Assessing the Efficacy of a Product Containing *Bacillus thuringiensis* Applied to Honey Bee (Hymenoptera: Apidae) Foundation as a Control for *Galleria mellonella* (Lepidoptera: Pyralidae)[1]

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Abstract Greater (*Galleria mellonella* L.) and lesser wax moth (*Achroia grisella* F.) larval feeding can cause significant damage in active honey bee (*Apis mellifera* L.) colonies and stored equipment. This damage may lead to significant material and financial losses. Traditional control methods use toxic chemicals that may leave residues in wax and honey and are potentially hazardous to bees and humans. In this study, we evaluated the use of a product (B401®; Vita [Europe] Ltd., Basingstoke, UK) that contains *Bacillus thuringiensis* Berliner, a bacterium that is not harmful to bees or humans. The main objectives of our research were to determine if B401 could be successfully applied to the midrib of foundation during its production and if combs constructed on the foundation were protected from wax moth damage. B401 significantly reduced the comb damage score that was given to combs on a scale from 0-10, with 0 equal to no damage and 10 equal to complete damage (B401 = 1.70 ± 0.39 and 0.45 ± 0.16, control = 8.55 ± 0.32 and 3.80 ± 0.71) and the proportion of larvae surviving at 6 wk (B401 = 0.69 ± 0.07, control = 0.95 ± 0.04). Yet, as administered in our study, the product did not eliminate all wax moth damage to combs.

Key Words *Bacillus thuringiensis*, biological control, wax moth, honey bee

The greater (*Galleria mellonella* L.) and lesser wax moths (*Achroia grisella* F.) cause damage in active honey bee (*Apis mellifera* L.) colonies and stored equipment, damage that may lead to significant material and financial losses. The wax moth's larval stage causes the most significant damage because it feeds on and builds silk-lined feeding tunnels in honey bee combs. The larvae feed on impurities present in wax, such as bee feces and cocoons of bee larvae; therefore, old, dark combs containing profuse bee larval cocoons are most at risk for moth-associated damage (Charrière and Imdorf 1999). Before pupation, the moth larva spins a cocoon on a firm support, a process that often damages the wooden infrastructure of the frames or other wooden hive components. In severe infestations, wax moth damage can render entire frames (combs and bars) useless.

Recommendations for controlling wax moth damage in beehives include: (1) maintaining strong colonies, (2) removing comb from unoccupied hives, and (3) replacing combs regularly (Charrière and Imdorf 1999). There are several technical, physical,

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and chemical methods that are practiced to limit moth damage to stored combs. Technical and physical methods include sorting old comb from new comb, storing in areas with light and drafts, cool storage, and frost or heat treatment (Charrière and Imdorf 1999). Chemical controls include several chemicals used as fumigants (such as paradichlorobenzine) all which pose safety and toxic risks to bees and humans. Some biological control methods have been explored. These include the parasitoid wasp *Trichogramma* and the bacterium *Bacillus thuringiensis* Berliner (*Bt*) (Bollhalder 1999, Cantwell and Shieh 1981).

From the late 1960s-1970s, Burges (1976a, b, 1977) and Burges and Bailey (1968) explored the potential use of *Bt* as a wax moth control. The *Bt* strains tested for wax moth control produce a toxin that is specific for moth larvae. The toxin affects the midgut thereby causing ceasation of larval feeding (Hoopingarner and Materu 1964). The products containing *Bt* must be ingested by the larvae, which are killed from a day to several weeks later, depending on their size and the amount of *Bt* they consumed (Burges and Bailey 1968). Burges and Bailey (1968) also found that a commercial formulation of *Bt* incorporated into cold wax foundation controlled both greater and lesser wax moths while not harming bees or leaving toxic residues dangerous to humans.

Cantwell and Shieh (1981) present results of a successful attempt to develop the product called Certan™ (Vita [Europe] Ltd., Basingstoke, UK) for the control of wax moths. The *Bt* subspecies *aizawai* (Aizawai serotype 7) was used in this product. The product is suspended in solution and sprayed to the outside of combs prior to their storage. Therefore, each side of each comb must be sprayed with the solution thus protecting the treated frames during storage. The product does not leave any residue in the wax or honey and is harmless to bee larvae and adults. McKillup and Brown (1991) confirmed the efficacy of Certan to control moth damage in stored combs. Negatively, however, the product is labor intensive and costly to use because frames need to be treated every year. Consequently, the product is no longer marketed in the U.S. but it is marketed internationally as B401®. The main objectives of our research were to determine if B401 can be successfully applied to the foundation midrib during foundation production and if combs constructed on the foundation are protected from wax moth damage. Pending good efficacy, this application method could allow beekeepers to purchase foundation that already contains the B401 product. This could reduce the need to spray individual combs each storage season, and allow combs to be protected from wax moth damage while in a honey bee colony.

