USE OF BT SPORE/CRYSTAL FORMULATIONS TO CONTROL INSECT PESTS: THEY'RE NOT DEAD YET!

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Abstract

Interest and use of *Bacillus thuringiensis* (Bt) formulations to control cotton insect pests has declined recently primarily due to the success of transgenic cotton expressing the Cry1Ac gene (Bt cotton) to control the tobacco budworm, *Heliothis virescens.* However, Bt cotton is less efficacious against other pests such as cotton bollworm, *Helicoverpa zea,* beet armyworm, *Spodoptera exigua,* and the fall armyworm, *S. frugiperda*. Additionally, widespread concern over the threat of insecticide resistance developing against these single Bt toxin plants allows for reconsideration of the positive attributes of current and future Bt formulated materials. This presentation will re-visit the positive attributes of Bt formulated products and discuss the more recent discoveries in Bt research which may allow for Bt formulations to fill an important role in cotton insect control.

Introduction

Transgenic cotton expressing the Cry1Ac gene from *Bacillus thuringiensis* (Bt) to primarily control the tobacco budworm, *Heliothis virescens* was introduced commercially in 1996. Control of this pest with Bt cotton was considered excellent, and acreage to be planted in 1997 with Bt cotton is predicted to increase. Bt cotton is a result of exploiting some of the positive attributes of Bt with that of using plant molecular biology to produce a plant which constitutively expresses the Bt toxin in most tissues throughout the plant. These plants produce more Bt toxin than can be efficiently applied to plant surfaces using commercial Bt formulations and conventional spray equipment, and that can persist at economically acceptable levels. However, expressing a single toxin within a plant presents several concerns. The first major concern with expressing a single Bt endotoxin is that each Bt endotoxin has its own host range. Therefore, maximizing control against one insect pest (e.g. tobacco budworm) would reduce efficacy against other insect pests that are more susceptible to other Bt toxins (e.g. *Helicoverpa zea*, or *Spodoptera exigua*). Most Bt formulations contain multiple endotoxins which should then have a broader host range than a transgenic plant expressing a single endotoxin. The second major concern with expressing a single Bt endotoxin is that resistance to a single toxin should develop quicker than when using multiple toxins with multiple modes of action. Most Bt formulations contain not only endotoxins (with potential different binding sites) but also secreted insecticidal toxins, as well as the bacterial spore which has been shown to synergize insecticidal activity of the endotoxin(s) against various lepidoptera and, therefore, reduce insecticide resistance in certain cases. This presentation will highlight the major advantages that Bt formulations have over current cotton cultivars expressing single Bt toxin genes in the hope that Bt formulations still will be considered for use in cotton to control certain insect pests, as well as one component in a resistance management system.

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Discussion

Bt endotoxins

Typical Bt formulations such as Dipel 2X contain Cry1Aa, Cry1Ab, Cry1Ac, Cry2a and Cry2b. The spectrum of activity of these toxins against *H. virescens*, *H. zea*, and *S. exigua* are shown in Table 1.
Therefore, a cotton plant expressing Cry1Ac only should be more efficacious against *H. virescens*, less efficacious against *H. zea*, and have little activity against *S. exigua*. Bt endotoxin concentrations in formulations such as Dipel 2X range from high concentrations of Cry1Ab, lower concentrations of Cry1Ac and Cry1Aa, to low levels of Cry2a and Cry2b. Therefore, based on endotoxin alone, Bt formulations such as Dipel would be expected to have relatively high activity against *H. zea* and *H. virescens* but little to no activity against *S. exigu*a. (Note: Only relative toxicities are discussed, not differences in quantities of endotoxins produced).

Compounds in Bt other than endotoxins

It has been known for years that many Bt strains produce other insecticidal compounds such as exotoxins, phospholipases, chitinases, etc. However, perhaps the most important contribution to the overall toxicity of Bt formulations other than endotoxins is the spore. Spores have been shown to synergize endotoxins against many insect pests such as *S. exigua*, and *H. zea*.

Several new secreted compounds have been recently identified from Bt with exciting insecticideal properties. The first compound is zwittermycin. This compound is found in many Bt formulated materials. This compound synergizes endotoxins by enhancing the affinity of endotoxin to the insect midgut. The sceond group of compounds are the Vegetative Insecticidal Proteins (VIPs). These proteins are produced during vegetative growth. About 10% of all Bt strains screened contained at least one VIP. VIPs have activity equivalent to endotoxins, with activity against many lepidopterous insects.

