NEW MECHANISM FOR Bt RESISTANCE IN CATERPILLARS

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Introduction

The application of insect-resistant transgenic crops is expanding at double-digit rates (Christou et al., 2006) and it appears this technology will remain as an important part of agricultural production in the foreseeable future. The insect toxins most often used in transgenic plants are derived from the bacterium, *Bacillus thuringiensis* (Bt). All existing evidence indicates that these proteins are safe to the environment, wildlife and human health. Also, new technologies are rapidly being developed for alternative proteins to the current Bt Cry toxins as well as approaches using double stranded RNA (dsRNA). There are many important benefits to the use of insect-resistant transgenic crops which include improved insect control, lower use of synthetic chemical insecticides and their reduced negative effects on non-target organisms, and the simplification of pest management which allows farmers to concentrate on other aspects of crop production.

The development of resistance to insecticides has been documented since 1914 (IRAC, 2005). Resistance to inorganic insecticides, organic insecticides and Bt sprays has developed within 2–20 years of being applied to populations of target insects (IRAC, 2005). Looking at the tobacco budworm, *Heliothis virescens*, in cotton as a case in point, populations of this insect have developed resistance to a succession of four classes of insecticides since the 1960s (Sparks, 1981; Elzen et al., 1992).

Mechanisms of Insect Resistance

In general, there are two approaches for insecticide application: (i) sprays, where the insecticide is applied directly to the insect and the insect in this case has no method to avoid contact with the pesticide and (ii) plant transgenic control, where the insect must choose to consume the plant material. In the case of the latter, which has been developed during the past few decades, there are obvious advantages of convenience to the farmer, of not having to prepare and apply the pesticide, or of deciding when to make an application. However, there are also special, potential disadvantages which have not received much attention. It is these potential disadvantages that we have been examining.

Phytophagous insects and plants have been in competition for millions of years prior to the introduction of insecticidal sprays and transgenic crops. The insect feeds on the plant, the plant develops defenses through natural selection mechanisms to prevent this feeding, and the insect develops approaches to circumvent the plant defenses. Mechanisms of resistance include those presented in Figure 1. One well known insect strategy in this conflict has been the avoidance of plant compounds by the development of behavior mechanisms to avoid contact and the absorption of plant toxins.

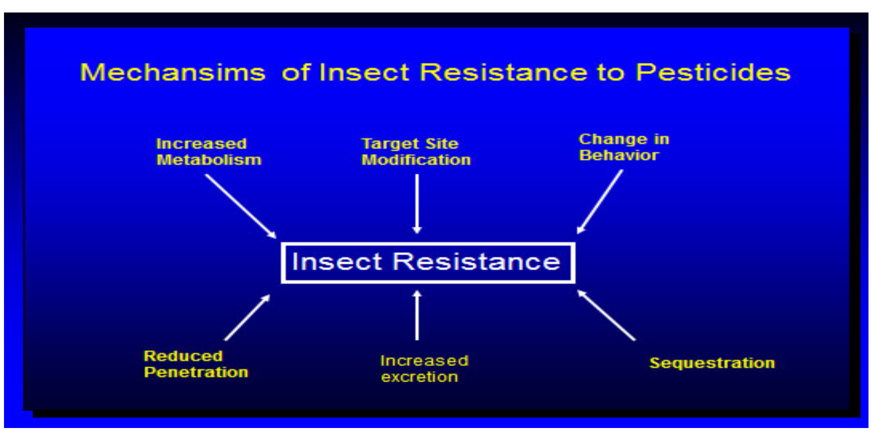

Figure 1. Mechanisms of insect resistance to pesticides.

Potential Vulnerability to Current Transgenic Approaches for Insect Control

Efforts to forestall the evolution of resistance to insect-resistant transgenic crops like cotton include the use of pyramided Bt toxin transgenes. In the future, plans are to expand pyramiding to include other protein toxins and RNAi technology. This strategy of pyramiding insecticidal transgenes can reduce the possibility of resistance based on all of the mechanisms shown in Figure 1 except for behavior resistance. Although the strategy of stacked genes is sound, it is aimed mostly at reducing the risk of target site resistance. The addition of an RNAi approach could also reduce the risk of increased metabolism, reduced penetration, reduced transport, increased excretion and increased sequestration because of the physiochemical differences inherent in nucleic acids versus proteins. However, mechanisms that allow the insect to avoid multiple killing agent(s) in the plant tissues could negate pyramiding efforts.

