compared to respective non-*Bt* isoline hybrids. The non-*Bt* hybrids had more ECB stalk tunneling (1.68 tunnels plant^{-1}) compared to their respective *Bt* isolines (0.05 tunnels plant^{-1}).

Even though *Bt* corn hybrids are more expensive than non-*Bt* hybrids because of the technology fees, they have been widely adopted across the US by producers since first commercially available in 1996. The rapid adoption is attributed to the economic and time saving benefits producers achieved with planting *Bt* hybrids compared to the application of an insecticide (Pilcher et al, 2002). The corn acreage in the U.S. planted to *Bt* genetics has risen from 8% in 1997 to 81% in 2015 (USDA, 2017). A Maryland survey in 2013 determined that some counties had adoption rates exceeding 90% (G.P. Dively personal communication, University of Maryland Entomologist, 2017). Besides controlling ECB, hybrids with multiple stacked and pyramided *Bt* genes are now produced that control a wide spectrum of insect pests including fall armyworm (*Spodoptera frugiperda , J.E. Smith)*, corn earworm (*Helicoverpa zea*, Boddie), and corn rootworm (*Diabrotica virgifera virgifera*, LeConte) (Bohnenblust et al., 2014, Brooks and Barfoot, 2017; Burkness et al., 2010,). *Bt* genes are also commonly bundled together with other traits, such as herbicide resistance to glyphosate (Roundup®) and/or glufosinate (Liberty Link®) to create double or triple stacked *Bt* hybrids to control multiple insect pests and improve weed control from common herbicides (Burkness et al., 2010, Fernandez et al., 2014, Que et al., 2010;).

Bt Hybrids Compared to Non-*Bt* Hybrids

After two decades of consistent and widespread use of *Bt* hybrids, the result has been greatly reduced populations of ECB, the major *Lepidopteran* target. In addition, many other corn insect pests are also controlled (Bohnenblust et al., 2014). Many farmers routinely report that they see little to no ECB moths or larvae. With this decreased insect population, farmers are questioning the economic benefit of routinely planting costlier *Bt* hybrids.

When no infestation pressure is present, studies comparing the performance of *Bt* and non-*Bt* hybrids have produced mixed results regarding which hybrid type is better. From a study conducted in Wisconsin, Stanger and Lauer (2007) found that *Bt* hybrids yielded greater than non-*Bt* hybrids across several planting populations. But, Stanger and Lauer (2007) concluded with an economic assessment that those better yields were not great enough to result in higher net return due to the greater seed cost associated with the *Bt* hybrids.

In a Maryland study comparing corn hybrids with multiple, single, and no *Bt* traits, Chen et al. (2010) found that the *Bt* hybrids did not always produce better yield. Furthermore, Chen et al. (2010) found that when ECB is the only pest present, there was no significant protection benefit attained with hybrids containing multiple *Bt* traits compared to those that contained a single *Bt* trait.

Studies comparing morphological and physiological characteristics for *Bt* hybrids and their non-*Bt* near-isolines have found some differences in growth, development, and yield potential that may be due to the *Bt* genetics. In a study conducted by Saxena and Stotzky (2001), it was reported that the insertion of *Bt* genes alters the cellular wall structure by producing up to 97% more lignin. In a greenhouse study, Ma and Subedi (2005) found that *Bt* hybrids took two to three days longer to reach physiological maturity (PM) compared to their non-*Bt* near-isolines. Later maturity would more likely result in a later harvest date. The concentration of nitrogen (N) in plant organs has been found to differ significantly between *Bt* and non-*Bt* hybrids; an outcome that may indicate different nitrogen demand for each hybrid type. From work conducted in Ottawa, Canada, Subedi and Ma (2007) found that *Bt* hybrids had higher content of N in kernels and leaves at physiological maturity compared to their non-*Bt* near isolines. They attributed this outcome to greater dry matter accumulation in the kernels and leaves of the *Bt* hybrids. Yanni et al. (2011) conducted a study in Quebec, Canada and reported that *Bt* hybrids had a higher concentration of N in their stems and roots compared to their non-*Bt* near isoline hybrids.

Nitrogen Management for Corn

The differences between the hybrid types in morphological and physiological characteristics and in N concentration/accumulation logically leads to the question: Do the hybrid types also differ for nitrogen use efficiency (NUE) and grain yield. If differences between hybrid type do exist for NUE, then N management may need to be adjusted to achieve optimum yields. NUE can be defined in different ways, but specifically for field corn production NUE is best described as pounds of N needed to produce 1 bu of corn. A common method to determine how much nitrogen is required for corn production is first to estimate a realistic yield. This estimate is usually based off of the average yield from serval growing seasons, the estimate is then multiplied by a factor to determine lb N A^{-1} Current recommendations for corn fertilizer N rate differ from state to state in the U.S. Some states use a simple yield based factor, such as 1.0 lb N per bushel of expected yield. Other states, particularly in the Midwestern Corn Belt, estimate corn N rates with an economic based N-response model termed the Maximum Return to Nitrogen (MRTN). Many states also use a field N-assessment to recommend corn N rates, which includes a yield-based N factor with adjustments for field-specific factors such as manure history, legume history, soil residual nitrate-N, soil N mineralization, irrigation water nitrate-N, etc. (Meisinger et al., 2008; Morris et al., 2018). In addition to the above pre-season N recommendation approaches, most states also suggest some type of in-season or post-season soil or crop evaluation, such as: the pre-sidedress soil nitrate test (PSNT), leaf chlorophyll meter monitoring, the corn stalk nitrate test (CSNT), aerial imagery, or crop N sensors to further evaluate and monitor the N status of the crop (Meisinger et al., 2008; Morris et al., 2018).

University of Maryland Extension recommends the use of the yield goal method for determining corn nitrogen rate (1 lb N bu^{-1} yield goal up to 250 bu A^{-1}) for field corn (McGrath, 2010) along with adjustments for previous manure applications, previous legume crops (primarily soybeans or alfalfa). In Maryland, nitrogen management is essential to preventing buildup of nitrogen in the Chesapeake Bay caused by leaching and/or runoff losses of nitrogen from fields. Producing corn by using only the necessary amount of N to optimize yield is not only important environmentally but it is also economically important to farmers so that they achieve optimum returns for their N investments.

If differences exist in N concertation/accumulation for corn hybrid type, these differences may also continue into NUE and grain yield. As a result, current N recommendations may need to be evaluated to include adjustments based on hybrid type. Therefore, one of the objectives of this research is to evaluate the yield-based NUE of the Bt hybrid and its near-isoline.

Agronomic and Economic Yield Assessments for Corn

For corn production, it is important to understand the extra cost of *Bt* technology in association with the effects the technology has on morphological and physiological characteristics and if those characteristics influence NUE for attaining grain yield. The approach used to determine optimum NUE for corn production can differ depending upon whether you have an environmental, economic, or agronomic perspective. One of the most common methods for predicting NUE for corn grain yield is the use of the quadratic regression plus plateau model (Bullock and Bullock, 1994; Cerrato and Blackmer, 1990; Meisinger et al., 2008). The quadratic plus plateau model has been shown to accurately predict maximum yield. In addition, it more accurately predicts optimum N rates compared to other popular models (e.g. quadratic, linear-plus plateau, and exponential regression) used for calculating maximum yield and its associated N rate (Bullock and Bullock, 1994; Cerrato and Blackmer, 1990; Nafziger et al., 2004). The quadratic plus plateau model uses a split formula SAS v 9.4 (SAS Institute Inc. Cary, NC).

Eq 3.1

$$\mathrm{E}[Y|x] = \begin{cases} \alpha + \beta x + \gamma x^2 & \text{if } x < x_0 \\ c & \text{if } x \geq x_0 \end{cases}$$

Where: Y = Yield, and x= N rate. If x is < the join point (x_o) than Y is represented by a quadratic regression and if x is $\geq x_o$ than Y is represented by a plateau value (c). The model predicts yield over a range of N rates, and can be interpreted using a two-step approach. First, a quadratic regression model determines the point where N is no longer considered limiting. The second step determines the plateau value. This quadratic plus plateau model is most useful for identifying the Agronomic Optimum Maximum Yield (AOMY), the Agronomic Optimum Nitrogen Rate (AONR), the

Economic Optimum Maximum Yield (EOMY), and the Economic Optimum Nitrogen Rate (EONR) (Bock and Hergert 1991; Meisinger et al., 2008; Lindsey et al., 2015). The two agronomic optimums (AOMY and AONR) do not consider the cost of N inputs that the two economic optimums (EOMY and EONR) do. Thus, the economic optimums for yield and N rate tend to be lower than their respective agronomic optimums and are more practical for producers. However, the agronomic optimums are beneficial for providing information on the yield potential of corn hybrids. Generally, AOMY and EOMY are similar, while the difference between AONR and EONR can be great. A study in Ohio, (Lindsey et al., 2015) found that EOMY was 1.3% less than AOMY, while EONR was 16% less than AONR.

Agronomic and economic optimums can determine how much nitrogen is needed to obtain a maximum yield and also identify the NUE for attaining that yield. The agronomic and economic NUE calculations for grain yield are AONR ÷ AOMY and EONR ÷ EOMY, respectively. These calculations show the ratio of lb N bu^{-1} of yield needed to obtain optimum agronomic and economic maximum yields. These calculations can help producers to determine how much N they need to obtain their yield potential or yield goal (Bock and Hergert 1991; Doberman et al., 2011; McGrath, 2010).

References

Bock, B. R., and G. W. Hergert. 1991. Fertilizer Nitrogen Management. In: R. F. Follett, D. R. Keeney, and R. M. Cruse, editors. Managing Nitrogen for Groundwater Quality and Farm Profitability, SSSA, Madison, WI. p. 139-164. doi:10.2136/1991.managingnitrogen.c7

Bode, W. M., and D. D. Calvin. 1990. Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. Journal of Economic Entomology 83(4): 1595-1603.

