Evaluation of the *Bacillus thuringiensis* based insecticide in the control of Lepidopterous caterpillars on golf courses

C. Guerrero; D. Pereira; and L. Neto

Abstract— *Bacillus thuringiensis* was tested as a biological insecticide in turfgrass. A field experimental work was carried out at the Benamor Golf course, in the Algarve region, where it was evaluated the lepidopterous larvae biological control effectiveness of an EIBOL product, designated RET-BT.

Best results, were obtained using the highest biological pesticide concentration (2 $L.ha^{-1}$).

Keywords — *Bacillus thuringiensis*, biological insecticide, golf-courses, integrated management, *Noctuidae*

I. Introduction

Insect pests are a serious problem in turfgrass management. There are species which may easily controlled with chemical agents. Most of the insects live underground and are difficult to control because chemicals do not reach their target and are broken down by the microbial fauna in the thatch layer [8].

In some parts of the Western Europe there are restriction or completely omit the use of chemical pesticides [4]-[8].

Biological control with, such as entomopathogenic nematodes, fungi and bacteria, and/or cultural practices, such as topdressing with silica sand, use of turfgrass selected species or cultivars, may overcome some of pest problems [3]-5]-[8].

The most important pest species occurring in turfgrass areas, such as golf courses, football pitches, gardens, belong to Noctuidae, Scarabaeidae, and Tipulidae [3].

Biological insecticides, such as entomopathogenic nematodes and *Bacillus thuringiensis*, can be used only if the preventive measures are inadequate [3]-[6].

Bacillus thuringiensis insecticides (BT) have several advantages in relation to conventional synthetic insecticides, mostly when insects need to be controlled in areas where a high human traffic around treated sites can create a risk of exposure to pesticides. This is the case of golf courses where players are exposed to pesticides residues when walking across the fields. Also, for golf courses managers would be essential to have pesticides which could be applied immediately before a player crossed the course. Also, players and golf course managers increasingly demand significant reductions in pesticide use. Several studies showed that BT insecticides have no toxicity to mammals [7], and even toxic volatile agents are not released during or after a spraying operation [9] which means that a player can walk trough golf courses immediately after a spraying on the area. BT is highly pest specific and is generally non-injurious to beneficial insects, therefore is a very attractive pesticide to golf course pest management in an environmental sound.

These microorganisms are natural inhabitants of soils, but their presence on plant organs where insect feeds can be rare or even absent if the insect feeds above ground. So they must be applied artificially to efficiently control insects. Although there are numerous strains of *B. thurigiensis*, only some are actually commercialized. The most widely used strain - *B. thurigiensis*var. *kurstaki* is effective to most caterpillars but some Noctuidae insects are not susceptible to these insecticide [1].

Noctuidae are important pest of golf courses in south Portugal and environmental sound strategies that efficiently control these pests are needed. A new BT based pesticide, trade name RET-BT (*Bacillus thuringiensis* var. *kurstaki*, Strain E-144, 16 million UI/mg, SC) was developed by EIBOL for the control of caterpillars. The objective of this study is to investigate the efficacy of this insecticide on the control of caterpillars in golf courses and the effect of the dose on its efficacy.

II. Material and Methods

The study was carried out in a 320 m^2 of a putting course of Benamor Golf course (Algarve, south Portugal). The spray schedule follow the practices usually carried out on golf by greenkeepers in Algarve courses.

The studied area was divided in 16 experimental units (4 x 5 m^2) and 4 treatments were carried out on this area.

1 – Control (water);

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2 – BTK 1 (RET-BT: concentration, 0.4 ml/L; 1.0 L of formulation/ha);
3 – BTK 1.5 (RET-BT: concentration, 0.6 ml/L; 1.5L of formulation /ha);
4 – BTK 2 (RET-BT**:** concentration, 0.8 ml/L; 2 L of formulation/ha).
RET-BT (Insecticide with *Bacillus thuringiensis* var. *kurstaki*, 16000 UI/mg, SC from EIBOL). Treatments were applied at 2500 $L.ha^{-1}$ to ensure pesticide penetration on canopy of turf and mixed with an acidifying agent (FIT-FAI, an EIBOL product) to lower the pH until 5,5-6, due to the high pH of water. Treatments were applied mixing a wetting agent at 100 $mL.5L^{-1}$ spray mixture. After each spray a 5 minutes irrigation was carried out. Treatments allocation to experimental units followed a completely randomised block design with four replications (Fig. 1). Spray applications were carried out at four dates - 24/06/06, 01/07/06, 08/07/06 and 18/07/06 with a hand sprayer.

