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Supplement to: Bt Corn & European Corn Borer: Long-Term Success Through Resistance Management, NCR-602

Regional Research Committee, NC 205
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Executive Summary

1. NC-205 is a regional research committee supported by Land Grant Universities, USDA-CSREES and ARS. It is comprised of scientists from 20 states, Mexico and Canada who have conducted research on stalk-boring pests since 1954.
2. The Committee re-examined many of the assumptions upon which our previous scientific assessments were based. This update http://ent.agri.umn.edu/ecb/nc205doc.htm summarizes our scientific understanding and recommendations for resistance management of Bt corn. Our initial recommendations were published in North Central Regional Publication 602 during 1997. An electronic version of NCR-602 is located at http://www.extension.umn.edu/Documents/D/C/DC7055.html.
3. The Committee reaffirmed, as a premise, the importance of prolonging the durability of Bt corn technology. Bt corn provides more effective and consistent control of European corn borer than insecticides, with less cost and fewer logistical, health, or environmental concerns. Bt corn has insurance value by reducing risk of yield loss from European corn borer.
4. We believe that resistance management using the high-dose/refuge strategy is possible. Recent data based on samples from three localities support a key assumption that major resistance genes are rare. Survival of resistant heterozygotes is assumed to be low. Additional data are needed to confirm both assumptions.
5. Providing susceptible mates for resistant survivors in the Bt crop is a crucial component of resistance management. A refuge of 20-30% of the larval population of European corn borer should be protected from exposure to Bt toxin on each farm. Recent data on non-random mating and regional genetic structure of European corn borer, coupled with new theoretical models, suggest that a 20% refuge is the minimum needed for resistance management. A 30% refuge provides a hedge for uncertainty in biological and operational assumptions.
6. Economic analyses suggest corn growers can benefit from planting refuges. Under plausible biological, genetic and economic conditions, and a 10-20 year planning horizon, economic models indicate that farmers capture most, if not all, of the benefits of Bt technology by planting 20-30% refuge.
7. A refuge of 20-30% of the larval population of European corn borer can be achieved by planting 20-30% of the corn on a farm to unsprayed non-Bt corn. This area should increase to 40% if the refuge is sprayed with insecticides. The non-Bt corn refuge should be planted within each 320-acre area that has Bt corn, at a similar time and with similar maturity characteristics as the nearby Bt corn.
8. Possible biological threats to successful resistance management are declines in toxin concentration early in the growing season, interactions between minor and major resistance genes, non-random mating or inbreeding of resistant individuals, and the effects of Bt corn on natural enemies of pests and other non-target organisms.
9. Until additional data are obtained, we suggest that these recommendations for European corn borer be applied in areas where other stalk-boring pests of corn occur.
10. Growers are key partners in managing insect resistance to Bt corn. Dissemination of consistent information to growers is essential.

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Introduction

NC-205 is a North Central Regional Research Committee supported by the Land Grant Universities and USDA-CSREES and ARS. This Committee has a long history (beginning in 1954) of addressing research issues on the "Ecology and Management of European Corn Borer and Other Stalk-Boring Lepidoptera." Participants in the program include representatives from 20 states, USDA-ARS, Mexico, and Canada. Collaborative efforts among members of the Committee have produced several hundred publications, including practical pest management guidelines for corn stalk-boring insects (Mason et al. 1996).

For the past three years, the NC-205 Committee has sponsored meetings with EPA and industry to discuss Bt corn, resistance management and associated issues. Recognizing the need of corn growers for more information, a publication entitled, ***Bt Corn & European Corn Borer: Long-Term Success Through Resistance Management***, (North Central Regional Publication 602) was produced in 1997 with more than 35,000 copies distributed. An electronic version is located at http://www.extension.umn.edu/Documents/D/C/DC7055.html. The NC-205 Committee met September 24-25, 1998 reviewed NCR-602, shared current research results, and developed this statement. These findings will be periodically reviewed and updated as new data become available.

Bt corn provides more effective and consistent control of European corn borer than insecticides, with less cost than an insecticide application and fewer logistical, health, or environmental concerns. Bt corn has shown to farmers what years of educational efforts could not; local evidence that European corn borer significantly reduces yield. Furthermore, this technology has insurance value by reducing risk of European corn borer infestations, thereby improving yield stability. However, Bt corn involves season-long expression of a control measure that can be expected to produce intense selection for resistance to Bt toxin in the corn borer population. Left unmanaged, this evolutionary pressure could limit the value of Bt corn technology as a pest-management tool, just as it has frequently limited the value of chemical pesticides applied by traditional means.

