Interaction between *Beauveria bassiana* and *Bacillus thuringiensis* var. *israelensis* for the control of house fly larvae and adults in poultry houses

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ABSTRACT Field trials were carried out in the poultry houses to determine if a spray formulation of *Beauveria bassiana* (Balsamo) Vuillemin isolate R444 would enhance the control potential of *Bacillus thuringiensis* var. *israelensis* (*Bti*) applied in chicken feed as a larvicide against house fly larvae and the emergence of adult house flies. The trials compared larval mortalities and adult house fly emergence that resulted from the *Bti* plus *B. bassiana* treatments with those resulting from a commercial larvicide, Larvadex, plus *B. bassiana* treatments. All treatments significantly reduced the house fly larval densities and adult house fly emergence compared with any of the agents acting alone. After 6 wk of application, house fly larvae decreased by 11% as a result of *B. bassiana* treatment alone, 41% for 250 mg·kg⁻¹ *Bti* alone, and 42% for 500 mg·kg⁻¹ *Bti* alone. Larval mortalities as a result of the combination treatments were 45 and 52% as a result of 250 mg·kg⁻¹ *Bti* plus *B. bassiana* and 500 mg·kg⁻¹ *Bti* plus *B. bassiana* treatments respectively. House fly larval mortalities as a result of Larvadex and *B. bassiana* were 30% for Larvadex alone and 38% as a result of Larvadex plus *B. bassiana*. The *Bti* treatments were more effective at inhibiting the emergence of adult house flies than Larvadex, even when Larvadex was applied together with *B. bassiana*. The interaction effects of *Bti* plus *B. bassiana* and Larvadex plus *B. bassiana* were additive. These trials suggested that in the control of house fly larvae, the efficacy of *Bti*, applied as a larvicide, may be improved with frequent spray applications of *B. bassiana* to the chicken manure.

INTRODUCTION

The house fly, *Musca domestica* L., is a key pest of poultry facilities and a vector of many metaxenic pathogens and can cause serious sanitary problems because of its high reproductive potential, feeding habits, and ability to disperse. Organic wastes from intensive poultry farms provide excellent habitats for the growth and development of this insect (Thomas and Skoda 1993). Control recommendations for the house fly are currently limited to use of chemical insecticides to kill the house fly adults and larvae. Due to the problems associated with the development of pesticide resistance by the house fly (Keiding, 1999; Scott et al., 2000; Kaufman et al., 2001), as well as other environmental and regulatory concerns, research toward developing alternative control strategies is warranted. *Beauveria bassiana* (Balsamo) Vuillemin and *Bacillus thuringiensis* Berliner (*Bt*), which occur naturally as pathogens of *M. domestica*, are some of the potential alternatives. *Bacillus thuringiensis* has been found to be toxic to the house fly (Hodgman et al., 1993). Several natural isolates of *Bt* have also been found that are active against larvae of the house fly (Johnson et al., 1998). Thuringiensin-containing preparations have been used to control larvae of *M. domestica* (Mullens et al., 1988; Mullens and Rodriguez, 1988). It has also been reported by Carlberg et al. (1991) that nuisance flies in cattle sheds, slaughter houses, and latrines could be successfully controlled by applying *Bt* var *thuringiensis* to larval breeding sites.

The entomopathogenic fungus, *B. bassiana*, is a ubiquitous and important entomopathogen of several insect pests (Feng et al., 1994; Inglis et al., 2001; Lacey et al., 2001) and can be used effectively to suppress house fly populations. One approach to controlling house flies with *B. bassiana* would be to target the adult house flies as they fly around the poultry houses or rest on the...
MATERIALS AND METHODS

Biopesticides

The Bti bran formulation and a wettable powder formulation of B. bassiana R444 were obtained from Plant Health Products (Pty) Ltd. (KwaZulu-Natal, South Africa). The Larvadex-treated commercial feed was obtained from the University Research Farm at Ukulinga, Pietermaritzburg, KwaZulu-Natal, South Africa.

Layers and Housing

For field evaluation experiments, 80 hens that were 51-wk-old commercial-type layers (Hy-Line Brown) were obtained from the University Research Farm. Layers were evaluated upon receipt for signs of disease or other complications that could affect the outcome of the study. After examination, the layers were randomly allocated into 80 hanging pens (440 × 420 × 610 mm) in 6 chambers. Four chambers had 10 birds each, whereas 2 chambers had 20 birds each. Pens were separated by wire mesh on all sides. Each pen was equipped with a removable feed trough and a nipple drinker. Each pen had a removable tray for individual manure collection. Layers were housed in environmentally friendly chambers fitted with fans and 16L:8D at the Poultry Section, University Research Farm.

