Sublethal effects of *Bacillus thuringiensis* Berliner on the diamondback moth, *Plutella xylostella* (L.), and its natural enemy, *Cotesia plutellae* Kurdjumov: implications for resistance management

by

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Summary

Natural enemies are often an important component of an integrated pest management (IPM) program. While much research has focused on the impact of insecticides on natural enemy conservation, little is known about the influence of natural enemies on the rate of evolution of insecticide resistance in the target pest. This research examined specific biological and behavioural interactions among the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), one of its natural enemies, *Cotesia plutella* Kurdjumov (Hymenoptera: Braconidae), and the microbial insecticide, *Bacillus thuringiensis* Berliner (*Bt*). The results were used to develop a deterministic simulation model that describes the influence of *C. plutella* on the evolution of resistance to *Bt* in a diamondback moth population, and examines various resistance management strategies that may also be adapted to other pest-natural enemy-insecticide systems.

In the diamondback moth, resistance to *Bt* is controlled by an incompletely recessive, autosomal gene, and thus heterozygous larvae will be partially resistant. After an application of *Bt*, some heterozygotes will be sublethally infected while resistant homozygotes will be largely unaffected. This differential host response may affect the biology and behaviour of natural enemies, and, depending on the level of discrimination between host genotypes, could affect selection for resistance to *Bt* in the pest population.

To mimic the heterozygote response, bioassays were performed to determine a concentration of *Bt* to sublethally infect larvae, and to examine its effect on diamondback moth biology. Sublethal infection of second instar larvae with *Bt* significantly reduced larval weight gain and initial leaf consumption, delayed larval and pupal development, and reduced pupal weight. Adult fecundity and survival were not affected by sublethal
infection during the second instar larval stage. A preliminary comparison of mortality at a range of Bt concentrations revealed that third and fourth instars were more susceptible to Bt than second instars while fourth instars were more susceptible than third instars.

Diamondback moth larvae were parasitised at various stages of infection to determine the effect of host sublethal infection on the biology of C. plutellae. The timing of a Bt application in relation to parasitoid oviposition can affect the development time of parasitoid larvae. Oviposition less than two days prior to sublethal infection of host larvae delayed pupation of parasitoid larvae, however, oviposition two days after host sublethal infection did not affect parasitoid development.

The level of parasitoid discrimination between host genotypes may have important consequences for the evolution of resistance to Bt in the pest. Therefore, the effect of host sublethal infection on searching behaviour and attack success of C. plutellae was investigated in a flight tunnel and under semi-field conditions. Differential plant damage as a result of feeding by sublethally infected or healthy host larvae, affected in-flight orientation of C. plutellae, with parasitoids preferentially flying to plants damaged by healthy larvae. Time spent walking/antesnating, pointing, and grooming on plants infested with sublethally infected and healthy larvae was not significantly different, however, searching parasitoids encountered healthy larvae more often than sublethally infected larvae. When present on the same plant, healthy and sublethally infected larvae were encountered equally as often by C. plutellae. Since diamondback moth larvae have a violent defensive reaction to contact, particularly older instar larvae, the proportion of these encounters that resulted in oviposition (or at least insertion of the ovipositor) was examined. Sublethally infected larvae were parasitised more often than healthy larvae 96 hours after infection with Bt, primarily as a result of less effective larval defensive behaviour. The presence of spray residues of Bt on plants was shown not to affect
parasitoid behaviour. Under semi-field conditions, parasitism of sublethally infected and healthy larvae did not differ, although increasing the duration of larval exposure to parasitoids could affect the result. These results suggest that searching behaviour and attack success of C. plutellae is affected by the presence of sublethally infected hosts, and could lead to differential parasitism of healthy and sublethally infected larvae. The overall impact of differential parasitism on the evolution of resistance to Bt in diamondback moth may depend on the relative fitness of larvae of each genotype, their spatial distribution and relative proportion, and the effect of aggregation on the functional response of searching parasitoids.

A deterministic simulation model was constructed to examine the effect of various biological and operational factors on the evolution of resistance to Bt when Bt is used alone, or in conjunction with parasitoids, to control diamondback moth. The model also compares the effect of applying Bt sprays with deployment of highly or partially resistant transgenic plants to control the pest. Empirical data revealed that when exposed to Bt, development of heterozygous larvae was delayed and they were parasitised more often than resistant homozygotes and unexposed susceptible homozygotes. Simulations indicated that these factors are important in delaying the onset of resistance when parasitoids are used together with Bt. Operational factors such as refuges also significantly reduced the rate of evolution of resistance. Deployment of highly resistant transgenic plants revealed similar results, with refuges in the form of non-transgenic plants playing an important role in delaying resistance development. Simulations using partially resistant transgenic plants revealed that the presence of parasitoids may accelerate the evolution of resistance through selective removal of susceptible insects, which in this case are able to survive, but are affected by Bt-toxins to a greater degree than heterozygotes. The results suggest that while the use of parasitoids in an IPM program may be useful in reducing the pest population, the specific interactions between pest, natural enemy and insecticide require careful
consideration to ensure that parasitoids themselves do not accelerate selection for resistance. The relevance of these results for other pest management systems is discussed.