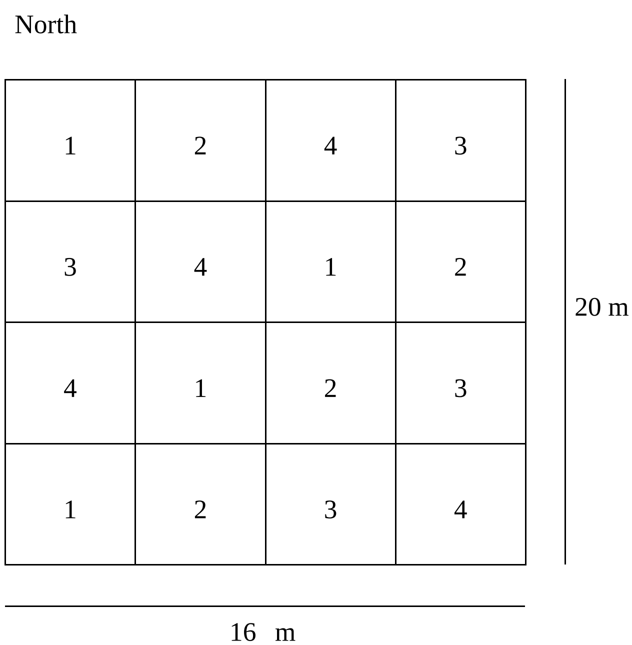

Fig. 1 – Treatments allocation to experimental units; 1-4: treatments: see text for description

To evaluate the relative efficacy of each treatment larvae of Noctuidae were sampled using the irritant drench technique [2] with 50 mL of liquid dishwashing detergent in 5L of water on a $1m^2$ area of each experimental unit and the number of medium to large-sized caterpillars appearing at the turfgrass surface within 5 minutes were counted. The numbers of larvae were compared between treatments using one-way analysis of variance (ANOVA), followed by multiple comparison Duncan's post test. Differences were considered significant when $p<0.05$. Normality of sample distribution and homogeneity of variances were verified before ANOVA [10]. Data was standardized by the logarithmic transformation.
Sampling was carried out at 5 dates: 08/07/06, 13/07/06, 15/07/06, 18/07/06 and 20/07/06 and the five sampling total was used on the statistical tests.

III. Results

The average number of larvae trapped on each treatment during the five sampling operations is presented on Fig. 2. The first larvae appeared on the 13^{th} July, mainly in plots were no insecticide has been applied (not shown). This result shows that 3 applications were carried out preventively. This fact has implications on the amount of pesticides that are sprayed on golf courses. A good sampling program would permit greenkeepers to decide when the best time to spray with BT pesticides reducing costs of control programs.

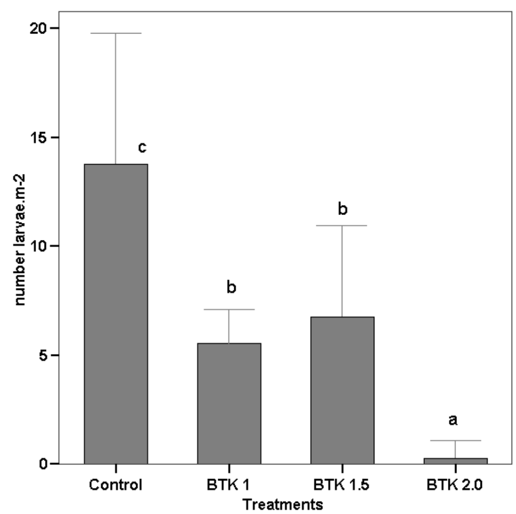

Fig. 2 – Average number of larvae of *Noctuidae* on 1 m^2 for each treatment. Averages with the same letter do not present significant differences at 95% (multi average comparison - Duncan test)

Control plots had significantly more larvae than insecticide treatments; BT insecticides are therefore efficacious on controlling Noctuidae larvae on turfgrass. This is important when greenkeeper needs to reduce the amount of synthetic insecticides for environmental reasons or public concern.

The treatment with RET-BT with 2,0 $L.ha^{-1}$ of spray mixture showed the best control action. This result shows that the amount of the spray mixture applied is crucial on BT effectiveness against soil born insects. As the insecticide must be ingested by insect caterpillars to be effective, a spray application must guarantee that the plant organs on which the insects feeds are covered by the bacteria. Therefore, if the insect feed on grass roots the application must ensure the adequate penetration of the insecticide. This result also shows the importance of a correct identification of the insects involved on the damage of turfgrass and its living habits in order to decide which spray doses are more adequate to the pest.

IV. Conclusions

These results show that BT insecticides, mainly *Bacillus thuringiensis* var. *kurstaki* can be an important alternative to synthetic pesticides when managing Noctuidae pests on golf courses. Curculionidae (curculionidae larvae) and Gryllotalpidae (mole crickets) can already be controlled by entomopathogenic nematodes [8]. Biological insecticides have low mammalian and environmental toxicity which are important characteristics when managing golf courses in an environmental sound approach. However, to be effective, it is necessary bear in mind that several factors can affect the performance of these pesticides. First, the time of application is crucial since to some insect pests, only the first and second larval instars are susceptible. To achieve the best application time a good sampling program is required and some phenological models can be either very useful. Results with *Bacillus thuringiensis* were strongly influenced by the larval stage and concentration. Against early instars in autumn between 74 and 83% control was achieved with 13 kg ha^{-1} *Bacillus thuringiensis* of 5,700 International Toxic Units (ITUs) and 20 kg ha^{-1} of 3,000 ITUs [6]. Applications in spring against third and fourth instars achieved between 0 and 32% reduction [6]. Second, the dose of pesticide mixture to apply must ensure the correct covered of the plant organs attacked and must be choose according the insect pest; therefore the correct identification of the insect is crucial. Third, several cultural practices, such as topdressing with silica sand, can help in the reduction of earthworms and cutworms [5].

The correct management of these variables will ensure an efficacy in pesticide application and will permit to reduce the number of applications, with obvious economic and environmental advantages.

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