Summary of Preliminary Evidence for Behavior Resistance to Bt Toxin

Unfortunately, we have preliminary evidence for new, behavioral based mechanisms for Bt toxin resistance with the potential for cross resistance to different protein toxins and RNAi. When the rate of food passage through the digestive system was artificially reduced in Bt susceptible tobacco budworms (TBW), we found that the susceptibility to Cry1Ac toxin increased. Furthermore, we have found natural differences in feeding rates in different TB populations and a 37% increase in the feeding rate of a laboratory strain of the TBW which is cross resistant to several different Bt toxins. We have also found that TBW have mechanisms to rapidly distinguish between Bt and non-Bt diet.

Variations in Food Consumption in Different Tobacco Budworm Populations

We have seen wide variations in the feeding rates of field-collected populations of TBW. Figure 2 shows the fecal production rate for 1st instars collected as eggs from tobacco growing in three NC counties (Cabrera et al., 2011). The eggs were separated from the tobacco, allowed to hatch in the laboratory and within 12 h of hatching, placed on artificial diet with no Bt. Fecal production was used as a convenient measure of food consumption. The more they eat, the more feces that are produced. Among these three populations, there was a 2.5-fold variation in feeding rate as measured in terms of fecal production.

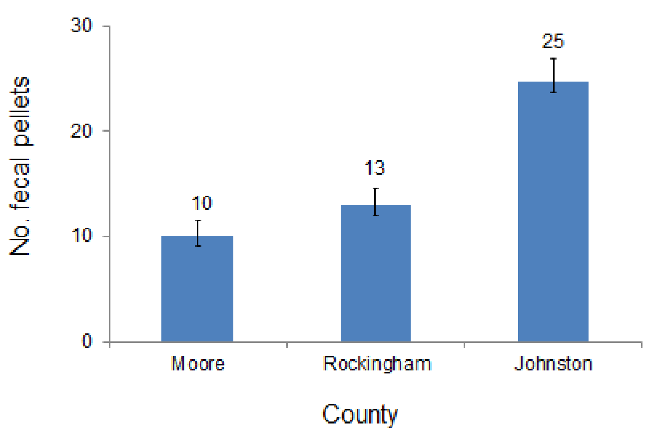

Figure 2. Variation in 24-hour average fecal production of TBW 1st instars (n = 64) collected as eggs from three North Carolina tobacco fields and fed FDT meal pads.

Effect of Artificial Changes in Food Consumption Rate on Susceptibility to Bt Toxin

The artificial reduction in feeding rate, achieved by a reduction in incubation temperature, increased TBW neonates susceptibility to Cry1Ac toxin in artificial diet. When we fed 1st instars of a lab strain of TBW at two different temperatures on artificial diet with no Bt, there was an expected lower feeding rate at the lower temperature as measured by the number of fecal pellets produced. (Figure 3). Fecal production at 20 °C was 42% of fecal production at 30 °C (Figure 3). These experiments were repeated with a dose of MVPII in the diet that reduced fecal production at 30 °C to 54% of the non-Bt control (Figure 4). Based on the observed effect of temperature alone on the feeding rate (Figure 3), expected fecal production for neonates of the same strain fed diet containing Bt at 20°C was 15 fecal pellets per larva (Figure 4). However, the actual fecal production rate observed was lower, 60% of the expected rate (Figure 4). The reduced rate of food ingestion and passage through the gut may have increased the insect's susceptibility to the Bt toxin. Other possible factors include changes in the rate of processing of the Bt toxin by the digestive system.