NC-205 members recognize the benefit of prolonging the commercial usefulness of Bt toxins, including their traditional use as an organic pesticide, and the growers' need for information on the stewardship of Bt corn. As farmers gain experience with Bt corn, we believe they will have strong motivation to preserve the benefits of Bt technology. Grower surveys indicate a willingness to embrace resistance management recommendations that are logistically feasible. Incentive-based programs and consistent educational messages from academic, extension, industry, and regulatory sources should enhance the acceptance of sound management practices, including the adoption of non-Bt corn refuges.

In the NCR-602 document, our basic goal was to communicate information about Bt corn and resistance management. We recognized the need to provide susceptible mates for resistant survivors in the Bt crop as a crucial component of resistance management. We recommended protecting 20 to 30% of each local European corn borer larval population from exposure to Bt toxins. Since the publication of NCR-602, additional research results have refined our understanding of the factors that affect management of resistance to Bt corn.

The information used to develop our initial recommendations in NCR-602 included the best available biological data and theoretical models assessing the interaction between European corn borer and Bt corn. We have re-examined many of the assumptions on which our previous scientific assessment was based. In the following paragraphs, we summarize current information relative to the high-dose/refuge resistance management strategy.

High-Dose/Refuge Strategy

The high-dose/refuge strategy, which the Environmental Protection Agency (EPA) (1997, 1998) and industry (Fishhoff 1996) also have advocated, involves exposing one portion of the pest population to Bt plants with an extremely high concentration of toxin, while maintaining another part of the population in a refuge where the pests do not encounter any Bt toxin. By maintaining the refuges in close proximity to the Bt corn, susceptible pests that survive in the refuge are expected to intermingle and mate with any toxin-resistant pests that survive on the Bt corn plants. The offspring from these matings are assumed to be unable to survive on Bt corn. Population genetic theory (e.g., Tabashnik and Croft 1982; Gould 1986; Mallet and Porter 1992; Alstad and Andow 1995; Onstad and Gould 1998b; Caprio 1998) and experiments (Tabashnik 1994) predict that this approach will substantially delay resistance, if it is appropriately implemented and its assumptions are met.

High-Dose/Refuge Strategy Has Three Essential Assumptions.

1. Major resistance genes must be sufficiently rare so that nearly all such genes will be in heterozygous individuals. (A heterozygous individual has only one copy of the resistance gene and is referred to as a RS heterozygote). A gene frequency of less than one in 1,000 for major resistance genes is needed for the high-dose/refuge strategy to be successful.
2. Resistance genes must be nearly recessive. In other words, the RS heterozygotes should have very low survival on the Bt crop. RS survival rates that are less than 5% of the expected survival of homozygous RR resistant individuals on Bt corn are needed for the high-dose/refuge strategy to be successful. (For an operational definition of 'high-dose' refer to EPA 1998 at http://www.epa.gov/pesticides/SAP/finalfeb.pdf).
3. Non-Bt refuges are needed to provide a source of susceptible pests to mate with the resistant ones so that their offspring will be RS heterozygotes. This requires random mating within the typical dispersal distances of the adults.

While these are the three critical assumptions, most of the theoretical models also have assumed that the pest population exhibits local random mating and no regional genetic isolation. Recent data and models that have been used to evaluate these assumptions are described below. In summary, the high-dose/refuge strategy can substantially delay resistance if (1) the frequency of major resistance genes is low, (2) RS heterozygote survival is low, and (3) there is random mating of adults within typical dispersal distances.

Insect Resistance Management: Current Issues

- **Frequency of Resistance Genes Suggests that Resistance Management is Possible.** Successful resistance management requires that resistance genes be rare in the insect population. When we made our first recommendation, the initial frequency of resistance to Bt toxins in the European corn borer population was unknown. Based on estimates from other insects, we assumed that the initial frequency would be low and initial results of empirical studies support this assumption. We now know that the initial frequency of resistance genes is probably less than 10^{-3} in parts of Minnesota, Iowa, and Illinois (Andow et al. 1998; unpublished; Andow and Hutchison 1998; Hutchison et al., unpublished; Pierce et al. 1998;). Statistical techniques (Andow and Alstad 1998) applied to samples from Iowa and Minnesota give an expected frequency of major resistance alleles of 8.93×10^{-4}, and a 95% confidence interval of $[0, 4.38 \times 10^{-3}]$. Collectively, this information suggests that resistance management is still possible if effective refuges are employed. Estimates of resistance gene frequency may be needed from other corn-producing areas.

