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RESISTANCE MANAGEMENT RATIONALE AND STRATEGY FOR WIDESTRIKE™ INSECT PROTECTION (CRY1F/CRY1AC) IN COTTON

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Abstract

A resistance management strategy for WideStrike Bt cotton has been developed based on the doses of the Cry1F and Cry1Ac Bt proteins against the key target pests, their mode of action, and the biology of the target pests. This paper describes the scientific basis of the IRM plan and the resistance management plan options available to growers of WideStrike Bt cotton. Computer simulation modeling was conducted to understand how the target pests may adapt to WideStrike in realistic agroecosystems where multiple crops are grown and multiple Bt traits are used. The model integrated our knowledge of insecticidal dose, cross-resistance potential, pest biology and likely use patterns. This modeling showed that the rate of adaptation is likely to be very slow. The use of standard refuge practices will provide additional assurance that WideStrike will remain durable for the long term.

Introduction

WideStrike Bt cotton recently received full registration by the US regulatory agencies and will be available to be grown commercially for the first time in 2005. WideStrike expresses two proteins from *Bacillus thuringiensis* (Bt): Cry1Ac from Bt var *kurstaki* and Cry1Fa from Bt var *aizawai* and provides season-long broad spectrum control important lepidopteran pests (Haile et al. 2004, Langston et al. 2004). An integral component of the management practices for WideStrike will be the implementation of measures designed to prevent or delay the evolution of resistance to the Bt proteins in populations of the key target pests: tobacco budworm (*Heliothis virescens*), bollworm (*Helicoverpa zea*), and pink bollworm (*Pectinophora gossypiella*). The insect resistance management (IRM) plan for WideStrike was developed based on a broad understanding of key target pest biology, resistance genetics, the pest-crop interactions and cotton agroecosystems.

The goal of insect resistance management for Bt crops is to reduce the survival of alleles conferring resistance to the Bt proteins (R-alleles) relative to the survival of alleles conferring susceptibility (S-alleles). This can be achieved through deploying Bt crops that are likely to present a lethal dose to insects heterozygous for R-alleles, by combining two Bt proteins in a single plant both of which having significant insecticidal activity against the pests of concern, and the use of non-Bt refugia. The refugia can be structured (for example, non-Bt cotton planted for the purpose of resistance management) or non-structured (for example, alternative host crops or non-crops). This paper explores the resistance risk profile for WideStrike Bt cotton and describes the steps to be taken to manage that risk.

Dose Against Key Target Pests

Bt crops expressing high doses of insecticidal proteins are thought to be at lower risk of pest adaptation than Bt crops expressing lower doses when planted with a non-Bt refuge (US Environmental Protection Agency 1998). This is because heterozygotes with one copy of an R-allele conferring adaptation to the Bt crop are also likely to be killed at a high rate, slowing the rate at which such alleles can become established in the population.

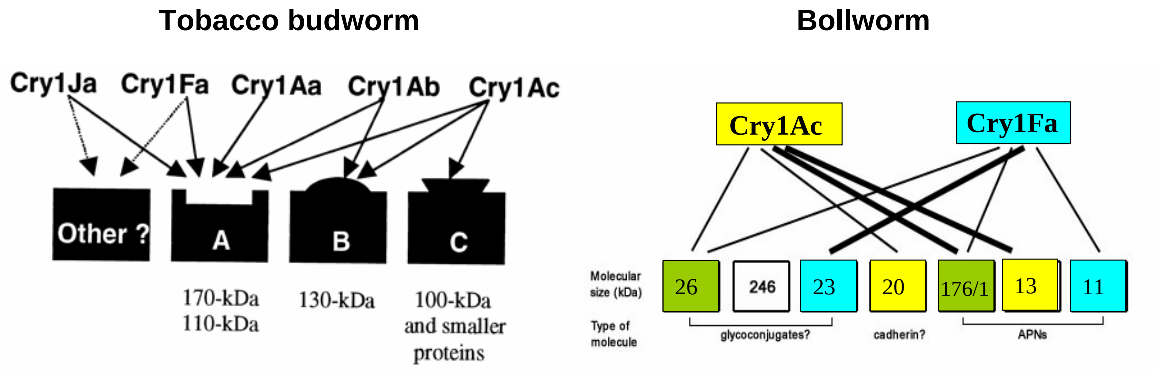
The level of the Cry1Ac component of WideStrike Bt cotton is sufficient to kill > 99.99% of *H. virescens* neonates, and is at least 25-fold higher than the level required to kill 99% of *H. virescens* neonates (Blanco et al. 2003). The level of Cry1Ac is sufficient to kill larvae that are 25 times more tolerant of the protein than neonates. By the same methods, we have shown that the Cry1Fa component in WideStrike is close to 25X the LC99 of susceptible *H. virescens* (Blanco et al. 2003).