Diet Preparation for Layers and Administration

The Bti-formulated bran was mixed into the layer mash [Nutrex, Nutrex KZN (Pty) Ltd., KwaZulu-Natal, South Africa] in varying quantities either of 250 or 500 mg·kg⁻¹ of layer mash, Larvadex-treated feed contained 0.5% of Larvadex·kg⁻¹ of layer mash. The controls did not contain any Larvadex or Bti-formulated bran. The rations were stored in trashcans lined with plastic bags until fed to the chickens. There were 10 birds per treatment. Feed and drinking water were provided ad libitum. Layers were fed their respective dietary treatments for 6 wk. Additionally, 3 chambers were treated with a wettable powder formulation of B. bassiana R444 isolate [1 g of powder (10⁸) in 1 L of water] at a rate of 250 mL per chamber (27m⁻³) once a week. Applications were made using a Dyna-Fog Cyclone Ultra-Flex ULV sprayer (Curtis Dyna-Fog Ltd., Westfield, IN). Posttreatment larval densities were monitored after every 7 d. Data were analyzed to measure reduction in the numbers of house fly larvae in comparison with the untreated controls.

Fly Larvae Sampling

Excreta from each bird was collected every 7 d. Sampling of house fly larvae began 1 wk after administering the different feed to give the layers time to adjust to the
different treatments. House fly larvae were monitored using a bulb planter (400 mL) to collect 2 manure cores from each tray/bird per diet. Larvae were extracted in Berlese funnels and enumerated. Manure was allowed to accumulate in each tray until the end of the trials. Larvae were maintained for 2 wk at 25°C in the treated or untreated manure until adult house fly emergence and numbers were recorded. Adult house fly emergence rate was then calculated taking into account the number of exposed larvae.

**Statistical Analysis**

Percentage mortality of larvae in the treated trays was calculated and corrected to the reduction, if any, in the control plots using the formula of Mulla et al. (1971) as given below:

\[
\% \text{Mortality} = \frac{C_1 - C_2}{C_1} \times 100,
\]

where \(C_1\) is abundance of larvae in untreated manure and \(C_2\) is abundance of larvae in treated manure.

Adult house fly emergence inhibition was calculated using the following formula, on the basis of determining adult house fly emergence from the number of larvae that were obtained:

\[
\% \text{Inhibition of adult house fly emergence} = \frac{C - T}{C} \times 100,
\]

where \(C\) is percentage of adult house flies emerging from the control manure and \(T\) is percentage of adult house flies emerging from treated manure.

The percentage values were normalized by square-root arcsine transformation. This data is presented in the tables together with the untransformed means and were used in statistical analysis. Treatments were also compared based on these transformed values. Repeated measures ANOVA were conducted using the GENSTAT statistical package (VSN International, Hemel Hempstead, UK). Analyses included nominal variables representing application or no application of Bti and B. bassiana or Larvadex and B. bassiana applications. The LSD method was used to find significant differences between the means of the treatments.

To determine the effects of Bti-B. bassiana and Larvadex-B. bassiana treatments, the observed mortality rates were compared with the expected mortality rates under the assumptions of an independent effect. The expected mortality (\(BB_E\)) for the Bti-B. bassiana treatment was calculated as follows:

\[
BB_E = B_T + B_B (1 - B_T),
\]

where \(B_T\) and \(B_B\) are the observed proportional mortalities caused by Bti and B. bassiana alone, respectively. Similarly, the expected mortality (\(BL_E\)) for the Larvadex plus B. bassiana combinations was calculated by:

\[
BL_E = B_L + B_B (1 - B_L),
\]

where \(B_L\) and \(B_B\) are the observed proportional mortalities caused by Larvadex and B. bassiana alone, respectively.

Results from an \(\chi^2\) test were compared with the \(\chi^2\) table values (\(df = 1, P \leq 0.05\)).

\[
\chi^2 = (O - E)^2 / E,
\]

where \(O\) is the observed mortality for either of the Bti doses plus B. bassiana or Larvadex plus B. bassiana treatments and \(E\) is the expected mortality for either of the Bti doses plus B. bassiana or Larvadex plus Beauveria treatments. Additivity would be indicated if \(\chi^2 < 3.84\). If \(\chi^2 > 3.84\), there would be reason to suspect that the interaction was nonadditive (i.e., synergistic or antagonistic between the 2 agents; Naylor, 1964). If \(O < E\), the interaction would be considered antagonistic. Synergism would be indicated if \(O > E\).