- **Will RS Heterozygote Survival Be Low Enough to Enable Resistance Management?** Survival of RS heterozygotes is still unknown because major resistance genes have not yet been found and characterized. This lack of knowledge requires us to make a critical assumption: that RS heterozygote survival is low enough to enable resistance management (i.e., is almost fully recessive). Two lines of indirect evidence suggest that RS survival is low despite this absence of direct evidence. Work on resistance to Bt toxin in other organisms has shown RS survival to be low, ranging from ~0 to 0.025 (Tabashnik et al. 1992; Gould et al. 1997; McGaughey 1985; McGaughey and Beeman 1988). In addition, because several searches for resistance in the field have yet to confirm resistant individuals in European corn borer populations (Pierce et al. 1998; Hutchison et al., unpublished), dominant major resistance genes may be rare. However, these searches have included only a minuscule part of the approximately 80 million acres of corn grown annually in the U.S. Additional research is needed to evaluate the assumption of low RS survival.

- **Significant Numbers of European Corn Borers Move Only Short Distances.** For the high-dose refuge strategy to be effective, refuge insects must mate with resistant insects surviving in the Bt corn. For this to occur, European corn borer moths must emerge from the refuge at the same time as resistant moths and be close enough to mate with resistant moths. Data from MN and NE indicate that significant numbers of European corn borers move only short distances under some conditions. Refuge corn adjacent to Bt corn sustains less borer damage up to 100 meters from the Bt corn, suggesting limited dispersal during the second flight (Alstad and Andow, unpublished). In a recent mark-release-recapture experiment conducted in Nebraska, almost all recaptures of unmated females were made within ca. 500 meters of the release point (Hunt et al., unpublished). Collectively, these data suggest limited adult European corn borer movement. To improve the probability of desired matings, NC-205 recommends that refuges should occur on each farm where Bt corn is planted and within each 320-acre area**.** In other corn production regions, where the landscape patterns differ, movement patterns of adult European corn borers also may be quite different.

- **European Corn Borer Populations Exhibit Local Non-Random Mating.** Non-random mating in local populations can lead to more rapid evolution of resistance, because RR homozygotes are more likely to mate with each other, fewer RS heterozygotes will be produced, and fewer resistance genes will be killed by Bt corn. Local non-random mating is measured by the ***Fis*** statistic (Wright 1965). When ***Fis*** = 0, local mating is random, and ***Fis*** > 0 implies that local populations contain fewer RS heterozygotes than expected under random mating. Electrophoretic analysis of three genetic markers revealed 15 of 45 European corn borer samples collected from 40 North American localities by NC-205 scientists to have ***Fis*** > 0.27 (NC-205, unpublished). With an initial R gene frequency of 10^{-4}, SS survival of 0, RS survival of 0.005, 20% refuge, 100% random mating, and no inbreeding, resistance is projected to evolve in 206 generations (Caprio 1998; Hutchison and Andow, in press). Holding the other parameters constant and either decreasing the population mating at random to 65% or increasing the inbreeding to 7%, and resistance evolves in <31 generations. A 30% refuge under these same conditions would extend the life of the technology to >45 generations. The observed values of ***Fis*** and ***Fst*** (below) suggest that the evolution of resistance will be faster than these simulations. Other studies have concluded that non-random mating can occur in local populations of European corn borer (Ni 1995, Mason unpublished).

- **European Corn Borer Populations Exhibit Regional Genetic Isolation.** Regional genetic isolation can lead to more rapid evolution of resistance (Peck et al. 1998; Caprio 1998). Resistance will develop faster in a subdivided population because resistance genes can become common in a sub-population by chance (random drift). Resistance genes from these isolated populations then could spread to other populations. Subdivision of a population is measured by ***Fst***. When ***Fst*** is 0, there is no subdivision, and when it is 0.2 there is substantial subdivision in the population. Model results suggest that when ***Fst*** > 0.05, resistance evolution occurs much more rapidly than when ***Fst*** < 0.02 (Caprio 1998). Recent empirical studies of the genetic structure (via electrophoretic analysis of enzymes) of European corn borer in North America found substantial regional genetic isolation. Forty-five samples provided by NC-205 cooperators from 40 North American localities show ***Fst*** values above 0.175 at three concordant genetic markers. These values demonstrate very high levels of genetic isolation (less than 1 migrant exchange per generation) between locations separated on average by 300 kilometers (NC-205, unpublished). This regional isolation could accelerate the rate of resistance evolution. Such a finding supports larger, rather than smaller, refuge proportions.