The Cry1Ac component of WideStrike cotton is sufficient to kill > 99.99% of *P. gossypiella* neonates, and is at least 25-fold higher than the level required to prevent the successful development of 99% of PBW neonates. The Cry1Fa component does not provide additional protection from *P. gossypiella*. For *P. gossypiella*, WideStrike, is a single-gene, high-dose, Bt cotton.

Field data indicate that the doses of either Cry1Fa or Cry1Ac alone do not cause >99% mortality of *H. zea* larvae, and therefore there is less expectation that heterozygote mortality will be high. However, the dose of the two proteins combined in WideStrike kills around 95% of susceptible larvae in the field

Cross-resistance Potential within WideStrike

All cases of high-level resistance to Bt insecticidal proteins have been receptor mediated (Ferre and Van Rie 2002). Patterns of cross-resistance are generally predictable from knowledge of binding sites (Ferre and Van Rie 2002). Because WideStrike expresses two insecticidal proteins with high levels of activity against both *H. virescens* and *H. zea*, it is important to understand the sites of action in the target insects and understand the potential for cross-resistance through midgut receptor mutations. Though these proteins are very similar, there are differences in binding patterns that are important from a product durability standpoint. Both ICPs bind to a range of different types of binding sites in both *H. virescens* and *H. zea*, with some receptor families binding Cry1Ac only, some binding Cry1F only, and others competitively binding both (Jurat-Fuentes and Adang, 2001, and Adang, unpublished; Fig. 1). Binding affinities in *H. zea* suggest that 60% of Cry1Ac binding is to a site shared with Cry1F and 40% is to independent sites (Sheets and Storer, unpublished). Based on the binding of Cry1Ac and Cry1Fa described in these studies, it is unlikely that a mutation in a gene for a single receptor will confer high levels of resistance to both ICPs. For example, the YHD2 colony of *H. virescens* is >300,000-fold resistant to Cry1Ac and only 130-fold resistant to Cry1F; at least four genes are involved in this resistance (Jurat-Fuentes et al. 2000) It is likely that for high survival, several genetic mutations will be needed. Insects bearing combinations of R-alleles are likely to be initially extremely rare, and, when coupled with a refuge, increase in proportion extremely slowly.


Tobacco budworm

Bollworm

Figure 1. Proposed binding maps for Cry1 proteins in midguts of tobacco budworm (Jurat-Fuentes and Adang, 2001) and bollworm (Adang, unpublished).

Cross-resistance Potential with other Control Technologies

WideStrike Bt cotton is entering a market where several technologies are used to manage lepidopteran pests. Other Bt cotton lines are available expressing Cry1Ac or Cry1Ac stacked with Cry2Ab. In addition, chemical insecticides from several classes are used to manage these pests. The addition of WideStrike Bt cotton to this mix will serve to lessen the selection pressure for resistance to any one technology; in turn, the use of these other technologies will limit the selection pressure for resistance to the Bt proteins in WideStrike.

Structured and Unstructured Refugia

A refuge is an area of host plants where Bt-susceptible individuals can be produced (i.e. there is no selective differential or susceptible genotypes are favored over Bt-resistant genotypes) that are available to mate with any individuals selected for Bt-adaptation in the Bt field. The offspring from such matings are susceptible homozygotes or heterozygotes, and both of these are controlled by the Bt cotton. To qualify as refugia therefore, the plants have to produce non-selected insects within the local population at the same time as selected insects are produced from Bt cotton. An example is a patch of non-Bt cotton planted close to Bt cotton and managed in the same way as the Bt cotton. Other nearby non-Bt hosts (be they crops or wild plants) that are attractive and suitable at the same time as the Bt cotton may also act as refugia. Gould et al. (2002) provided evidence that only a relatively small proportion of *H. zea* adults emerge from C3 plants (including but not restricted to cotton), and that a significant proportion emerge from C4 plants (mostly monocotyledonous plants). Over the last two years, considerable data have been generated documenting the role of several other crops, including corn, sorghum, peanut, and soybean as hosts for *H. zea* (e.g. Adamczyk et al. 2003, Diffie et al. 2004, Gore et al. 2004, Hardee et al. 2003, Jackson et al. 2004, Peters et al. 2004).

In addition susceptible populations may originate from other areas and subsequently become interspersed with local populations. These areas serve to reduce overall population-wide selection pressure and are an important factor to consider in understanding resistance risks. Given that the highly migratory nature of *H. zea* especially, the role of alternate hosts, both crops and non-crops, across the entire geographic range is relevant.

