

## UTILISATION OF THE GREEN MUSCARDINE, *METARHIZIUM ANISOPLIAE*, TO CONTROL THE SUGARCANE LONGHORN STEM BORER *DORYSTHENES BUQUETI* GUERIN (COLEOPTERA: CERAMBYCIDAE)

By

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### Abstract

THE GREEN muscardine, *Metarhizium anisopliae* is used to control the sugarcane longhorn stem borer, *Dorysthenes buqueti*, in infested areas of sugarcane fields in Thailand. Fields are treated by dispersing a fresh culture of *M. anisopliae* colonising cooked rice ( $2.7 \times 10^8$  spores/g). Single applications of *M. anisopliae* at the rate of 80 kg/ha were made in three locations at Suphanburi and Kanchanaburi Province in 2008. The effect of green muscardine on the larvae of longhorn borer as well as on sugarcane production was evaluated. The results revealed that, after applying the fungi, the green muscardine killed on average  $35.09 \pm 8.68\%$  of the borer larvae in all treated plots and  $4.49 \pm 1.64\%$  in all non-treated plots. The highest average percent of infested larvae was  $41.67 \pm 36.03\%$  at Rang Sa Ri district, Kanchanaburi Province. The sugarcane production averaged  $135.61 \pm 4.67$  t/ha in treated plots while, in non-treated plots, yields averaged  $72.11 \pm 10.51$  t/ha. These findings indicate that the *M. anisopliae* was an effective biological control agent in controlling sugarcane longhorn stem borer in Thailand.

### Introduction

The sugarcane longhorn stem borer, *Dorysthenes buqueti* (Coleoptera: Cerambycidae), is considered as one of the most serious insect pests of sugarcane in many areas of sugarcane production in Thailand (Charernsom and Suasaard, 1994). Infestations frequently cause economic losses. In 1998, in some areas of the North-Eastern and the Eastern region, 100% of the sugarcane fields surveyed were infested by *D. buqueti*. Outbreaks have now spread to all sugarcane growing areas including the lower Northern and the Central regions of Thailand. Sommartya *et al.* (2007) reported that all entomopathogenic fungi infecting *D. buqueti* larvae collected from sugarcane fields throughout Thailand were identified as *Metarhizium anisopliae* var. *anisopliae*, Family Monilaceae, Order Moniliales, and Class Deuteromycetes. *M. anisopliae* is considered the most effective natural enemy to control *D. buqueti*. It infects all stages of *D. buqueti* killing 100% of infected larvae in the laboratory 14 days after inoculation with a suspension of  $1 \times 10^7$  conidia per mL. In the greenhouse, 80–100% of larvae were killed within 20 days after application. Attempts to utilise *M. anisopliae* as a biological control agent for controlling insect pests was first recorded in 1879 (Cloy, 1999) and is utilised in the field in many countries such as Trinidad, Grenada, Mexico, Guatemala and Canada (Cloy, 1999; Hernandez-Velazquez *et al.*, 2003; Kabaluk and Ericsson, 2007).

The objective of this study was to evaluate the utilisation of *M. anisopliae* var. *anisopliae* as a biological control agent of sugarcane longhorn stem borer, *D. buqueti* under field conditions in Thailand.

## Materials and methods

### Mass production of *M. anisopliae*

*D. buqueti* infected larvae were collected from sugarcane fields from several locations and the pathogen was isolated and identified. A pure culture of *M. anisopliae* was used for initial stock culture. Mass production of *M. anisopliae* was carried out by colonisation of cooked rice at the National Biological Control Research Center (NBCRC) Central Regional Center, Kamphaeng Saen, Nakhon Pathom.

About 3000 kg of fresh culture on cooked rice were produced monthly for application and evaluation in the fields.

### Field application and assessment

Three test sites each were established in the Rang Ngoen, Pho Ngoen, Suphanburi Province and Rang Sa Ri Districts of Kanchanaburi Province in the central region of Thailand. *D. buqueti* is a serious pest in these areas.