Economic Assessment

Economic analysis of refuge size can provide additional insight into resistance management considerations. The refuge size depends on the biological and genetic information discussed previously, along with the planning horizon. Under these conditions (initial r allele frequency = 0.0001, RS survival rate = 0.025, random mating, and no inbreeding) and a 10-20 year planning horizon, economic models suggest that farmers capture most, if not all, of the benefits of Bt technology by planting 20-30% refuge (Hurley et al., submitted). This model is sensitive to underlying biological and genetic uncertainties at low levels of refuge. Onstad and Guse (unpublished) found that with an initial r allele frequency of 0.0001-0.001, RS survival rates of 0.0-0.025, and a 15-20 year time horizon, a 20% refuge level was usually superior economically. In extreme cases of pest density and crop value one could project an effective refuge ranging from 10 to 30%.

Risk analysis shows that the cost to farmers of planting too much refuge is less than the cost of planting too little refuge. For example, under the conditions stated previously for the Hurley et al. model, and a 15-year planning horizon, increasing refuge from 10% to 20% is expected to decrease the value of the Bt technology by less than 1%, while reducing the probability of resistance developing from 47% to less than 1%. However, reducing refuge from 10% to 5% is expected to increase the value of the technology by less than 1%, while increasing the probability of resistance developing from 47% to 79% (Hurley et al., unpublished). Therefore, economics and uncertainties about important model parameters suggest larger rather than smaller refuges.

Refuge Recommendations

The scientific evidence suggests that sufficient refuges, properly placed in space and time, have high potential to delay European corn borer resistance to Bt corn. After considering the implications of this research, the Committee unanimously reaffirmed its previous recommendation that refuges should prevent Bt protein exposure to 20-30% of the European corn borer larval population.

Implementation Issues

- **Non-Bt Corn Refuge Size.** A refuge of 20-30% of the larval population of European corn borer can be achieved by planting 20-30% of the corn on a farm to unsprayed non-Bt corn. This non-Bt corn refuge should increase to 40% if the refuge will be sprayed with insecticides. The non-Bt corn should be planted within each 320-acre area that has Bt corn. The non-Bt corn refuge should be planted at a similar time and should exhibit similar maturity characteristics as the nearby Bt corn.

- **Bt Corn Protection Extends Into Adjacent Non-Bt Corn Refuges.** Damage from European corn borer was reduced in non-Bt corn adjacent to Bt corn. Under high borer pressure, up to 50% reduction in damage occurred in refuge corn within 5-10 meters of Bt corn. Damage increased gradually with distance from the Bt corn. Some reduction of damage continued out to 80 meters from the Bt corn, but was undetectable beyond 80 meters (Andow & Alstad, unpublished). Theoretical simulation models also predict this phenomenon (Alstad and Andow 1995; Onstad and Guse, unpublished). Refuge corn planted in narrow strips within a field of Bt-corn experiences less damage than blocks of refuge (Andow and Alstad, unpublished). Simulations show that refuge strips 6-12 rows wide are effective at delaying resistance and can provide similar economic return as a separate block refuge established adjacent to the Bt corn field (Onstad and Guse, unpublished). Consequently, by positioning the refuges near Bt corn, producers could extend the protection benefits of Bt corn into the refuge.

- **Non-Field Corn Refuges.**
  Sacrificial Refuges. High-density popcorn can produce substantial numbers of European corn borer, which could considerably reduce the percentage of land required to produce refuge insects (Hellmich, unpublished). The logistics and feasibility of this strategy have not been investigated.

  Weeds, natural vegetation, and alternative crops. Many plants serve as aggregation areas and hosts for European corn borer and may provide refuges to conserve susceptibility in certain geographic areas (Hellmich et al. 1998). However, it is unknown whether these habitats will produce enough unselected individuals at the right time and whether their proximity to Bt corn allows for random mating. Until the contributions of these alternative hosts as refuges are known, refuge recommendations are being based solely on non-Bt corn (Hellmich, unpublished; Whalen et al., unpublished; Dively, unpublished; Losey et al., unpublished).

- **Impacts on Natural Enemies and Other Non-Target Organisms.** Recommendations regarding the size and distribution of non-Bt corn refuges have been made primarily to preserve susceptibility of the pest insects to Bt-toxins. Less attention has been paid to the potential effects of Bt corn on natural enemies in agricultural ecosystems (Orr and Landis 1997, Pilcher et al. 1997) and of the effects on other non-target organisms.