Simulation Modeling of Adaptation to WideStrike

The spatially-explicit stochastic model of Storer et al. (2003) was adapted to simulate *H. zea* adaptation to the complex environment where multiple Bt Cry proteins are expressed in cotton (Cry1Ac, Cry1F, and Cry2Ab). The model allows for three independent midgut receptors for these proteins, one that binds Cry1Ac only, one that binds both Cry1Ac and Cry1F, and one that binds Cry2Ab only. This is a conservative representation of Cry1 protein binding, since in reality there appear to be between four and six binding sites involved (Figure 1). The agroecosystem includes alternate crop and weed hosts as used by *H. zea* in the North Carolina coastal plain (Figure 2a) and in the Mississippi Delta (Figure 2b). The model simulates the change in population fitness on WideStrike Bt cotton over time. However, due to the variability and uncertainty inherent in pest population dynamics and pest management practices, the output is not regarded as predictive in an absolute sense; rather it enables comparison of resistance risks under different scenarios and under different resistance management practices.

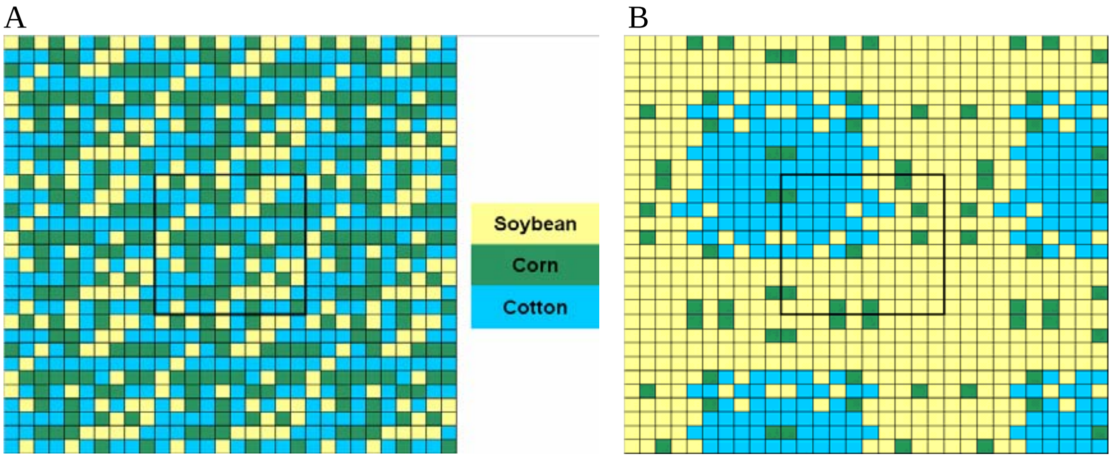


Figure 2. Crop distribution for two agroecoystems. North Carolina (A) and the Mississippi Delta (B) are depicted. In each, the center 10 x 10 fields are actually modeled, while the surrounding area is assumed to be identical for North Carolina and a mirror image for the Delta.

The model suggests that there will be very little change in population fitness on WideStrike over a 15-year time horizon, with or without a structured refuge (Figure 3). Larger structured refuges decrease the change in fitness compared with smaller ones. The ability to spray structured refuges for *H. zea* only slightly reduces their effectiveness. The properties of WideStrike (particularly the need for multiple receptor mutations to allow high larval survival) coupled with the pest's biology (especially extensive use of alternate hosts and long-distance migration) mean that the relative survival of R-alleles over S-alleles is not great.

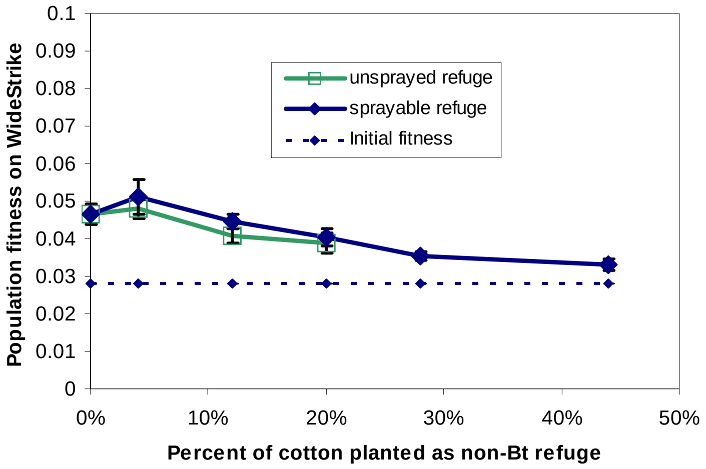


Figure 3. Simulated effect of refuge size on the change in population fitness on WideStrike in 15 years for the North Carolina agroecosystem with a 20% non-Bt cotton refuge. Bt cotton was planted to a combination of traits (50% WideStrike, 25% Bollgard and 25% Bollgard II). Each data point is the average (with standard deviation) of 5 model runs. Changes in population fitness were smaller in the Delta agroecosystem.