At each site, 2 plots (ca.10 ha) were selected for treated and non-treated (control) *M. anisopliae* plots. Approximately 80 kg of fresh culture of *M. anisopliae* mixed with 1000 kg of biofertiliser (filter cake) was applied per hectare by spreading into the row at planting (Figure 1). Water was applied as furrow irrigation in every plot.



Fig. 1—Application of the green muscardine, *Metarhizium anisopliae*.

Field assessments were made at two week intervals beginning one month after planting and until harvest of sugarcane. The total number of *D. buqueti* larvae, the number of infected larvae, and the number of infested stalks in one stool were counted and recorded as one sample. Fifty samples were done in each plot.

The yield of sugarcane in each plot was evaluated. These data were used for evaluating the success of *M. anisopliae* to control *D. buqueti*.

## Results and discussion

### Field assessment of utilization of *M. anisopliae*

Comparisons between percent infested larvae of *D. buqueti* by *M. anisopliae*, percent infested sugarcane stalks by *D. buqueti* and yield of sugarcane in treated and non-treated plots were indexed to evaluate the success in utilizing *M. anisopliae*.

The efficacy of *M. anisopliae* in controlling *D. buqueti* was obvious when the population of *D. buqueti* was evaluated. The population dynamics of *D. buqueti* in treated plots were of a similar pattern at every location.

Populations decreased one month after application of *M. anisopliae* especially in Rang Ngoen and Rang Sa Ri while the population in non-treated plots increased. It was apparent that the population of *D. buqueti* in treated plots was lower than those in nontreated plots at every location as shown in Figure 2.