Bohnenblust, E.W., J.A. Breining, J.A. Shaffer, S.J. Fleischer, G.W. Roth, and J.F. Tooker. 2014. Current European corn borer, Ostrinia nubilalis, injury levels in the northeastern United States and the value of Bt field corn. Pest Manage. Sci. 70:1711–1719. doi:10.1002/ps.371

Brookes, G., and P. Barfoot, 2017. Environmental impacts of genetically modified (GM) crop use 1996–2015: impacts on pesticide use and carbon emissions. GM Crops & Food, 8(2), 117-147.

Bullock, Donald G., and David S. Bullock. 1994. Quadratic and quadratic-plus-plateau models for predicting optimal nitrogen rate of corn: A comparison. Agronomy Journal 86, no. 1: 191-195.

Burkness, E.C., W. D. Hutchison, R. A. Weinzierl, J. L. Wedberg, S. J. Wold, and J. T. Shaw. 2002. Efficacy and risk efficiency of sweet corn hybrids expressing a Bacillus thuringiensis toxin for Lepidopteran pest management. Midwestern U.S. Crop Protection 21(2): 157-169.

Burkness, E.C., G. Dively, T. Patton, A.C. Morey, and W D. Hutchison. 2010. Novel Vip3A Bacillus thuringiensis (Bt) maize approaches high-dose efficacy against Helicoverpa zea (Lepidoptera: Noctuidae) under field conditions: Implications for resistance management. GM Crops 1(5): 337-343.

Chen, G., Hooks, C. R., Patton, T. W., Kratochvil, R., and Dively, G. 2016. Tolerance to stalk and ear-invading worms and yield performance of Bt and conventional corn hybrids. Agronomy J. 108(1): 73-84. doi:10.2134/agronj15.0139

Cerrato, M. E., and A. M. Blackmer. 1990. Comparison of Models for Describing; Corn Yield Response to Nitrogen Fertilizer. Agron. J. 82:138-143. doi:10.2134/agronj1990.00021962008200010030x.

Dillehay, B. L., G. W. Roth, D. D. Calvin, R. J. Kratochvil, G. A. Kuldau, and J. A. Hyde. 2004. Performance of Bt Corn Hybrids, their Near Isolines, and Leading Corn Hybrids in Pennsylvania and Maryland. Agron. J. 96:818-824. doi:10.2134/agronj2004.0818

Dobermann, A., C.S. Wortmann, R.B. Ferguson, G.W. Hergert, C.A. Shapiro, D.D. Tarkalson, and D.T. Walters. 2011. Nitrogen response and economics for irrigated corn in Nebraska. Agron. J. 103:67–75

Fernández, F.G., E.D. Nafziger, S.A. Ebelhar, and R.G. Hoeft. 2009. Managing nitrogen. Illinois Agronomy Handbook. Univ. Illinois Coop. Ext. Serv., Urbana-Champaign: 113-132.

Fernandez-Cornejo, J., S. Wechsler, M. Livingston, and L.W. Mitchell. 2014. Genetically engineered crops in the United States. Econ. Res. Rep. 162. USDA Econ. Res. Serv., Washington, DC.

Gianessi L.P., and J.E. Carpenter.1999. Agricultural Biotechnology: Insect Control Benefits. Washington, DC: Natl. Cent. Food Agric. Policy. 98 pp.

Jacobsen, J., G. Jackson, and C. Jones. 2003. Fertilizer guidelines for Montana crops. Publ. no. 161. Montana State Univ. Ext. Serv.

Kocourek, F., and J. Stara. 2012. Efficacy of Bt maize against European corn borer in central Europe. Plant Prot. Sci. 48:S25–S35.

Lindsey, A. J., P. R. Thomison, D. J. Barker, and R. W. Mullen. 2015. Drought-Tolerant Corn Hybrid Response to Nitrogen Application Rate in Ohio. Crop, Forage & Turfgrass Management 1:2015-0168. doi:10.2134/cftm2015.0168.

Ma, B., and K. Subedi. 2005. Development, yield, grain moisture and nitrogen uptake of Bt corn hybrids and their conventional near-isolines. Field Crops Research 93(2-3): 199–211.

McGrath, J.M. 2010. SFM-1. Agronomic crop nutrient recommendation goals based on soil tests and yield goals. Univ. of Maryland Ext., College Park.

Meisinger, J.J., J.S. Schepers, and W.R. Raun. 2008. Crop nitrogen requirement and fertilization. p. 563–612. In J.S. Schepers et al. (ed.) Nitrogen in agricultural systems. Agron. Monogr. 49. ASA, CSSA, and SSSA, Madison, WI.

Morris, T. F., T. S. Murrell, D. B. Beegle, J. J. Camberato, R. B. Ferguson, J. Grove et al 2018. Strengths and Limitations of Nitrogen Rate Recommendations for Corn and Opportunities for Improvement. Agron. J. 110:1-37. doi:10.2134/agronj2017.02.0112

Nafziger, E.D., J.E. Sawyer, and R.G. Hoeft. 2004. Formulating N recommendations for corn in the Corn Belt using recent data. p. 5–11. In Proc. 20th North Central Extension-Industry Conf., Des Moines, IA. 17–18 Nov. 2004. Vol. 20. Int. Potash and Phosphate Inst., Brookings, SD.

Pilcher, C.D., M.E. Rice, R.A. Higgins, K.L. Steffey, R.L. Hellmich, J. Witkowski, et al. 2002 Biotechnology and the European corn borer: measuring historical farmer perceptions and adoption of transgenic Bt corn as a pest management strategy. Journal of Economic Entomology 95(5): 878-892.

Que, Q., M.D.M. Chilton, C.M.D. Fontes, C. He, M. Nuccio, T. Zhu, et al. 2010. Trait stacking in transgenic crops: Challenges and opportunities. GM Crops 1(4): 220–229.

Saxena, D., and G. Stotzky. 2001. Bt Corn Has a Higher Lignin Content than Non-Bt Corn. American Journal of Botany 88(9): 1704.

Stanger, T. F., and J. G. Lauer. 2007. Corn Stalk Response to Plant Population and the Bt–European Corn Borer Trait. Agron. J. 99:657-664. doi:10.2134/agronj2006.0079.

Subedi, K. D., and B. L. Ma. 2007. Dry Matter and Nitrogen Partitioning Patterns in Bt and Non-Bt Near-Isoline Maize Hybrids. Crop Science 47, no. 3: 1186.

University of Georgia. 2008. Fertilizer recommendations by crops, categorized. Univ. of Georgia. In: Kissell, D.E. and L. Sonon, editors, Soil test handbook for Georgia. SB-62. Georgia Coop. Ext., College of Agric. & Environ. Sci., Univ. of Georgia. http://aesl.ces.uga.edu/publications/soil/STHandbook.pdf (accessed 16 Feb. 2018).

United States Department of Agriculture (USDA). 2017. Adoption of Genetically Engineered Crops in the U.S. https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/. (accessed 5 Jan. 2018).

Witkowski, J. F., J. L. Wedberg, K. L. Steffey, P. E. Sloderbeck, B. D. Siegfried, and M. E. Rice. 2016. Bt Corn and European Corn Borer: Pest Management: Corn Production: University of Minnesota Extension. http://www.extension.umn.edu/agriculture/corn/pest-management/bt-corn-and-european-corn-borer/. (accessed 26 Sept. 2016).

Yanni, S. F., J. K. Whalen, and B. Ma. 2011. Field-Grown Bt and non-Bt Corn: Yield, Chemical Composition, and Decomposability. Agron. J. 103:486-493. doi:10.2134/agronj2010.0367

Chapter 2: Performance of a *Bt* Corn Hybrid Compared to its non-*Bt* Near Isoline over Multiple N Rates

Objectives of Research

The objective of this study was to evaluate the agronomic and economic performance of two near-isoline corn hybrids (with and without *Bt* genetics) over a range of nitrogen rates in an environment where little to no ECB pressure exists. Results obtained from this research were used to 1) assess if differences in performance exist between the two hybrid types; 2) identify the agronomic and economic optimum nitrogen rates for both hybrid types; 3) calculate NUE for each; and 4) compare profitability for both.

Materials and Methods

Experimental Design

Over a period of two years (2013 and 2014), six field experiments were conducted at three University of Maryland Agricultural Experiment Station farms located in the Piedmont and Coastal Plain regions of Maryland. Table 3.1 describes the field locations and their respective soil classification information. Two corn hybrids with 111-day relative maturity, DeKalb brand DKC 61-88 containing *Bt* genetics (GENVT3P®) and its non-*Bt* near isoline, DeKalb brand DKC 61-86 RR2 (DeKalb, St. Louis MO), were evaluated across six nitrogen rates (0, 50, 100, 150, 200, and 275 lb N A^{-1}). The experiment had a factorial arrangement of treatments

(Factor A was Hybrid and Factor B was Nitrogen rate) in a randomized complete block design. Factor A contained two levels and depending on the measurement, Factor B had two, four, or six levels. Each factorial treatment was replicated either four or five times (Table 3.2). Each plot consisted of six corn-rows (designated rows one through six) spaced 30 in. apart. Plot length varied per location (Table 3.2). The two center rows (rows three and four) were used for grain harvest, rows two and five were used for assessing ECB damage (method described below), and the outside rows (rows one and six) served as borders. The border rows for each plot were planted to a non-study hybrid using only the outside rows of a six-row corn planter. The four inner rows of each plot were no-till planted to the appropriate hybrid as defined by the site randomization using a four-row John Deere 1750 planter (Moline, IL) equipped with coulters and trash-wheels and with modified seed distribution units for planting small research plots (Clewell Precision Machine, Inc., Milton, PA). The planter was set to deliver 31,000 seeds A^{-1}. All planting dates were within the normal planting window for Maryland (Table 3.2).