**RESULTS**

Larval mortalities were significantly affected by the applications of Bti and B. bassiana treatments (\(F = 48.99; P < 0.001\), time (\(F = 45.37; P < 0.001\)), and the treatment \(\times\) time interaction (\(F = 13.71; P < 0.001\)). The 3 factors, treatment (\(F = 84.88; P < 0.001\), time (\(F = 278.38; P < 0.001\)), and time \(\times\) treatment (\(F = 21.91; P < 0.001\)) were also significant when Larvadex plus B. bassiana were applied.

The effects of applying Bti alone and when applied together with B. bassiana spray applications are shown in Figure 1. Combinations of the 2 treatments resulted in higher larval control than either of the 2 treatments applied individually. However, there was no significant difference between the 250 mg·kg\(^{-1}\) and 500 mg·kg\(^{-1}\) feed when these were used alone. These treatments resulted in 41 and 42% larval mortality, respectively. The same treatments plus Larvadex resulted in significantly higher larval mortality than the 250 mg·kg\(^{-1}\) plus B. bassiana treatment (Figure 1). Larval mortalities as a result of the combined applications of 250 mg·kg\(^{-1}\) plus B. bassiana and 500 mg·kg\(^{-1}\) plus B. bassiana treatments were 45 and 52%, respectively (Figure 1). Use of B. bassiana alone resulted in low levels (11%) of larval control after 6 wk. Application of Larvadex together with B. bassiana spray applications resulted in significantly higher larval mortalities than either of the 2 treatments individu-
ally (Figure 1). After 6 wk of application, Larvadex plus Beauveria treatments resulted in >15% more larval mortality than Beauveria alone and 7% more than Larvadex alone (Figure 1).

Regression lines of mortalities of larvae obtained as a result of the Bti, B. bassiana, and Larvadex treatments applied individually or in combination are presented in Figure 1. Larval mortalities as a result of the Bti treatments plus B. bassiana achieved better control than mortalities obtained as a result of Larvadex alone or when integrated with B. bassiana (Figure 1). Larval mortalities obtained as a result of using B. bassiana alone were the lowest. The 250 mg·kg⁻¹ and 500 mg·kg⁻¹ Bti feed treatments were more effective (in terms of larval mortalities) than the Larvadex treatment integrated with B. bassiana spray applications (Figure 1).

Although addition of B. bassiana to both Bti and Larvadex resulted in significantly higher larval control than any of the agents acting alone, the interaction between Bti and B. bassiana and between Larvadex and B. bassiana was additive and was based on the 3 agents acting independently for the entire 6-wk trial (Table 1).

The emergence of house flies was significantly affected by Bti and B. bassiana treatments ($F = 194.39; P < 0.001$), time ($F = 193.87; P < 0.001$), and treatment × time ($F = 5.34; P < 0.001$). The 3 factors treatment ($F = 102.13; P < 0.001$), time ($F = 64.93; P < 0.001$), and treatment × time interaction ($F = 4.40; P < 0.001$) had significant effects on the emergence of adult house flies when Larvadex and B. bassiana were applied together or singly.

**Figure 1.** Percent mortality of larvae in manure sprayed with or without Beauveria bassiana (Bb) and layers fed with Larvadex (Lv), 250 mg·kg⁻¹ Bti (Bt1), or 500 mg·kg⁻¹ Bti (Bt2). Larvadex obtained from University Research Farm at Ukulinga, Pietermaritzburg, KwaZulu-Natal, South Africa. Bti = Bacillus thuringiensis var. israelensis.

**Table 1.** Effect of combining *Bacillus thuringiensis* var. *israelensis* (Bti) and Larvadex¹-formulated feeds integrated with spray applications of *Beauveria bassiana* on the mortality of *Musca domestica* larvae