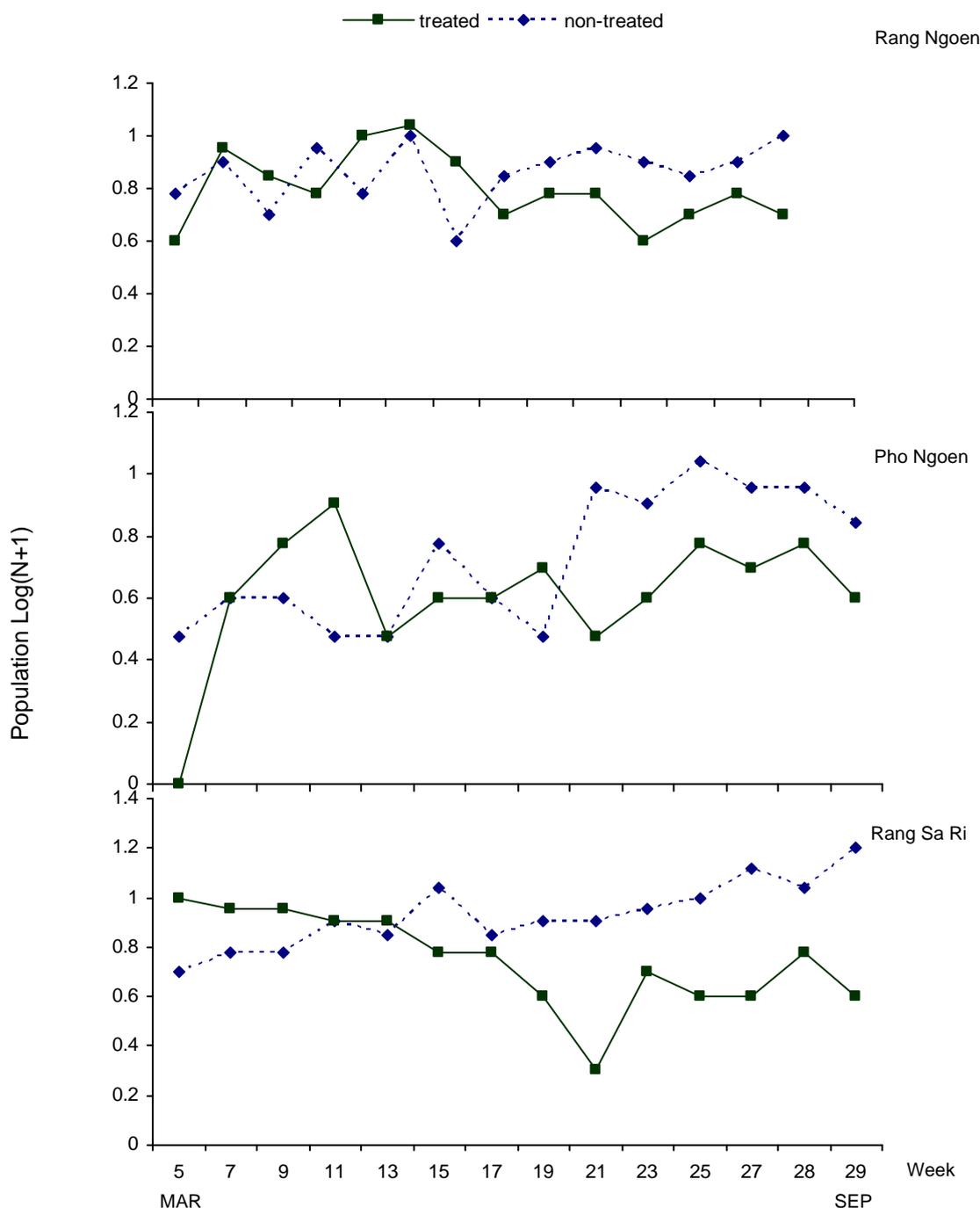


Fig. 2—Population of sugarcane longhorn stem borer, *Dorysthenes buqueti* in the treated and non-treated plots of the green muscardine, *Metarhizium anisopliae* plots at Rang Ngoen, Pho Ngoen and Rang Sa Ri districts in 2008.

The percent infested larvae of *D. buqueti* in treated and non-treated plots at every location were evaluated and results are illustrated in Figure 3.

The figure shows that the percent of infested larvae in treated plots was clearly higher than those in non-treated plots during the investigation period March to September 2008 at every location. Infested larvae of *D. buqueti* in treated plots increased after application of *M. anisopliae*, while the infested larvae in non-treated plots did not increase. The highest percent infested larvae was 100% in treated plots at Rang Sa Ri district, Kanchanaburi Province.