All sites received pre and post-emergence herbicide applications for weed control. Farm crews at the sites applied necessary non-nitrogen fertilizers to meet nutrient management recommendations. Source of nitrogen used as both the starter fertilizer and the sidedress treatments was UAN (30% N as urea-ammonium nitrate solution). Each plot received, approximately 25 lb A^{-1} of starter nitrogen applied with the planter at planting with the exception of the 0 lb A^{-1} treatment. The rest of the nitrogen to meet each treatment rate was supplied with a sidedress application during growth stages V5-V7 (Abendroth et al., 2011). The sidedress nitrogen treatments were applied using a custom-built six-row applicator that placed the nitrogen via sub-surface injection approximately 4 inches deep and mid-way between each set of two rows. A Spray Mate II Automatic Rate Controller (Micro-Trak Systems Inc. Eagle Lake, MN) mounted on the applicator differentiated each sidedress nitrogen treatment.

Measurements

Seedling emergence was measured by counting number of plants in the two center rows of each plot approximately two-weeks post planting to verify uniformity of stand and to ensure the stand was within an acceptable +/- 10% of the seeding rate. At black layer formation (growth stage R6), stand counts were again taken from either row three or four to determine harvest population. At the same time, number of lodged plants (plants either leaning at a 45^{o} or greater angle or plants that have broken stalk below the ear) in each plot were counted.

To assess ECB damage, ten consecutive and representative plants (five each from the non-harvest rows two and five) at four N rate treatments (0, 100, 150, and 275 lb N A^{-1}) were selected following black layer formation at all Site-years except one (Queenstown-2013). These plants were cut at the mid-point of the inter-node between the brace roots and first node. The ten stalks for each plot had their ears removed and leaves stripped before they were bundled together by plot and stored until the ECB damage assessment. When ECB damage was assessed, each stalk was split length-wise (base to tassel) by a Ryobi ban saw (Hiroshima-ken, Japan) and then assessed visually for number of tunnels stalk^{-1} and when tunnels were found the length of each was measured.

Yield measurements were collected when the center two rows (rows three and four) of each plot were harvested with a Massey Ferguson 8 XP plot combine (Kincaid Equipment, Colwich, KS) equipped with a HM800 Classic GrainGage system (Juniper Systems, Logan, UT) for measuring grain weight and grain moisture. Individual plot data was saved on an Allegro Field PC (Juniper Systems, Logan, UT). Weight for each plot was converted into yield (bu A $^{-1}$) at 15.5% moisture.

Statistical Approach

The statistical analysis software system, JMP PRO 12.2.0, (SAS Institute 2015) was used to analyze the dependent variables plant population (plants A^{-1}), lodging (%), and ECB damage (tunnels stalk^{-1}). For the analyses of these variables, Hybrid, N rate, and Site-year were considered fixed effects, while Replication at each Site-year was considered a random effect. Additionally, these three measurements were subjected to the Fit Y by X procedure in JMP PRO 12.2.0, (SAS Institute 2015) to analyze the strength of the correlation between the measurements and yield for each Hybrid. A mixed model analysis of variance (ANOVA) in JMP PRO 12.2.0, (SAS Institute 2015was used to compute generalized least squares estimates of the fixed effects parameters on the dependent variables of harvest grain moisture content, ECB damage (tunnels stalk^{-1}), and grain yield. The ANOVA for grain moisture content and grain yield did not include all six N rates. Instead, only the two N rates (150 and 200 lb N A^{-1}) which are closest to those that Maryland producers would employ were used. For these analyses, an F-test ≤ 0.05 for a fixed effect was considered significant. Mean comparisons differed depending on which fixed effects were significant. The differences between the two hybrids were determined by comparing the Least Square Means (LS Means) with a Student's t test, while, Tukey's Honestly Significant Difference (HSD) was used for multiple LS Means comparison when analyzing differences due to N rate, Hybrid X N rate, and N rate X Site-year.

All six N rates (0, 50, 100, 150, 200, and 275 lb N A^{-1}) were used to determine the AOMY, AONR, EOMY, and EONR, using the PROC NLIN procedure in SAS 9.4 (SAS Institute, 2013) to perform the quadratic plus plateau analysis. The agronomic and economic optimums for yield and nitrogen rate were calculated for each Hybrid at each Site-year. The AOMY for each Hybrid X Site year was the yield represented by the intersection of the quadratic and plateau components. The associated AONR was the nitrogen rate at which the intersection occurred. Economic optimum calculations used a grain selling price of \$3.75 bu^{-1} and a N price of \$0.50 lb N^{-1}. In addition, the seed cost A^{-1} for each hybrid was the MSRP $unit^{-1}$ less a 15% discount which a typical producer would receive from a seed dealer (B. Dillehay, personal communication, 2017, Regional Agronomist for Monsanto®) and was set at \$108.50 A^{-1} (*Bt*) and \$98.50 A^{-1} (non-*Bt*) when planting 31,000 seeds A^{-1}. The input costs of each hybrid and N rate was subtracted from the gross returns for yield for each Hybrid X N rate combination to obtain the net return. Net return was analyzed using the quadratic plus plateau model in SAS® v9.4 (SAS Institute Inc. Cary, NC) to determine the maximum return and EONR. Using the plateau regression output for the AOMY and AONR, the y-intersection, linear coefficient, and quadratic coefficient parameters were used to obtain EOMY. These parameters were used to calculate the EOMY by inserting the EONR in the equation, EOMY = y-intersect + linear coefficient x EONR + quadratic coefficient x $EONR^2$. For this study, agronomic NUE (ANUE) for grain yield was the ratio between AOMY and AONR (AONR÷ AOMY). The economic NUE (ENUE) was the ratio between EOMY and EONR (EONR÷EOMY).

Results and Discussion

Weather

Rainfall totals for each site and growing season are presented in Table 3.3 and the LS mean yield over two N rates (150 and 200 lb N A^{-1}) for each Site-year are used to show the effect that rain and temperature may have had on yield. Each of the site-years were within +/-15% of their corresponding 30-year average for rainfall (1981-2010) (NOAA, 2018). The rainfall amounts during the study indicate that there was neither extreme drought nor excessive rainfall totals during the growing seasons. However, there were some months/multiple months when the amount of rainfall received may have affected corn performance. For example, Beltsville 2013 had a three-month period (July-September, when it received 5.42 in. or only 48% of average for that period (Table 3.3). This reduced rainfall was simultaneous with the grain fill period and may be a major reason why yield was only 133 bu A^{-1} at the site. Furthermore, without the 7.77 in. rainfall during June that provided good soil moisture at least through the pollination period, yield at Beltsville 2013 may have been even lower. Grain yield at Queenstown 2013 averaged only 134 bu A^{-1}, an amount that is approximately 25% less than the average expected yield for this site. Rainfall during the last two growing season months at this location was only 45% of average (Table 3.3); an amount that likely caused a yield reducing effect during the R3-R5 stages of grain fill. Upper Marlboro 2013 also received below average rainfall (67% of average) during the last two months of the growing season (Table 3.3). However, average yield (~153 bu A^{-1}) at Upper Marlboro 2013 was considered normal for that site. This yield outcome is likely due to the over 200% of normal rainfall that occurred during June and July providing adequate soil moisture for the corn during pollination and the first half of the grain fill period. Contrary to the yield limiting rainfall amounts just described, Beltsville 2014 was the recipient of timely and above-average rainfall during much of the 2014 growing season (Table 3.3). This is likely the cause for the nearly 206 bu A^{-1} yield, an amount approximately 40% greater than the average yield for the site.

Temperatures at all six Site-years were mostly average or below compared to the 30-year average (1981-2010) (NOAA, 2018) (Table 3.4). The most notable temperature discrepancies occurred at Queenstown 2013 and 2014 where monthly averages both years were consistently below the average. Furthermore, , the July and August temperatures at Queenstown 2014 were 8.7^{o}F and 6^{o}F below average, respectively. There was also above average rainfall during these same two months at Queenstown 2014 (Table 3.3). Grain yield at this site averaged nearly 148 bu A^{-1}, an amount approximately 15% less than the average yield. It is likely that maximization of growing degree units (GDU) did not occur during this period because of the cooler temperatures resulting in a lower yield response. The most outstanding difference in days $\geq$ 90^{o}F was at Beltsville 2013 and 2014.Although monthly temperatures were similar, the number of days $\geq$ 90^{o}F was twice as many for Beltsville 2013 compared to 2014. Beltsville 2013 accumulated most of its days $\geq$ 90^{o}F from July-September, with almost half coming during July (14). These days $\geq$ 90^{o}F may have stressed the plant at the end of pollination and during the grain fill period during 2013 causing a large discrepancy in yield compared to Beltsville 2014 which had only half as many days $\geq$ 90^{o}F (Table 3.4). Queenstown 2013 and 2014 had the lowest number of days $\geq$ 90^{o} F compared to the other site years. While the number of 90^{o}F days were low both years at Queenstown, Queenstown in 2013 had three times as many days $\geq$ 90^{o}F (6) compared to Queenstown-2014, with 5 of the days $\geq$ 90^{o}F in 2013 occurring during one week in mid-July. The timing of the 2013 days $\geq$ 90^{o}F may have stressed the early grain fill period for corn at Queenstown 2013. The days $\geq$90^{o}F at Upper Marlboro was relatively similar from 2013-2014 with both years accumulating almost half of their total days $\geq$ 90^{o}F in July.