Materials and Methods

Comb preparation. In March 2006, we poured pure beeswax and manually pressed it into thin sheets of 23.178 cm deep foundation using a foundation mold. Only one side was embossed with a hexagon cell pattern resulting in each sheet having one smooth side and one imprinted or embossed side. Following label instructions, we applied a 1:20 solution of B401:water to the smooth side of each sheet of cooled foundation and allowed it to dry. We assembled 40 frames using 2 sheets of foundation per frame by putting the foundation into the frames with the smooth sides of both sheets facing each other on the inside, hence embedding the product in the midrib of the foundation. We assembled 40 control frames similarly but without applying B401. Established honey bee colonies received the frames. We initially fed colonies sugar syrup to encourage the bees to draw the foundation and to rear brood in

the combs. In May 2007, we moved combs above the queen excluder to allow brood to emerge. After 1 mo, we removed the test combs and left them in the apiary for an additional week to allow bees to remove any remaining honey. Following this, we took the empty combs to the laboratory to test the efficacy of B401.

Laboratory experiment. Laboratory methods were modified after Burges (1976b). We collected wax moth pupae from weak or dead honey bee colonies. We placed the pupae in 950-ml glass containers having screen wire lids. Pupae were kept in an incubator at 32°C until eclosion. Six unsexed adults were placed into an empty 950-ml glass container. To provide a substrate in the container for laying eggs, we introduced 7 pieces of wax paper that had been folded and paper clipped into a fan-like shape. Containers were returned to the incubator, and adults were allowed to lay eggs on the paper for 24 h.

From the control and treated combs, we cut 10 cm^2 sections of empty, dark comb where brood had been reared and placed the sections individually into 17 × 20 cm plastic bags. We placed a sheet of approx. 100 eggs (0-24 h old) into a shallow plastic cup on top of a single comb section, sealed the plastic bag with adhesive tape, and placed it into a 15 × 15 × 5 cm plastic container having a double-screen section in the lid for ventilation. This procedure was replicated 10 times for both treatments. The plastic containers were kept in the incubator at 32°C. Remaining combs were stored at 5°C for use in a second trial.

On 7 d, we counted the total number of eggs and the number of eggs that did not hatch. We calculated hatch rate (or proportion of hatched eggs) by dividing the number of hatched eggs by the total number of eggs. We removed the plastic bags and returned combs to the incubator. On 21 d, the comb section was scored for damage on a scale from 0-10, with 0 equal to no damage and 10 equal to complete damage. We counted the surviving wax moth larvae and removed them from the container. We returned combs to the incubator and repeated the process of counting surviving larvae approx. every 7 d, 4 additional times. The proportion of larvae surviving from egg was calculated by dividing the total number of surviving larvae collected over the trial by the number of hatched eggs. We performed a second trial using the same methods and timeline as trial one.

Statistical analysis. Arcsin square root transformations were performed on values for hatch rate and proportion of surviving larvae. However, we use untransformed means for purposes of reporting. All response variables were analyzed for treatment (B401 comb or untreated comb) and trial effects, with interactions, with the general linear models procedure (SAS Institute 2002-2003). The main effects trial and treatment were tested against their interaction term. Where necessary, means were compared using Tukey's test.

Results and Discussion

For hatch rate, there were no significant effects of treatment ($F = 12.37$; df = 1, 1; $P = 0.1763$), trial ($F = 6.75$; df = 1, 1; $P = 0.2339$), or trial × treatment ($F = 0.29$; df = 1, 36; $P = 0.5918$). The proportion of hatched eggs for the B401-treated and control treatments were similar (Table 1). Because the product must be ingested by larvae to be effective, we did not expect to observe differences in hatch rates between the treatments.