The model further shows that in the competitive market where growers can choose different types of Bt cotton (such as varieties expressing Cry1Ac and Cry2Ab), adaptation to WideStrike will occur even more slowly than if WideStrike were the only Bt cotton option (Figure 4). This illustrates that the presence of competing insecticidal traits in the market place will help reduce the resistance risk for each.

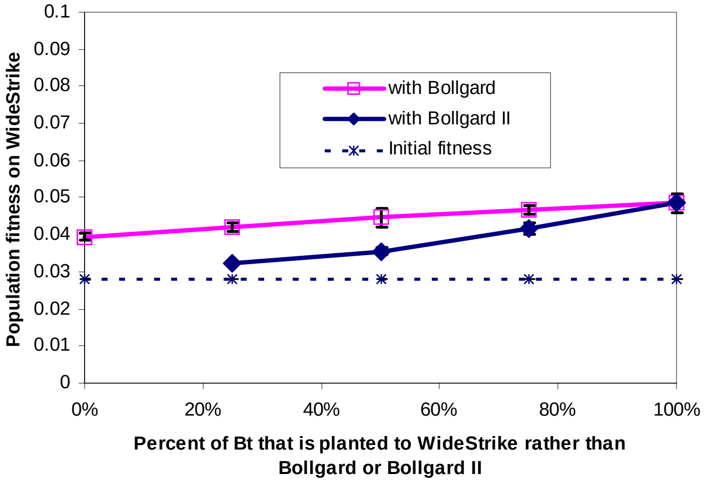


Figure 4. Simulated effect of market share of WideStrike with either Bollgard or Bollgard II on the Change in Population Fitness in 15 Years in the North Carolina agroecosystem with a 20% non-Bt cotton refuge. Each data point is the average (with standard deviation) of 5 model runs. Changes in population fitness were smaller in the Delta agroecosystem.

The Peck et al. (1999) model of *H. virescens* adaptation to Bt cotton showed that the resistance risk for a single-gene, high dose, Bt cotton with a 20% refuge is low. By extension, the resistance risk for WideStrike with two high dose Bt proteins, even with the potential for limited cross-resistance between Cry1Ac and Cry1F, is even lower.

Resistance Management Options for WideStrike

Given the low risk of adaptation by the key target pests, the use of standard refuge practices will provide additional assurance that WideStrike will remain durable for the long term. The IRM plan for WideStrike Bt cotton in 2005 is the same as the plan for other Bt cottons.

External unsprayed refuge option.
5% of a farmer's cotton must be planted to non-Bt cotton refuge and not be treated with any lepidopteran-control technology. The refuge must be at least 150 ft wide, and within ½ mile of the Bt cotton.

External sprayable refuge option.
20% of cotton must be planted to non-Bt cotton refuge, and can be treated at threshold with lepidopteran-active insecticides (or other control technology) except for microbial Bt formulations. The refuge must be within 1 mile of the Bt cotton.

Embedded refuge option.
5% of a cotton field must be planted with a non-Bt variety. This must be planted as a block or blocks within the Bt cotton field at least 150 ft wide and can be treated with lepidopteran-active insecticides only if the entire field is treated at the same time. Multiple small fields can be combined and managed as one large field.

Embedded refuge option for PBW.
One single row of a non-Bt cotton variety must be planted for every 6 to 10 rows of Bt cotton. This can be treated with lepidopteran-active insecticides (or other control technology) only if the entire field is treated at the same time.

Community refuge option.
Several growers can combine their fields so that one field can act as the 20% sprayable refuge or the 5% unsprayed refuge for several farms.

Conclusions

The risk of adaptation to WideStrike Bt cotton by target pests is inherently low for several reasons. WideStrike exposes the key target pests to two insecticidal proteins simultaneously. Doses of the two proteins against *H. virescens* are very high. Dose of the two proteins combined against *H. zea* is high. Dose of Cry1Ac against *P. gossypiella* is very high. There is limited potential for cross-resistance between these two proteins in the heliothine pests. The biology of the *H. virescens* and *H. zea* (as well as other target pests) in the cotton agroecosystem reduces the selection pressure for alleles conferring adaptation to WideStrike. When coupled with the significant structured refugia required to be planted with Bt cotton, the rate of pest adaptation is expected to be very small. Furthermore, the introduction of WideStrike Bt cotton is expected to reduce the selection pressure for pest adaptation to other pest management technologies, including other Bt cotton lines and chemical sprays.

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