Harvest Population

To assure that harvest population did not unduly influence yield responses, correlation analysis was conducted between harvest population and yield for each hybrid at each Site-year (Table 3.5). One Site-year (Queenstown-2013) had a significant negative correlation between yield and plant population for the non-*Bt* hybrid. Although significant, the correlation was weak at -0.44 (Table 3.5). The average plant population for this Site-year (Table 3.5) was higher than the planting rate of 31,000 seeds A^{-1} for both hybrids and was likely due to an incorrect setting for seed delivery. Population at this site was approximately 1000 plants A^{-1} higher for the *Bt* hybrid compared to the non-*Bt* which may have been an influence on the better yield attained by the *Bt* hybrid. All the remaining site-years had no significant correlations with plant population and yield and were within at least 90% of the planting rate (Table 3.5).

Lodging

There was very low incidence of lodging during the study. The combined analysis of variance (Table 3.6) indicated that hybrid treatment was not significant, while Site-year and N rate each had a significant effect on lodging but there was also a significant Site-year X N rate interaction. A Tukey's (HSD) multiple means comparison determined that the Site-year X N interaction was the result of only two N rates (0 and 275 lb N A^{-1}) at Queenstown 2014. These two N rates had significantly more lodging (4.85% and 6.31% lodging, respectively) than any other

Site-year X N rate combination; all other Site-year X N rate combinations had lodging amounts that were not significantly different (data not shown). Increased lodging in these two N rate treatments at Queenstown-2014 is most likely due to deer damage that was identified as a factor when lodging assessments were conducted.

ECB Damage

Although the overall damage caused by ECB was low, the combined ANOVA (Table 3.7) indicated that Hybrid significantly influenced the number of tunnels plant^{-1} but this outcome was confounded by N rate X Hybrid and Site-year X Hybrid interactions (Table 3.7). The N rate X Hybrid interaction (Fig. 3.1) shows that non-*Bt* hybrid in combination with the 275 lb N A^{-1} treatment had the most tunnels plant^{-1}. One Site-year (Upper Marlboro 2013) had significant differences in tunnels stalk^{-1} due to Hybrid, with the non-*Bt* plots experiencing 0.73 tunnels plant^{-1} compared to 0 tunnels plant^{-1} for the *Bt* hybrid (Fig. 3.2). When tunnels were present, the non-*Bt* hybrid had mean tunnel length of 2.94 inches compared to 0.25 inches for the *Bt* hybrid. However, presence of tunnels occurred in only 19 plots (16 non-*Bt* and 3 *Bt*) of the total 216 plots sampled. And, 8 of the 19 plots with ECB damage came from Upper Marlboro-2013. The low ECB infestation in this study could be attributed to a combination of factors including the high efficacy of *Bt* hybrid for ECB control (Kocourek F., Stará J. 2012), areawide suppression of ECB populations in the northeast (Dively et al. 2018, Bohnenblust et al., 2013), and the proximity of *Bt* hybrids to the non-*Bt* treatments in and around this study.

Harvest Moisture

Combined analysis of variance (Table 3.8) indicated that grain harvest moisture was affected by Hybrid, N rate, and Site-year. There were no interactions among those three fixed effects. The *Bt* hybrid had lower harvest grain moisture (19.79%) than the non-*Bt* hybrid (20.62%). These results contradict other studies (Ma and Subedi, 2005; Dillehay et al., 2004) who reported *Bt* hybrids had higher harvest grain moisture content compared to their non-*Bt* near isolines. Both studies attributed their results to higher ECB infestations present in the non-*Bt* hybrids resulting in more rapid grain dry down after physiological maturity. Although there was some significantly higher amount of ECB damage for the non-Bt hybrid in this study, the low amount of damage likely had no influence on the overall moisture content and performance of either hybrid. Thus, our results did not provide any evidence as to why the non-*Bt* hybrid had approximately 0.8% higher moisture at harvest than its *Bt* counterpart. The 200 lb N A^{-1} treatment had higher harvest moisture (20.95%) than the 150 lb N A^{-1} treatment (19.81%). A similar study in Ottawa, Canada found grain moisture did not differ between a 0 and 107 lb N A^{1} rate two out of three years (Ma and Subedi, 2005). Although the two hybrids tested have the same genetic background, it should be noted that they are not 100% isogenic, so differences in moisture levels could be attributed to slight differences in their growth and maturation patterns.

There were differences among the Site-years for grain harvest moisture (Table 3.8; Figure 3.3). The range for the grain harvest moisture content was between 18%

and 22% and typical for grain moisture content for the dates of harvest for this study (Table 3.2). This outcome is not surprising primarily because harvest dates, rainfall events near harvest time, soil type, temperature, and other environmental factors will cause harvest moisture differences among sites.

Grain Yield

The combined Site-years ANOVA for grain yield is presented in Table 3.9. To attain a more realistic comparison of performance for the two hybrids, this analysis only included data for the 150 and 200 lb N A^{-1}, the two N rate treatments that most closely represented N rates used by Maryland farmers. Significant N rate and Site-year responses were observed along with a significant Hybrid X Site-year interaction (Table 3.9). There was only one of the six Site-years, Queenstown 2013, where the two hybrids differed for yield (Fig 3.4). At that location, the *Bt* hybrid produced nearly 23% more than the non-*Bt* hybrid. Unfortunately, this Site-year did not have ECB damage assessed so it is not possible to ascertain whether ECB damage was the reason for the difference. One possible explanation for the yield difference is that the Queenstown location is a hotspot for ECB infestation and that significant ECB damage did occur in the non-*Bt* hybrid. To support this premise, Chen et al., (2016) compared *Bt* and non-*Bt* hybrids at the same Queenstown farm and found infestation rates in non-*Bt* hybrids to be 66.3, 71.8, and 97.9% in 2010, 2011, and 2012, respectively. They partly attributed these high rates of infestation to the planting of non-*Bt* corn around their study. However, for our study most of the corn planted near and around the study site were *Bt* hybrids. Other studies conducted in environments with ECB infestations that had yield differences between near-isoline hybrids (with and without Bt) found yield to decline 2-6% for each ECB larvae $plant^{-1}$ (Dillehay et al.,2004; Bode and Calvin, 1990). In order to explain the yield differences for our study at Queenstown 2013 in terms of ECB infestation, the ECB infestation for the non-Bt hybrid should have been at least 3 larvae $plant^{-1}$. Even though there were no assessments of ECB damage at Queenstown 2013, the presence of three larvae $plant^{-1}$ would have been noticed when lodging assessments were made. There were population differences at Queenstown-2013 with the *Bt* hybrid having a higher population (~1,000 plants A^{-1}) compared to the non-*Bt* (Table 3.9). This may have contributed to the yield advantage for the *Bt* hybrid. A third possible explanation for the better yield performance for the *Bt* hybrid was the late season reduced rainfall (August and September) that may have caused some crop stress that the *Bt* hybrid was better able to tolerate.

The remaining five site-years with no differences in yield between the two hybrids were similar to results found by Subedi and Ma (2007), who found that *Bt* hybrids yielded similarly or up to 12% less than non-*Bt* hybrids when ECB infestation levels were considered low to moderate.

Yield differences among the Site-years was expected, considering the different environmental factors and plant density stands. However, the Site-year that produced the best yield (Beltsville 2014) is surprising. This Site-year produced 210 bu A^{-1} and was over 30% greater yield than the next best location (Upper Marlboro 2013). Beltsville is a location where corn yield is typically 140 bu A^{-1}. The excellent yield during 2014 is attributed to adequate and timely rainfall during the growing season accompanied by excellent growing season temperatures. And finally, for the N Rate response, the 200 lb N A^{-1} rate produced 7.2 bu A^{-1} more (157.6 bu A^{-1}) than the 150 lb N A^{-1} rate (150.4 bu A^{-1}).

Agronomic Optimum Assessments – Yield, Nitrogen Rate, and Nitrogen Use Efficiency

The quadratic plus plateau analysis was used to identify the AOMY and AONR. The AOMY is defined as the yield corresponding to the intersection between the curve representing the quadratic response and the horizontal line representing the yield plateau. The AONR is defined as the nitrogen rate at the junction point where the quadratic curve and the plateau meet. Thus, using the quadratic-plateau model to summarize the N response provides a method to estimate the AOMY and the AONR for each hybrid at each Site-year. Figure 3.5 shows the results of the quadratic-plateau model for the non-*Bt* hybrid at Beltsville-2013. The remaining AOMY and AONR estimates for each hybrid are listed in Table 2.10 for the other five Site-years along with the ANOVA for yield across all six N rates for both hybrid types at each site-year. The estimates for all the parameters and their confidence limits for each corresponding hybrid and site-year are presented in Table 2.11. The hybrids comparisons for AOMY and AONR at each site-year have overlapping confidence limits and are most likely not significantly and comparisons for this section represents possible trends that may occur. A comparison between the two hybrids for AOMY at each site-year found that the *Bt* hybrid had better yield at the three 2013 Site-years. During 2014, the non-*Bt* hybrid produced better at two Site-years and the two hybrids had the same AOMY at one Site-year (Table 3.10). During 2013, the three Site-years had a common late season weather pattern; rainfall was less than average during the latter stages of grain fill (August and September) (Table 3.3). At Beltsville in particular, there were almost twice as many days $\geq 90^{o}$ in 2013 than in 2014. The 2013 outcome indicates that the *Bt* hybrid may have been able to withstand a weather related stress better than the non-*Bt* hybrid.