<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Week</th>
<th>Observed (%)</th>
<th>Expected (%)</th>
<th>$\chi^2$</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt1 and B. bassiana</td>
<td>1</td>
<td>12.74</td>
<td>19.86</td>
<td>0.025</td>
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</tr>
<tr>
<td></td>
<td>2</td>
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<td>26.98</td>
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</tr>
<tr>
<td></td>
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<td>24.17</td>
<td>24.62</td>
<td>0.008</td>
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</tr>
<tr>
<td></td>
<td>4</td>
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<td>34.78</td>
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<td>Additive</td>
</tr>
<tr>
<td></td>
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<td>41.28</td>
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</tr>
<tr>
<td></td>
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<td>44.64</td>
<td>47.67</td>
<td>0.193</td>
<td>Additive</td>
</tr>
<tr>
<td>Bt2 and B. bassiana</td>
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<td>20.39</td>
<td>0.04</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>28.15</td>
<td>0.01</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>26.96</td>
<td>24.48</td>
<td>0.04</td>
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</tr>
<tr>
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<td>37.47</td>
<td>35.90</td>
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</tr>
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<td>44.73</td>
<td>0.001</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
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<td>51.54</td>
<td>48.45</td>
<td>0.197</td>
<td>Additive</td>
</tr>
<tr>
<td>Larvadex and B. bassiana</td>
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<td>19.11</td>
<td>0.04</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.34</td>
<td>26.27</td>
<td>0.01</td>
<td>Additive</td>
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<tr>
<td></td>
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<td>23.22</td>
<td>0.007</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>28.37</td>
<td>30.86</td>
<td>0.003</td>
<td>Additive</td>
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<td>32.71</td>
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<tr>
<td></td>
<td>6</td>
<td>37.91</td>
<td>37.99</td>
<td>1.68</td>
<td>Additive</td>
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</tbody>
</table>

¹Obtained from University Research Farm at Ukulinga, Pietermaritzburg, KwaZulu-Natal, South Africa.

²Bt1 = 250 mg·kg⁻¹ Bti; Bt2 = 500 mg·kg⁻¹ Bti.
The effects of applying Bti alone and when applied together with B. bassiana are shown in Figure 2. The Bti treatments whether applied individually or together with B. bassiana significantly inhibited the emergence of adult house flies (Figure 2). Application of B. bassiana alone inhibited the emergence of adult house flies by 24% after 6 wk of application. Combining 250 mg of Bti plus B. bassiana inhibited the emergence of adult house flies by approximately 30% more than B. bassiana alone and 6% more than Bti alone. The 500 mg·kg⁻¹ Bti feed application reduced the emergence of adult house flies by 55%. This was only 4% less than when the 500 mg·kg⁻¹ feed treatment was combined with B. bassiana spray application (Figure 2).

No significant differences were observed between the 250 mg·kg⁻¹ Bti feed application and 250 mg·kg⁻¹ plus B. bassiana treatment with respect to reducing adult house fly emergence for the first 3 wk of application. Similar observations were made between the 500 mg·kg⁻¹ Bti feed application and 500 mg·kg⁻¹ Bti plus B. bassiana spray application in the first 2 wk of application (Figure 2). However, significant differences were observed thereafter between these treatments.

Use of Larvadex alone produced no substantial reduction in adult house fly emergence during the first 3 wk of the study. However, Larvadex applied together with B. bassiana reduced adult house fly emergence by 48% after 6 wk (Figure 2), 10% more than Larvadex alone. The B. bassiana formulation applied alone had the lowest effect on the emergence of adult house flies (Figure 2). The Bti treatments were more effective than Larvadex even when Larvadex was applied together with spray applications of B. bassiana. The most effective combinations were Bti combined with B. bassiana spray applications (Figure 2).

The interactions between Bti and B. bassiana and between Larvadex and B. bassiana were additive effects during the entire study period (Table 2).

**DISCUSSION**

Addition of B. bassiana spray applications to Bti or Larvadex feed treatments offered some advantage to both agents in terms of larval mortality and inhibition of adult house fly emergence. Moreover, throughout the study, integrations of B. bassiana spray applications with either Bti or Larvadex feed treatments resulted in better larval control than either Bti or Larvadex acting individually. Furthermore, there was no evidence of antagonistic interaction between B. bassiana with Bti or Larvadex. On the other hand, B. bassiana on its own was not very effective in controlling larval populations or inhibiting adult house fly emergence. Our results confirm observations that weekly applications of B. bassiana are inadequate to achieve effective insect larval control in the field (Wright and Ramos, 2002, 2005). Moreover, the fact that time of exposure was a significant factor suggests that repeated applications would be more effective in controlling larval densities.

A few studies (Johnson et al., 1992; Lobo-Lima et al., 1992; Johnson and Goettel, 1993; Inglis et al., 1996) have also reported inconsistent results of B. bassiana with some insect hosts in the field, despite good control being obtained in the laboratory. In our earlier studies, B. bassiana isolate R444 sprays resulted in >90% fly mortality within 2 d in the laboratory.

The potential of B. bassiana in the field may be limited by high temperatures (Carruthers et al., 1985; Feron et al., 1991; Fargues et al., 1992; Vestergaard et al., 1995; Ekesi et al., 1999). Also, sunlight is known...
to rapidly inactivate B. bassiana conidia (Inglis et al., 1997b), and house flies are known to thermoregulate, raising their body temperatures above ambient level (Watson et al., 1993), which may enable them to rid themselves of infection by basking in sunlight (Inglis et al., 1997b).