There was a significant trial × treatment effect on the comb damage score ($F = 15.50$; df = 1, 36; $P = 0.0004$); therefore, we analyzed treatment by trial. There were

no overall effects of treatment ($F = 8.49$; df = 1, 1; $P = 0.2104$) or trial ($F = 2.94$; df = 1, 1; $P = 0.3362$). For trial one, there was a significant treatment effect ($F = 181.05$; df = 1, 18; $P < 0.0001$). The average damage score for the control combs was significantly higher than that of the B401-treated combs (Table 1, Fig. 1). For trial two, there also was a significant treatment effect ($F = 21.12$; df = 1, 18; $P = 0.0002$). Again, the average damage score for the control combs was significantly higher than that of the B401-treated combs (Table 1). Hence, the trial × treatment interaction was a result of magnitude differences between the B401-treated and the control in the two trials.

Treatment ($F = 8{,}220.15$; df = 1, 1; $P = 0.0070$) and trial ($F = 1075.12$; df = 1, 1; $P = 0.0194$) significantly affected the proportion of surviving larvae whereas trial × treatment ($F = 0.00$; df = 1, 36; $P = 0.9678$) did not. For each trial, the proportion of surviving larvae for the control treatment was significantly greater than that for the B401-treated group (Table 1).

B401 significantly reduced the proportion of surviving larvae, but it did not provide complete control. The mortality rate was only about 30%. Some requirements for *Bt* to work effectively are that the insect must be at a susceptible stage of development and the bacterium must be ingested in sufficient quantity (Hoffman and Frodsham 1993). The first requirement was met by our study by starting with eggs on the combs. Ali et al. (1973) found that larval susceptibility to *Bt* treated foundation was greatest for early instars. Because wax moth infestations begin with female adults laying eggs, it is most important to control the first instar of larval development. Whether the second requirement was met is unknown. If less than a lethal dose of *Bt* is eaten, then growth may be retarded (Hoffman and Frodsham 1993). This may have occurred in our study. We continued to collect surviving larvae throughout the 6 wk period in both trials and observed that the larvae were smaller in the B401-treated combs, although this observation was not quantified.

Burges (1977) also observed similar problems with applying other formulations of *Bt* to the midrib of the foundation and noted the following: (1) the concentration of *Bt* possibly was diluted by addition of wax by adult bees and by accumulation of cocoon material from bee larvae, (2) the last portion of the comb to be consumed was often the midrib of foundation, and (3) sometimes larvae did not die until they were large, by which time they had caused severe damage. We also observed that larvae preferred to consume the drawn comb portion first and only tunneled through the midrib portion

Table 1. Results from B401® wax moth control study. Values are mean ± SE (*n*). Columnar means with different letters are different at $\alpha \leq 0.05$. Means were compared using Tukey's test

	Comb damage (Score 0 = no damage to 10 = 100% damage)		Proportion of hatched eggs	Proportion of surviving larvae during trial
Treatment	Trial One	Trial Two	Overall	Overall
B401-Treated	1.70 ± 0.39 (10) a	0.45 ± 0.16 (10) a	0.91 ± 0.16 (20) a	0.69 ± 0.07 (20) a
Control	8.55 ± 0.32 (10) b	3.80 ± 0.71 (10) b	0.83 ± 0.03 (20) a	0.95 ± 0.04 (20) b

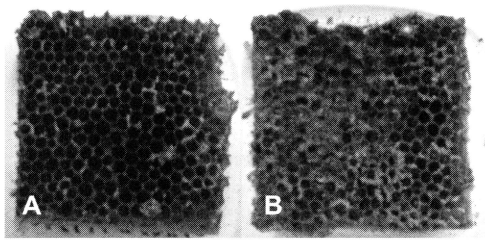

Fig. 1. Examples of wax moth damage to B401-treated comb (A) and control comb (B).

to move from one side to the other. It could be possible that larval feeding on the midrib is minimal and not enough bacteria were consumed. Consequently, the product's efficacy might be enhanced if applied on the foundation surface as well.

Currently, as used in our study, the product did not provide total control of wax moth damage to combs. Our data suggest that at the recommended application and dilution rates, it would not be profitable to invest in pretreated foundation for use in wax moth control. In order for B401 applied to the midrib of foundation to be an effective product, the concentration and application rates and methods should be adjusted to increase the concentration of *Bt*. Also observations on the larval feeding behavior may be helpful to determine if it is possible for the larvae to be exposed to a sufficient amount of the product. B401 reduced comb damage and larval survival; therefore, it is possible that, with adjustments and future testing, a method of midrib application could be successfully developed.

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