At each of the Site-years, with the exception of Upper Marlboro 2014, the hybrid with the greater AOMY also had a greater AONR to attain that yield (Table 3.10). This is possibly misleading because all it indicates is that the better yielding hybrid required more nitrogen to attain its optimum yield. The strong emphasis upon nutrient management in the region has producers interested in attaining the best yield with the best hybrid with the fewest pounds of nitrogen, i.e. nitrogen use efficiency (NUE). For this study, one definition of agronomic NUE (ANUE) is the ratio between AONR and AOMY. At four of the six Site-years, the hybrid that had the better yield had the larger ANUE value (i.e. less efficient), an indication that the better yielding hybrid was also the one that was the least efficient (Table 3.10). The one site-year where the hybrid with the better yield (*Bt* hybrid) also had lower ANUE was Queenstown 2013. And, at the remaining Site-year, Upper Marlboro 2014, the two hybrids had comparable maximum yield but the *Bt* hybrid accomplished the same yield with lower ANUE (Table 3.10). In summary, the *Bt* hybrid had the lower ANUE (was more efficient) at four Site-years but it produced better yield at only two of those Site-years and the non-*Bt* hybrid had lower ANUE at two Site-years, and at neither of those Site-years did it produce the better yield. These agronomic assessments do not provide any indication that one hybrid type was consistently better than the other hybrid.

Economic Assessment of Optimum Yield and Nitrogen Rate

In all situations, the EOMY for a hybrid will not be the same as AOMY, because the Law of Diminishing Returns requires that the EONR will be less than the AONR. The EONR is the point where the marginal returns from buying N fertilizer and seed equals the marginal benefits in grain yield, i.e. the last cent spent to by fertilizer N and seed is paid for by a grain yield increase of one cent. But in addition to fertilizer N costs, one must also account for other cost differences for the *Bt* and non-*Bt* hybrids. In this study, the main input costs between the two hybrids were for hybrid seed cost and the cost of nitrogen fertilizer to attain the EOMY for each hybrid, which differed at each site-year (Table 2.10). As described earlier (See Agronomic Optimum Assessment) the quadratic regression plus plateau analysis was used to find the EOMY and its corresponding EONR. Described earlier (see Materials and Methods), the economic assessment used the quadratic parameter estimates from the agronomic assessment to convert the maximum return for each hybrid into yield, as such EOMY and EONR are most likely not significantly different between hybrids because of overlapping confidence limits in table 2.11. As with the agronomic assessment, the economic assessment exhibited similar trends for hybrid performance. In 2013 all sites favored the *Bt* hybrid for EOMY over the non-*Bt* with Queenstown-2013 having the largest difference with the *Bt* hybrid yielding 26.4 bu A^{-1} more than the non-*Bt* hybrid (Table 3.10). In 2014, two sites had EOMY that favored the non-*Bt* hybrid (Beltsville and Queenstown), while at Upper Marlboro the EOMYs were nearly identical. As discussed earlier the *Bt* hybrid may have been better suited to handle less favorable growing conditions in 2013. Growing conditions were more favorable during 2014 and the non-*Bt* hybrid had better yield at 2-3 Site years. As with the AONR the hybrid with the greater EOMY also had a higher EONR at all site-years except Upper Marlboro-2014. For this study, better EOMY did not translate into a better ENUE. At four site-years the hybrid with the higher EOMY also had the higher ENUE (less efficient) than its near-isoline. For the remaining two site-years, the *Bt*hybrid had better ENUE. At Queenstown-2013, the *Bt* hybrid had a better (lower) ENUE and higher EOMY than the non-*Bt* while at Upper Marlboro-2014, where both hybrids had similar EOMY, the *Bt* hybrid had better (lower) ENUE (Table 3.10). For these economic assessments, neither hybrid proved to be consistently better.

The comparisons between the agronomic optimums and the economic optimums show that similar yields were attained with, in some cases, drastically different N rates. In this study, the EOMYs were never more than 2.7% lower than the AOMY, while the difference between AONR and EONR was up to 21.4% less N needed for attainment of the EONR. These results are similar to Lindsey et al., (2015) who found the AOMY was 1.3% more than the EOMY, while EONR was 16% less than AONR.

No clear indication about which hybrid had better NUE can be determined with the information obtained in this study, What is clear from this research is that the application of an economic assessment is going to identify a different NUE value compared to the use of only an agronomic assessment. In our study, the ANUE was 1.35 lb N bu^{-1} compared to an ENUE of 1.18 lb N bu^{-1}. Both these amounts raise questions about the reliability of Maryland's current yield goal approach for determining amount of nitrogen to supply corn. That recommendation calls for 1 lb N bu^{-1} of expected yield. Let's now assume that the EOMY for each hybrid and Site-year in Table 3.10 serves as the yield goal for a field. To determine the N rate for each of those examples, the EOMY is multiplied by 1 (1 lb N bu^{-1} of yield goal) which makes the N rate to achieve the yield goal the same as the EOMY. Let's now employ a comparison between the N rate (EOMY) and the EONR for each hybrid at each Site-year (Table 3.10). For 7-10 comparisons, the EONR is greater than the yield goal N rate (associated EOMY). This is an indication that the current yield goal method that uses 1 lb N bu^{-1} yield goal for corn in Maryland should be re-evaluated.

Summary and Conclusions

The overall performance of the *Bt* corn hybrid compared to its non-*Bt* near isoline produced no evidence that either hybrid type was agronomically superior. Differences in ECB damage between the two hybrids occurred but were attributed primarily to one site-year, while all other sites had very low ECB pressure. Harvest grain moisture was slightly lower for the *Bt* hybrid, a result that was contrary to other similar studies that have reported consistently lower grain moisture in the non-*Bt* hybrids tested. It is generally agreed that the stalk tunneling caused by ECB tends to accelerate the dry down phase of corn maturity. Previous studies were conducted under higher ECB pressure than the levels experienced in this study., As mentioned previously, both hybrids tested had the same genetic background, but were truly 100% isogenic, so differences in moisture levels could be attributed to slight differences in their growth and maturation patterns. Assessments of both hybrids based strictly on grain yield found yield was comparable at five of six site-years.. However, yield comparisons did not fully assess the performance of the two hybrids as per the objectives of this study. Additional assessments were done to evaluate agronomic and economic optimums for yield and nitrogen use. The agronomic optimum assessment determined that the *Bt* hybrid produced better during the 2013 season when rainfall was 33-50% below average during the latter stages of grain fill. This suggests that the *Bt* hybrid was better able to handle the weather induced stress. The economic assessments indicated that the *Bt* hybrids had a higher EOMY than the non-*Bt* hybrids at all three sites in 2013, while in 2014 the non-*Bt* hybrid was the superior hybrid in two of the three site-years with one site-year having similar EOMY. At four of the six site-years, the *Bt* hybrid had better ANUE and ENUE but at two of those site years, it produced less yield than the non-Bt hybrid. Overall, the *Bt* hybrid in this study did not consistently perform differently than its non-*Bt* near isoline. Even when considering the higher cost for seed for the *Bt* hybrid, there was no consistent difference in EOMY between the two hybrids.

The results of this study may leave producers with questions about whether they should be planting the more expensive *Bt* hybrids in the mid-Atlantic region where ECB populations are now significantly lower. A recent study (Dively et al. 2018) reported that average nightly moth captures of ECB in the mid-Atlantic region declined from 6.8 during 1976–1995 to 1.9 during 1996–2016, a net decline of 72% that was significantly related to Bt corn adoption. Similarly, mean sweet corn ear damage by ECB significantly declined from 50% during 1984–1995 to 15% since Bt corn introduction. If ECB is the primary target of the Bt technology, then the economic threshold concept of integrated pest management would dictate that planting Bt hybrids is not economically justifiable. Furthermore, although producers may consider it an economically reasonable preventative approach, use of Bt hybrids can exert unnecessary selection pressure on ECB populations which could ultimately lead to pest resistance. However, Bt corn, particularly hybrids with the more recent pyramided traits, control other insect pests of economic importance, such as corn root worm, western bean cutworm, fall armyworm and corn earworm. There are other regions in the US where Bt corn use is economically justified based on the expected yield losses caused by these pests. Also, all Bt corn types express herbicide tolerance traits that may not be available in the non-Bt counterparts. Finally, much of what is stated above is a moot point because the corn seed industry simply does not produce enough high-yielding, non-Bt hybrids to allow producers change their Bt corn practices.

Tables and Figures

Equations 2.1 Formula used to calculate a quadratic plateau regression model. Where Y = Yield, x= N rate. If x is < the join point (x_o) than Y is represented by a quadratic regression and if x is $\geq x_o$ than Y is represented by a horizontal plateau value (c).

$$E[Y|x] = \begin{cases} \alpha + \beta x + \gamma x^2 & \text{if } x < x_0 \\ c & \text{if } x \geq x_0 \end{cases}$$

Tables 2.1 The global addresses and pertinent soil classification information for the Maryland sites where two corn near-isoline hybrids were compared during 2013 and 2014.

Site	Year	Latitude	Longitude	Soil taxonomy	Soil series	Soil Type
Beltsville	2013	39° 1' 37” N	76° 50' 14" W	Aquic Hapludults	Russett-Christiana	Sandy loam
	2014	39° 1' 36" N	76° 50' 10" W	Aquic Hapludults	Russett-Christiana	Sandy loam
Upper Marlboro	2013	38° 51' 37" N	76° 46' 47" W	Aquic Hapludults	Annapolis	Sandy loam
	2014	38° 51' 29" N	76° 46' 20" W	Aquic Hapludults	Donlonton	Sandy loam
Queenstown	2013	38° 54' 43" N	76° 8' 47" W	Typic Hapludults	Nassawango	Silt loam
	2014	38° 54' 40" N	76° 8' 39" W	Typic Hapludults	Nassawango	Silt loam

Tables 2.2 Plot sizes, number of replications and the respective dates for planting, sidedress nitrogen applications and harvest at the Maryland sites (identified by site-year) where the two corn near-isoline hybrids were compared.