Future Bt formulations will be optimized to express these numerous native compounds. Increased expression should not only increase the insecticidal activity of these formulations, but also may help increase the time required for the development of Bt resistance.

Interactions between spores and endotoxins

A major concern with Bt formulations is that mortality occurs 2-3 days after ingestion, unlike traditional insecticides that kill within 24 hr. However, even this 2-3 day toxicity is a result of interactions between the endotoxin(s) and other compounds, especially the spore. As Tables 2 and 3 show against *S. exigua*, formulations (e.g. Xentari) containing endotoxins and spores are not only much more toxic than formulations containing only endotoxins (e.g. Mattch), the time required to induce mortality is significantly reduced (2 days vs. 5 days). Although endotoxins without spore often are less efficacious, ingestion of endotoxins without spore usually results in severe larval stunting which still may be enough to reduce crop damage to acceptable limits. However, another concern with use of endotoxins without spore (e.g. Bt cotton or formulations such as Mattch) is that evaluation of insecticide efficacy may need to be delayed several days. This may result in missing the window for alternative insecticide treatments and/or withstanding increased damage.

Resistance Management Considerations

Resistance to Bt formulations only has occurred in situations where Bt formulations were used so often, that fields were essentially "continuously" selecting for resistance, not unlike the scenario with Bt cotton. Therefore, the threat of resistance to Bt (formulations or transgenic plants) will increase with an increase in acreage treated and increased numbers of applications used.. The advantages of Bt formulations are that 1) a Bt formulation can be used only when needed, and be rotated in with other control stratagies throughout the growing season; 2) Bt formulations contain numerous compounds with multiple mechanisms of action which not only should help reduce resistance against the Bt formulation itself, but also should help reduce resistance to other insecticide treatments, even transgenic plants expressing single Bt endotoxins.

Environmental Persistence

One of the biggest concerns for the use of Bt formulations in cotton is that of lack of persistence. Persistence of Bt on cotton has been demonstrated to be from less than one day to several days, both of which are usually unacceptable. Considerable research has been undertaken recently to address these concerns. Tamez-Guerra et al. (1996) have demonstrated that the addition of lactic or citric acid with starch/flour materials greatly increased persistence of Bt technical powders against *Ostrinia nubilalis* on cotton leaves, even after 8 hr. of simulated sunlight exposure.

Summary

Many advances have taken place with Bt formulations recently, and this trend will continue for the foreseeable future. These advances should result in more toxic strains expressing multiple toxins (some of which have different mechanisms of action) which will persist longer on cotton foliage. Therefore, although Bt cotton currently looks outstanding, the use of Bt formulations in cotton may need to be reconsidered in order to control other insect pests, as well as to help reduce the threat of Bt endotoxin resistance.

References

Tamez-Guerra, P., M. R. McGuire, H. Medrano-Roldan, L. J. Galan-Wong, B. S. Shasha and F. E. Vega. 1996. Sprayable granule formulations for *Bacillus thuringiensis*. J. Econ. Entomol. 89: 1424-1430.

Table 1. Activity profile of common endotoxins

Endotoxin	TBW	CBW	BAW
Cry1Aa	+	++	-
Cry1Ab	++	+++	-
Cry1Ac	+++	+	-
Cry2a	++	++	++
Cry2b	++	++	-

Table 2. Toxicity of Mattch vs. Xentari against neonate *S. exigua*

Treatment (μg/g diet)	**Mortality**	
	slope (±SEM)	LC_{50} (±95%FL)
Xentari	2.84 (0.159)	26.4 (19.8 - 33.1)
Mattch	2.63 (0.250)	10,200 (6,990 - 12,650)

For Mattch, assuming a density similar to water, 1 μl of Mattch formulation equals 1 mg of formulation.

Table 3. Mean time of 50% mortality between Mattch vs. Xentari against neonate *S. exigua*

Treatment	Day (± SD)
Xentari	
Conc. (μg/g diet)	
20 (approx. LC_{50})	2.7 (1.2)
40	2.0 (0.0)
60	2.0 (0.0)
80	2.0 (0.0)
Mattch	
Conc. (μg/g diet)	
10,000 (approx. LC_{50})	5.0 (0.0)
15,000	4.0 (0.0)
20,000	5.0 (1.0)
25,000	4.3 (0.5)

Assuming a density similar to water, 1 μl of Mattch formulation equals 1 mg of formulation.