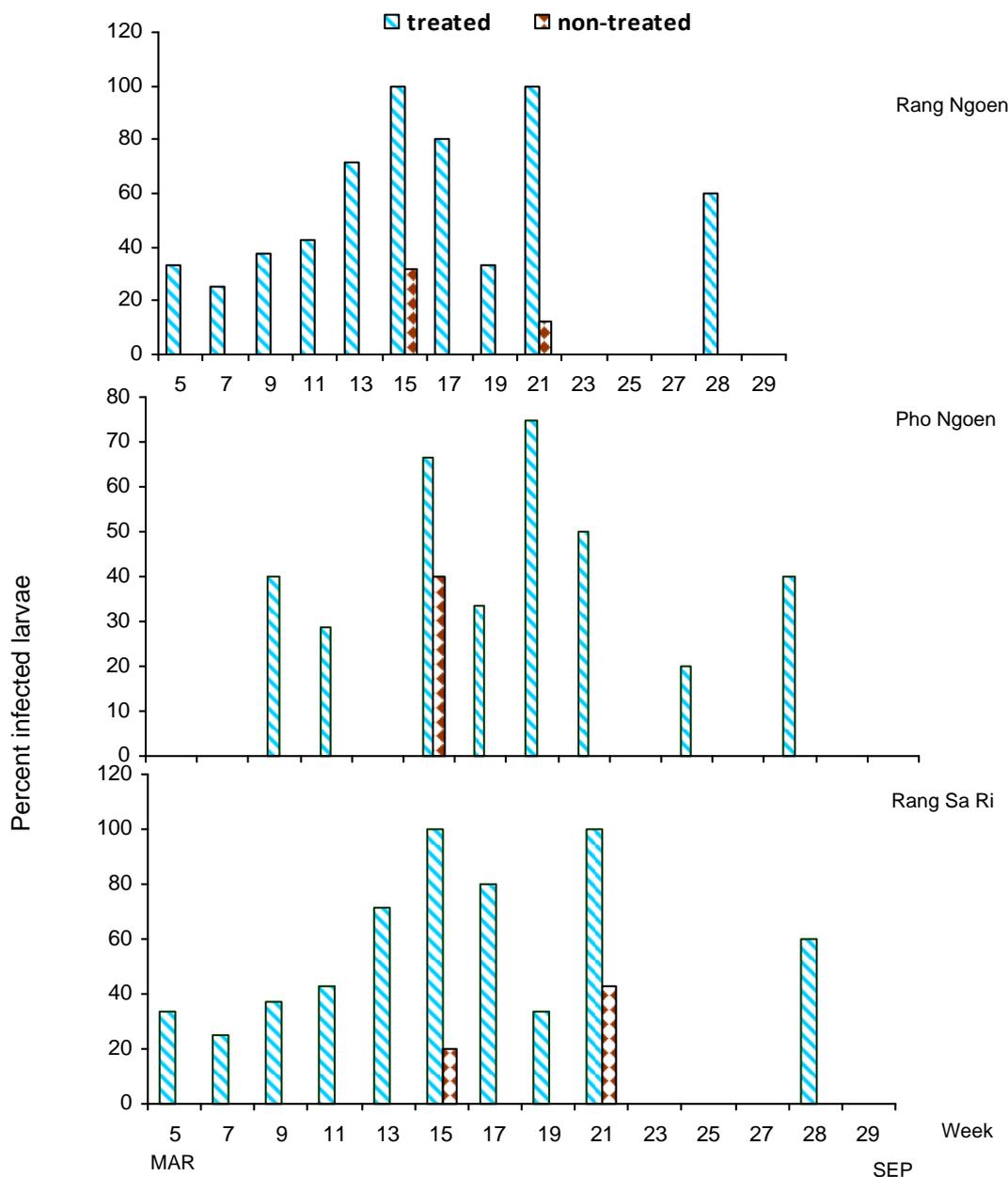


Fig.3—Percent infected larvae of sugarcane longhorn stem borer, *Dorysthenes buqueti* by the green mascardine, *Metarhizium anisopliae* in treated and non- treated plots at Rang Ngoen, Pho Ngoen and Rang Sa Ri districts in 2008.

The average percent infested larvae of *D. buqueti* in treated/non-treated plots at Rang Ngoen and Pho Ngoen, Suphanburi Province were 38.63/6.13 and 25.25/2.86.

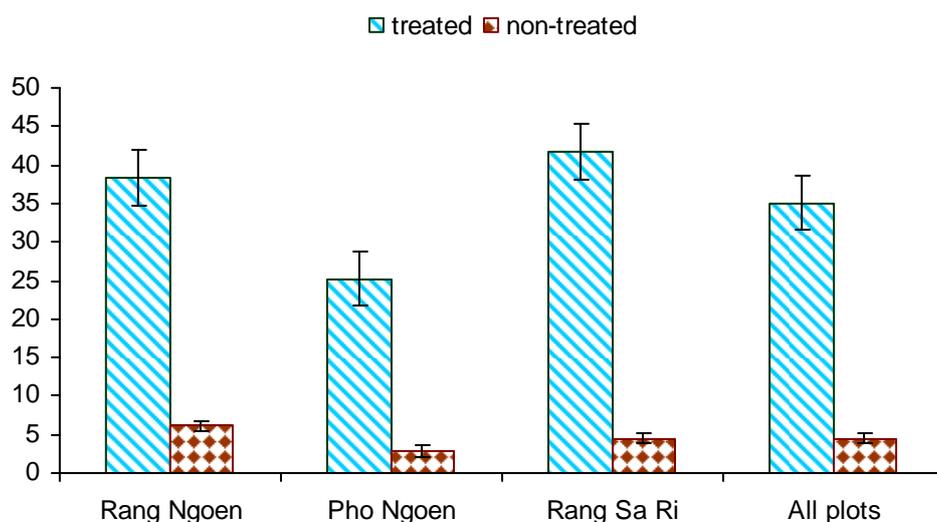
In Rang Sa Ri, Kanchanaburi Province these values were 41.67 and 4.49 for treated and non-treated plots respectively.