Site-year	Planting Date	Sidedress Treatments	Harvest Date	Replications	Plot Size (ft^2)
Beltsville 2013	22 May	20 June	25 Sept.	5	750
Beltsville 2014	12 May	16 June	1 Oct	5	900
Upper Marlboro 2013	14 May	14 June	25 Sept.	4	825
Upper Marlboro 2014	13 May	11 June	30 Sept.	5	750
Queenstown 2013	6 May	6 June	23 Sept.	4	600
Queenstown 2014	9 May	9 June	29 Sept.	4	900

Tables 2.3 Rainfall amounts for the growing season months and the 30-year averages (1981-2010) from the National Data Climate Center for each Site-year.

	Beltsville			Upper Marlboro			Queenstown		
	2013	2014	30 year average	2013	2014	30 year average	2013	2014	30 year average
Month	---Rainfall (in)---								
April	2.76	6.04	3.35	2.77	4.63	3.55	4.66	5.19	3.86
May	4.15	4.94	4.32	2.4	5.7	4.32	1.92	3.65	4.19
June	7.77	4.59	3.70	9.05	3.29	4.07	9.8	2.76	3.86
July	1.72	2.89	3.94	7.74	4.31	4.02	5.04	5.58	4.37
August	2.27	3.86	3.27	2.46	4.44	3.72	2.42	6.75	4.27
September	1.43	2.18	4.08	2.73	2.57	3.99	1.32	2.62	4.01
Total	20.10	24.50	22.66	27.15	24.94	23.67	25.16	26.55	24.56

Tables 2.4 Average growing season monthly temperatures and comparable 30-year average monthly temperatures (1981-2010).

Month	Beltsville 2013 (number of days ≥ 90^0)	Beltsville 2014 (number of days ≥ 90^0)	Beltsville 30 year Average	Upper Marlboro 2013 (number of days ≥ 90^0)	Upper Marlboro 2014 (number of days ≥ 90^0)	Upper Marlboro 30 year Average	Queenstown 2013 (number of days ≥ 90^0)	Queenstown 2014 (number of days ≥ 90^0)	Queenstown 30 year Average
	----------Degrees Fahrenheit----------								
April	55.4 (1)	52.5 (0)	53.7	55.0 (1)	53.4 (0)	54.3	54.9 (0)	53.1 (0)	56.3
May	62.6 (2)	64.0 (0)	63.4	63.0 (1)	64.9 (0)	63.5	63.9 (0)	64.8 (0)	65.4
June	73.4 (4)	72.3 (3)	72.9	73.9 (2)	73.0 (2)	72.7	73.4 (0)	72.0 (2)	74.5
July	77.0 (14)	74.7 (8)	77.3	78.4 (8)	75.9 (7)	77.0	78.1 (6)	70.9 (2)	78.6
August	71.6 (6)	71.5 (1)	75.8	73.6 (3)	72.5 (1)	75.0	72.7 (0)	70.9 (0)	76.9
September	66.2 (5)	67.8 (4)	68.4	69.4 (4)	69.1 (3)	67.8	66.0 (0)	67.5 (2)	70.2
Average	67.7 (32)	67.1 (16)	68.6	68.9 (19)	68.2 (13)	68.4	68.2 (6)	66.5 (2)	70.3

Tables 2.5 Correlation analysis of the covariates plant population and yield and average plant population for both hybrids at each site-year.

Site-year	Hybrid	Correlation coefficient	Prob.	Average
Beltsville-2013	Bt	0.29	0.11	29243
	Non-Bt	-0.04	0.83	29245
Beltsville-2014	Bt	-0.07	0.70	29564
	Non-Bt	-0.16	0.38	28516
Upper Marlboro-2013	Bt	0.03	0.88	28149
	Non-Bt	<0.01	0.98	27969
Upper Marlboro-2014	Bt	<0.01	0.98	30598
	Non-Bt	<0.01	0.99	30030
Queenstown-2013	Bt	-0.23	0.26	32742
	Non-Bt	-0.44	0.03	31773
Queenstown-2014	Bt	0.27	0.20	29850
	Non-Bt	0.12	0.58	29601

Tables 2.6 ANOVA for testing the lodging response to the fixed effects of the study.

	Percent of Lodging			
Source	Numerator df	Denominator df	F Ratio	Prob > F
N rate	5	229.5	3.16	0.0089
Hybrid	1	229.5	1.12	0.2901
N rate*Hybrid	5	229.5	0.22	0.9517
Site-year	5	20.43	9.62	<.0001
N rate*Site-year	25	229.5	2.83	<.0001
Hybrid *Site-year	5	229.5	0.28	0.9264
N rate*Hybrid *Site-year	25	229.5	0.69	0.8668

Tables 2.7 ANOVA for testing the European corn borer (ECB) response (measured as number of ECB tunnels plant^{-1} to the fixed effects in the study.

	ECB Tunnels Plant^{-1}			
Fixed Effects	Numerator df	Denominator df	F Ratio	Prob > F
N rate	3	126	1.70	0.1704
Hybrid	1	126	10.07	0.0019
N rate*Hybrid	3	126	2.90	0.0376
Site-year	4	18	2.03	0.1329
N rate*Site-year	12	126	1.60	0.0984
Hybrid *Site-year	4	126	3.58	0.0083
N rate*Hybrid *Site-year	12	126	1.29	0.2297

Figure 2.1 European corn borer (ECB) damage (measured as number of tunnels plant^{-1}) for two near isoline corn hybrids (*Bt* and non-*Bt*) at four N rates at five Maryland locations during 2013 and 2014. Bars (means and standard error) with the same lowercase letter are not significantly different at *P* < 0.05.

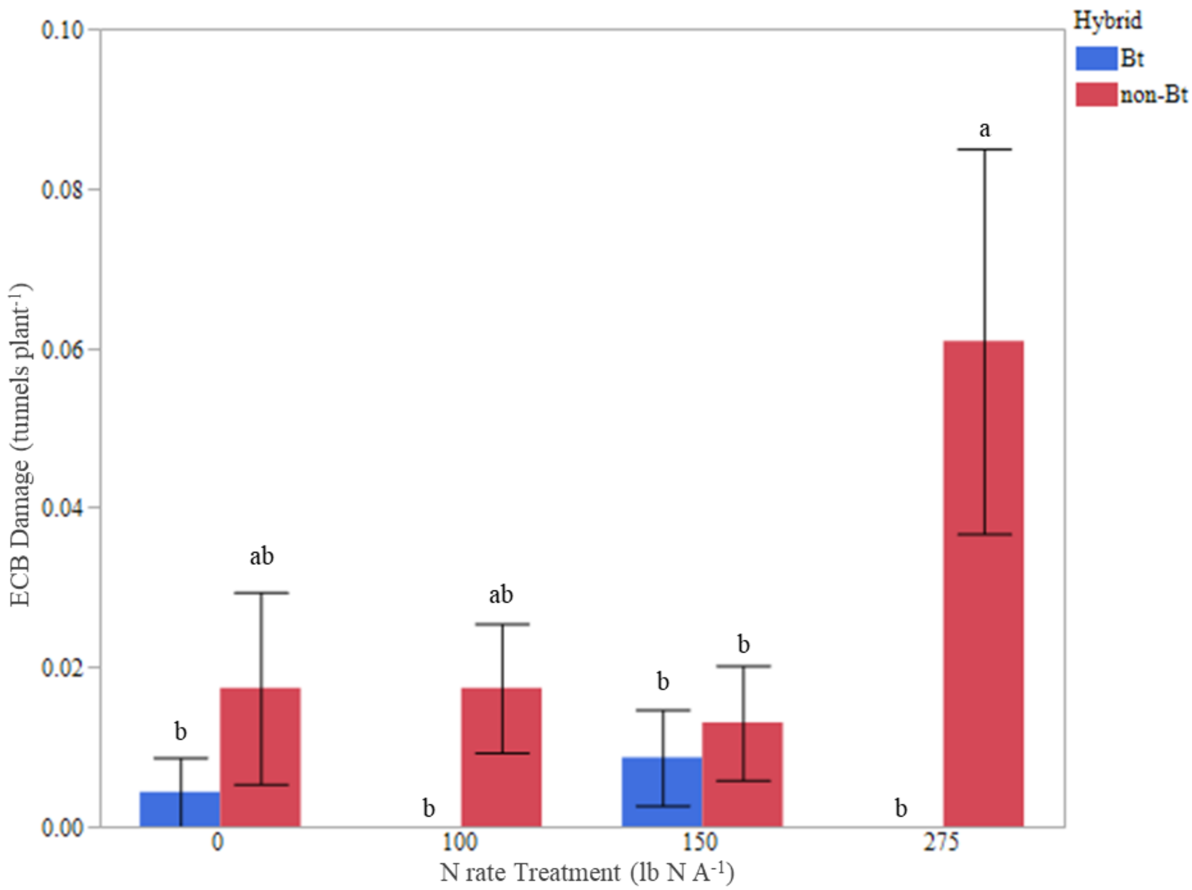
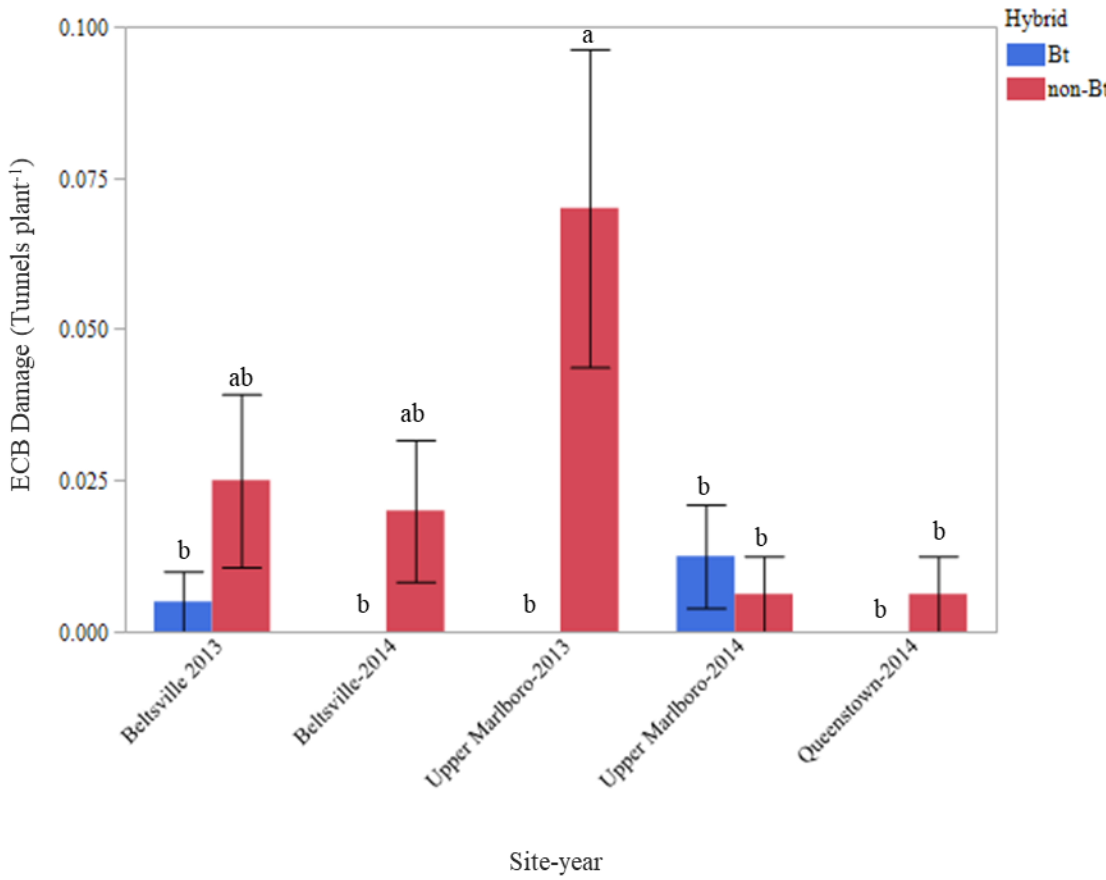