  Because of the extensive acreage that may be planted to Bt corn in the near future, this technology has potential to have widespread and lasting impacts on beneficial insects. One concern involves the effect of substantial local or regional declines in the natural enemy prey base that could result from widespread adoption of Bt corn. Additionally, direct Bt toxicity to natural enemies has recently been suggested (Hilbeck et al. 1998a, 1998b). These effects could ripple through other crops and habitats in unpredictable ways. While it is unclear if 20-30% refuge is sufficient to mitigate negative impacts on natural enemies in the long term, in the short-term, refuges of at least this size are prudent. A significant refuge should minimize negative impacts on beneficial insects that control other pests.

- **New Genes and Gene Combinations.** New transgenic technologies, including gene stacks, introduction of other Bt toxins, and registration of novel toxins are under development and their resistance management implications need to be evaluated. When based on other Bt toxins, cross-resistance is an important issue (Bolin 1998). Cross-resistance among Bt toxins that share a common binding receptor is well documented, especially among those classed as Cry1 toxins (McGaughey and Oppert 1998). Novel non-Bt toxins may interact with Bt receptors, and each technology will need examination for resistance management implications. Gene stacks with other pest management traits (e.g., herbicide resistance) need to be examined for impacts on European corn borer resistance management. The commercial availability and viability of these new technologies is unknown, which reinforces concerns about durability of existing strategies.

- **Incentive-Based Options.** A voluntary insurance program or discounts on the purchase of non-Bt seed might help "level" the perceived differences in economic returns associated with Bt-corn net revenue and non-Bt corn refuge acres.

- **Southwestern Corn Borer and Other Stalk Borers.** Southwestern corn borer, southern cornstalk borer, and (common) stalk borer also attack corn in parts of the U.S. Our biological information on these borers is limited. We recognize that there could be many differences between other borers and European corn borer that will influence insect resistance management strategies. Until we have additional information on how the relevant parameters are affected, we suggest that recommendations developed for European corn borer should be employed in areas where these other borers occur. We recognize that the sprayed refuge option is more likely to be used in areas infested with southwestern corn borer because of the serious losses associated with this insect.

- **Education.** Growers are key partners in resistance management. Dissemination of information to growers is essential for effective implementation and extending the durability of the technology. Economic and scientific reasons must be combined with practical deployment strategies for this educational message (particularly the refuge component) to be embraced widely by growers (Rice and Ostlie 1997; Rice and Pilcher 1998). Revision of NCR-602 is underway to reflect new events, and new information on Bt corn performance and resistance management.

Potential Threats to the High-dose Strategy.

- **Changes in toxin concentration throughout the growing season.** All current and future transgenic hybrids should be measured for toxin concentration throughout the season under a wide range of environmental conditions (e.g., soil, weather, irrigation). Toxin concentrations can decline after pollen shed in some Bt hybrids (Walker 1998), jeopardizing the high-dose strategy (Onstad and Gould 1998a).

- **Minor Bt-Resistance Traits are Common in European Corn Borer.** Laboratory selection programs for Bt resistance have shown increases in Bt tolerance of 20 to 80 fold (Huang et al. 1997; Keil et al. 1997; Bolin 1998; Keil and Mason, unpublished). These results demonstrate that minor resistance genes are common enough to be included in all of the original selection stocks, and there is substantial genetic variability for resistance in wild European corn borer populations. To date, however, survival of selected strains has not been documented on transgenic Bt corn hybrids. When major resistance genes are found, they are likely to occur in populations and genotypic combinations with minor traits that may increase their relative dominance, threatening the high-dose strategy (Alstad and Andow 1996).

Conclusions

Collectively, the new scientific information reinforces the basic principles of our 1997 resistance management statement. The premise of the NC-205 position is to prolong the practical benefits associated with Bt transgenic corn technology. We support a high-dose/refuge strategy for management of resistance to Bt corn. Based on current data, modeling, and scientific interpretation, these recommendations are that under a high-dose/refuge strategy, refuges should protect 20 to 30% of the European corn borer larval population from exposure to Bt toxins. In a practical sense, this suggests 20 to 30% of the corn acreage should be planted to non-Bt corn. This non-Bt percentage should be increased to 40% if the refuge will be sprayed. We also recommend refuges and Bt plantings be established in close proximity, such that the refuge always occurs within the same half-section (320 acres) wherein Bt corn is planted.

In summary, we find the scientific evidence leads us to reaffirm our 1997 recommendations. As additional information becomes available, we will continue to reassess these recommendations.

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