The efficacy of B. bassiana could be enhanced by making applications in the evening (Delgado et al., 1999) because B. bassiana has been reported to achieve better control in cool temperatures (Inglis et al., 1997a), owing to the prolonged life of exposed conidia, conceivably leading to germination and cuticle penetration by a greater number of conidia per insect and a higher effective inoculum (Delgado et al., 1999). Late evening applications of B. bassiana would allow more time for house flies to become exposed to fungal conidia through feeding on or contact with treated shed walls, or both.

There have been numerous studies on the effect of combinations of microbial agents for insect control. Although the mechanism is not clearly understood, infections by more than 1 agent usually lead to an increase in mortality of the host population, especially when the 2 infections are spatially separated (Jacques and Morris, 1981). Our findings of an additive interaction between B. bassiana and Bti confirm the results of Sander and Cichy (1967), Lewis and Bing (1991), Costa et al. (2001), and Ma et al. (2008). This interaction may be the result of the different routes of infection by the 2 pathogens (Ma et al., 2008). In our study, B. bassiana was sprayed in the chicken houses to reduce the number of breeding adult flies, whereas Bti was ingested by the larvae through the chicken manure.

A few authors have reported synergistic interactions between Bt and B. bassiana. For instance, Lewis et al. (1996) reported that application of Bt to maize (Zea mays) enhanced the suppression of European corn borer by B. bassiana and Wraight and Ramos (2005) reported synergistic interactions between B. bassiana strain GHA and Bt tenebrionis when applied in combination against larval populations of the Colorado potato beetle. The results of Lewis et al. (1996) and Wraight and Ramos (2005) may have been as a result of Bt prolonging of the interval of time between molts, therefore providing B. bassiana more time to penetrate the cuticle before being shed off (Wraight and Ramos, 2005). Also, larvae feeding on B. bassiana may acquire infection via the gut (Ma et al., 2008). Miranpuri and Khachatourians (1991) found germinated B. bassiana conidia in the gut of larvae of the mosquito Aedes aegypti 24 to 48 h after exposure.

On the other hand, a study by Furlong and Groden (2003) suggested that starvation might increase the susceptibility of larvae to B. bassiana infection, by prolonging the intermolt period. This has been the suspected mechanism that would explain the increase in effect of Bt plus B. bassiana mixed treatments. However, it was also found that the insect growth regulator Larvadex increased intermolt period without significantly affecting susceptibility to the fungus (Furlong and Groden, 2001) and it was concluded that some unknown effect of starvation on host physiology was responsible for the change in susceptibility.

Despite not being able to understand the exact mechanism of Bti and B. bassiana interaction, this study has shown that enhancement of Bti efficacy by B. bassiana is possible and warrants further study to understand the effect of combining various dosage levels of each pathogen. Furthermore, microbial pesticides are costly and any strategies allowing for reductions in the dosage levels make them attractive to large-scale users. Beauveria bassiana is affected by environmental factors and takes a long time to act. However, with frequent evening applications, B. bassiana might accumulate in the manure or even infect enough house flies to start an epi-

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**Table 2. Effect of combining Bacillus thuringiensis var. israelensis (Bti) and Larvadex1-formulated feeds with spray applications of Beauveria bassiana on the emergence of Musca domestica adult flies**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week</th>
<th>Observed (%)</th>
<th>Expected (%)</th>
<th>χ²</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bt1 and B. bassiana</td>
<td>1</td>
<td>22.75</td>
<td>31.42</td>
<td>2.39</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>36.87</td>
<td>1.88</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>32.55</td>
<td>40.22</td>
<td>2.65</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>37.04</td>
<td>49.92</td>
<td>3.32</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>46.89</td>
<td>56.57</td>
<td>1.66</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>52.11</td>
<td>63.60</td>
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</tr>
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<td>47.89</td>
<td>60.40</td>
<td>2.50</td>
<td>Additive</td>
</tr>
</tbody>
</table>

1 Obtained from University Research Farm at Ukulinga, Pietermaritzburg, KwaZulu-Natal, South Africa.

2 Bt1 = 250 mg·kg⁻¹ Bti; Bt2 = 500 mg·kg⁻¹ Bti.
zootic. This would result in long-term control, whereas the short residual Bti would provide rapid short-term control of larvae (Wraith and Ramos, 2005).

ACKNOWLEDGMENTS

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