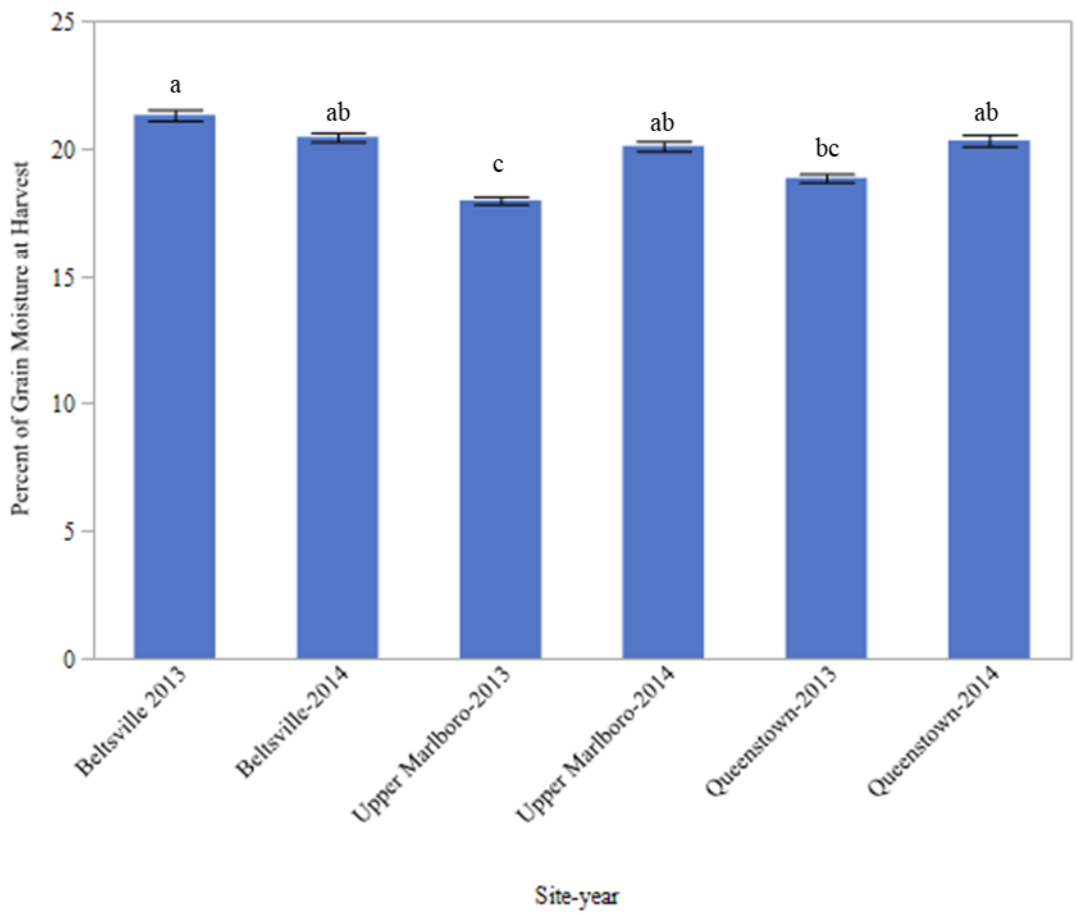
Figure 2.2 European corn borer (ECB) damage (measured as number tunnels plant^{-1}) for two near-isoline corn hybrids (*Bt* and non-*Bt*) at five Maryland locations during 2013 and 2014. Bars (means and standard error) with the same lowercase letter are not significantly different at $P < 0.05$.



Tables 2.8 ANOVA for testing grain harvest moisture content response to the fixed effects of the study. For the fixed effect of N rate, only the 150 and 200 lb N A^{-1} rates were included in the model.

	Percent Grain Moisture at Harvest			
Fixed effects	Numerators df	Denominators df	F Ratio	Prob > F
N rate	1	63	25.00	<.0001
Hybrid type	1	63	28.08	<.0001
N rate*Hybrid type	1	63	0.33	0.5632
Site-year	5	21	6.89	0.0006
N rate*Site-year	5	63	1.37	0.2463
Hybrid type*Site-year	5	63	1.95	0.0975
N rate*Hybrid type*Site-year	5	63	0.48	0.7893

Figure 2.3 Harvest grain moisture content at the three locations (Beltsville, Upper Marlboro, and Queenstown) during 2013 and 2014. Bars (means and standard error) with the same letter are not significantly different at $P < 0.05$.



Tables 2.9 ANOVA for testing the grain yield response to the fixed effects in the study. For the fixed effect of N rate, only the 150 and 200 lb N A^{-1} rates were included in the model.

Grain Yield				
Fixed Effects	Numerator df	Denominator df	F Ratio	Prob > F
N rate	1	63	8.92	0.0040
Hybrid	1	63	2.93	0.0914
N rate*Hybrid	1	63	<0.01	0.9219
Site-year	5	21	46.00	<.0001
N rate*Site-year	5	63	0.91	0.4761
Hybrid *Siteyear	5	63	4.16	0.0025
N rate*Hybrid *Site-year	5	63	0.58	0.7125

Figure 2.4 Grain yield for two near-isoline corn hybrids (*Bt* and non-*Bt*) at the three locations for the study during 2013 and 2014. Bars (means and standard error) with the same letter are not significantly different at $P < 0.05$.

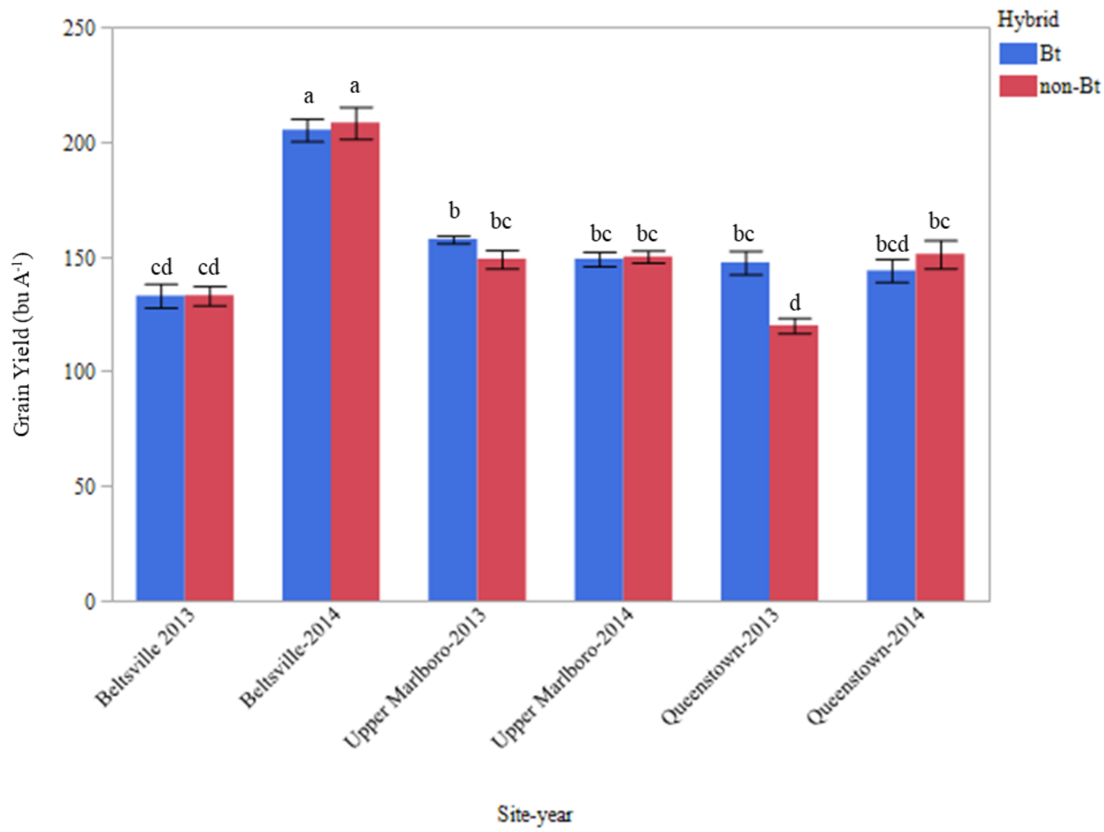
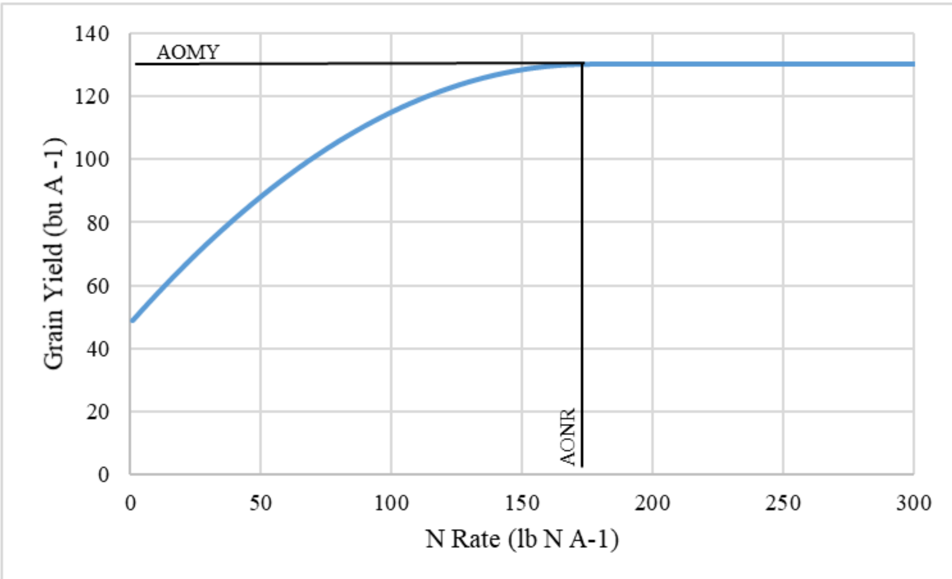