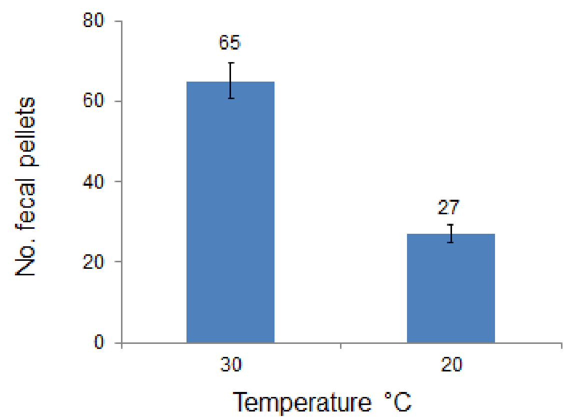

Figure 3. 24-hour average fecal production per control (NO BT) TBW 1st instar (n = 45) fed FDT meal pads at 30 °C & 20 °C.

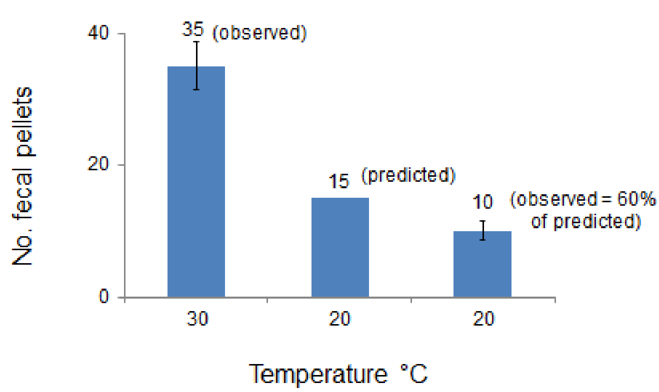

Figure 4. 24-hour average fecal production per TBW 1st instar (n = 45) fed MVPII Bt proteins in FDT meal pads at 30 °C & 20 °C.

Differences in Feeding Rates between Bt Susceptible and Bt Resistant TBW Strains

Interestingly, differences in feeding rate were found between the parent and resistant lab strains of the TBW. When we did a side-by-side test of the feeding rates of Bt-susceptible and Bt-resistant lab strains of TBW fed on artificial diet with no Bt toxin, the feeding rate for the resistant strain was 37% greater than for the susceptible strain (Figure 5). One explanation for these results is that the increased rate of food movement through the gut may be one component of the resistant mechanism for Bt in this strain. The extent of this mechanism in resistance, if correct, cannot be determined.

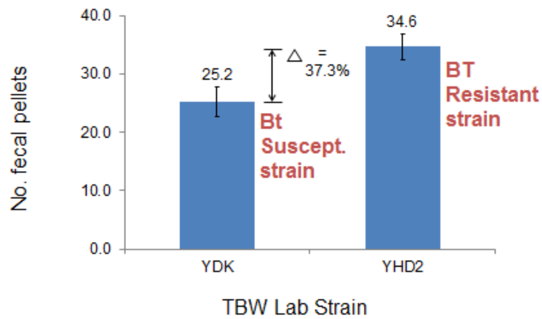

Figure 5. 24-hour average fecal production (n = 56-64) of Bt susceptible (YDK) vs. resistant (YHD2) TBW L1s fed FDT meal pads.

Summary

We present evidence that one potential mechanism for resistance to Bt toxins may be changes in the rate of insect feeding. When feeding rate was reduced by temperature, the apparent susceptibility to Bt toxin increased and increased feeding rate was found in a Bt resistant lab strain of the TBW as compared to the parent susceptible strain in the absence of Bt in the diet. We also found significant variation in the feeding rates of natural populations of the TBW. Not shown were greater feeding rates for the cotton bollworm and much lower susceptibility to Cry1Ac, Cry1F, and Cry1Ab in CB compared to TBW (van Kretschmar et al., 2011). Further work is needed to better understand the potential effect of feeding rate on susceptibility of caterpillars to Bt toxins, on potential cross resistance to RNAi approaches for their control, and the ability of caterpillars to make favorable choices to avoid Bt in their diet.

References

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