Figure 2.5 Quadratic plus plateau response for the non-*Bt* at Beltsville-2013. The two lines represent the agronomic optimum maximum yield (AOMY) and agronomic optimum N rate (ANOR).



Tables 2.10 ANOVA for yield across six N rates, it's corresponding *P* value Agronomic optimum maximum yield (AOMY), the agronomic optimum N rate (AONR), agronomic N use efficiency (ANUE), economic optimum maximum yield (EOMY), economic optimum N rate (EONR), and economic N use efficiency (ENUE) for each site-year and hybrid type.

Site-year	Yield (bu A^{-1})	*P* value	Hybrid	AOMY	AONR	ANUE	EOMY	EONR	ENUE
				bu A^{-1}	lb N A^{-1}	lb N bu^{-1}	bu A^{-1}	lb N A^{-1}	lb N bu^{-1}
Beltsville-2013	109.1	0.3959	Bt	136.9	223	1.62	134.8	188	1.39
	107.0		Non-Bt	130.3	175	1.34	129.1	154	1.19
Beltsville-2014	170.3	0.1285	Bt	210.5	194	0.92	207.1	163	0.79
	178.1		Non-Bt	236.7	310	1.31	235.7	281	1.19
Upper Marlboro-2013	124.1	0.1158	Bt	168.9	271	1.60	167.8	242	1.44
	118.8		Non-Bt	160.9	251	1.56	159.0	220	1.38
Upper Marlboro-2014	125.2	0.1206	Bt	147.3	131	0.89	143.2	103	0.72
	121.2		Non-Bt	147.0	148	1.01	143.0	120	0.84
Queenstown-2013	115.9	<0.001	Bt	150.3	202	1.34	148.4	175	1.18
	96.3		Non-Bt	125.7	199	1.58	122.0	160	1.31
Queenstown-2014	108.7	0.5467	Bt	155.9	219	1.40	153.5	192	1.25
	111.3		Non-Bt	169.5	269	1.59	168.4	247	1.47

Tables 2.11 Quadratic parameter estimates, approximate standard error, and confidence limits used in the quadratic regression plus plateau for two near-isoline corn hybrids (*Bt* and non-*Bt*) at six site-years for their agronomic and economic assessments. The corresponding plateau values (AOMY) and join points (AONR) are located in table 2.10.

Site-year	Hybrid	Parameter	Estimate	Approximate Standard Error	Approximate 95% Confidence Limits	
Beltsville-2013	Bt	alpha	54.7	5.9	42.6	66.9
		beta	0.74	0.12	0.48	0.99
		gamma	-0.0017	0.00053	-0.00274	-0.00056
	Non-Bt	alpha	31.2	5.4	19.9	42.5
		beta	0.95	0.13	0.69	1.21
		gamma	-0.0024	0.00058	-0.00358	-0.00119
Beltsville-2014	Bt	alpha	79.2	8.4	61.9	96.6
		beta	1.35	0.20	0.94	1.77
		gamma	-0.00349	0.00094	-0.00543	-0.00155
	Non-Bt	alpha	99.8	9.8	79.8	119.8
		beta	0.88	0.17	0.54	1.22
		gamma	-0.00142	0.00058	-0.00261	-0.00024
Upper Marlboro-2013	Bt	alpha	53.50	6.28	40.62	66.39
		beta	0.85	0.11	0.63	1.08
		gamma	-0.00157	0.00039	-0.00236	-0.00078
	Non-Bt	alpha	46.6	5.2	35.8	57.3
		beta	0.910	0.098	0.709	1.111
		gamma	-0.00181	0.00037	-0.00258	-0.00104
Upper Marlboro-2014	Bt	alpha	54.8	4.6	45.3	64.5
		beta	1.41	0.17	1.07	1.76
		gamma	-0.0054	0.0011	-0.0079	-0.0030
	Non-Bt	alpha	46.7	3.9	38.7	54.7
		beta	1.35	0.12	1.10	1.61
		gamma	-0.00457	0.00074	-0.00611	-0.00303
Queenstown-2013	Bt	alpha	40.7	6.1	27.9	53.4
		beta	1.09	0.14	0.79	1.38
		gamma	-0.00270	0.00064	-0.00403	-0.00137
	Non-Bt	alpha	31.2	5.4	19.9	42.5
		beta	0.95	0.13	0.69	1.21

		gamma	-0.00239	0.00057	-0.00358	-0.00119
Queenstown-2014	Bt	alpha	14.5	6.2	1.5	27.5
		beta	1.29	0.13	1.01	1.57
		gamma	-0.00294	0.00058	-0.00414	-0.00174
	Non-Bt	alpha	18.7	7.4	3.3	34.0
		beta	1.12	0.13	0.86	1.39
		gamma	-0.00209	0.00046	-0.00305	-0.00113

References

Abendroth, L.J. 2011. Corn growth and development. Iowa State University, University Extension, Ames, IA.

Bode, W. M., and D. D. Calvin. 1990. Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. Journal of Economic Entomology 83(4): 1595-1603.

Bohnenblust, E.W., J.A. Breining, J.A. Shaffer, S.J. Fleischer, G.W. Roth, and J.F. Tooker. 2014. Current European corn borer, Ostrinia nubilalis, injury levels in the northeastern United States and the value of Bt field corn. Pest Manage. Sci. 70:1711–1719. doi:10.1002/ps.371

Chen, G., Hooks, C. R., Patton, T. W., Kratochvil, R., & Dively, G. 2016. Tolerance to stalk and ear-invading worms and yield performance of Bt and conventional corn hybrids. Agronomy J. 108(1): 73-84. doi:10.2134/agronj15.0139

Dillehay, B. L., G. W. Roth, D. D. Calvin, R. J. Kratochvil, G. A. Kuldau, and J. A. Hyde. 2004. Performance of Bt Corn Hybrids, their Near Isolines, and Leading Corn Hybrids in Pennsylvania and Maryland. Agron. J. 96:818-824. doi:10.2134/agronj2004.0818

Dively, G. P., Venugopal, P. D., Bean, D., Whalen, J., Holmstrom, K., Kuhar, et al.,. 2018. Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. Proceedings of the National Academy of Sciences, 115(13), 3320-3325.

Kocourek, F., and J. Stara. 2012. Efficacy of Bt maize against European corn borer in central Europe. Plant Prot. Sci. 48:S25–S35.

Lindsey, A. J., P. R. Thomison, D. J. Barker, and R. W. Mullen. 2015. Drought-Tolerant Corn Hybrid Response to Nitrogen Application Rate in Ohio. Crop, Forage & Turfgrass Management 1:2015-0168. doi:10.2134/cftm2015.0168.

Ma, B., and K. Subedi. 2005. Development, yield, grain moisture and nitrogen uptake of Bt corn hybrids and their conventional near-isolines. Field Crops Research 93(2-3): 199–211.

NOAA (National Oceanic and Atmospheric Administration). 2010. Climate Data Online Search. https://www.ncdc.noaa.gov/data-access/land-based-station-data/land-based-datasets/climate-normals/1981-2010-normals-data. (accessed 1 Feb. 2018).

SAS Institute. 2013. The SAS system for Windows. Release 9.4. SAS Inst., Cary, NC.

SAS Institute. 2015. The JMP PRO system for Windows. Release 12.2.0. SAS Inst., Cary, NC.

Subedi, K. D., and B. L. Ma. 2007. Dry Matter and Nitrogen Partitioning Patterns in Bt and Non-Bt Near-Isoline Maize Hybrids. Crop Science 47, no. 3: 1186.