The average percent infested larvae in treated plots was significantly different from those in non-treated plots at every location as shown in Table 1 and Figure 4. *M. anisopliae* killed on average 35.09±8.68% of the larvae of *D. buqueti* in all treated plots and 4.49±1.64% in all non-treated plots.

**Table 1**—Average percent infected larvae of sugarcane longhorn stem borer, *Dorysthenes buqueti* by the green mascardine, *Metarhizium anisopliae* in treated and non-treated plots at Rang Ngoen, Pho Ngoen and Rang Sa Ri districts in 2008.

Location	Average infected larvae by green mascardine (%)	Range
Rang Ngoen		
treated	38.36±31.74 <sup>a</sup>	0–80.00
non- treated	6.13±13.42 <sup>b</sup>	0–40.00
Pho Ngoen		
treated	25.25±26.55 <sup>a</sup>	0–75.00
non- treated	2.86±10.69 <sup>b</sup>	0–40.00
Rang Sa Ri		
treated	41.67±36.03 <sup>a</sup>	0–100
non-treated	4.49±12.26 <sup>b</sup>	0–42.85
All plots		
treated	35.09±8.68	
non- treated	4.49±1.64	

Means followed by different letter were significantly different at the 95% probability level.



**Fig. 4**—Average percent infected larvae of sugarcane longhorn stem borer *Dorysthenes buqueti* by the green mascardine, *Metarhizium anisopliae* in treated and non-treated plots at Rang Ngoen, Pho Ngoen, Rang Sa Ri districts.in 2008

Yield evaluation from treated and non-treated plots confirms the success of *M. anisopliae* in controlling *D. buqueti*. The yields of sugarcane in treated and non-treated plots from all locations are shown in Table 2. It clear that the yield in treated plots was higher than those in non-treated plots and that these differences were significant at every location. The average yield in all treated plots was 135.6 t/ha while, in all non treated plots, was 72.1 t/ha. The highest yield was 146.3 t/ha at Pho Ngoen district, Suphanburi Province as illustrated in Figure 5. Although the average yields in all treated plots were higher than in non-trerated plots, these were still lower than the average yield in the areas not damaged by *D. buqueti*. These findings suggest that further studies in the development of formulations and application of *M. anisopliae*, including identifying important environmental factors affecting the system, are necessary.

**Table 2**—The yield of sugarcane in treated and non-treated plots at Rang Ngoen, Pho Ngoen and Rang Sa Ri districts in 2008.

Location	Yield (t/ha)	Range (t/ha)
Rang Ngoen		
treated	137.8±12.2 <sup>a</sup>	126.9–144.9
non- treated	83.6±9.9 <sup>b</sup>	72.4–98.8
Pho Ngoen		
treated	138.8±8.4	130.6–146.3
non- treated	69.7±7.2 <sup>b</sup>	62.4–78.1
Rang Sa Ri		
treated	130.3±17.8 <sup>a</sup>	115.6–141.3
non- treated	63.0±6.7 <sup>b</sup>	63.1–70.6
All plots		
treated	135.6±4.7	
non- treated	72.1±10.5	

Means followed by different letter were significantly different at the 95% probability level.

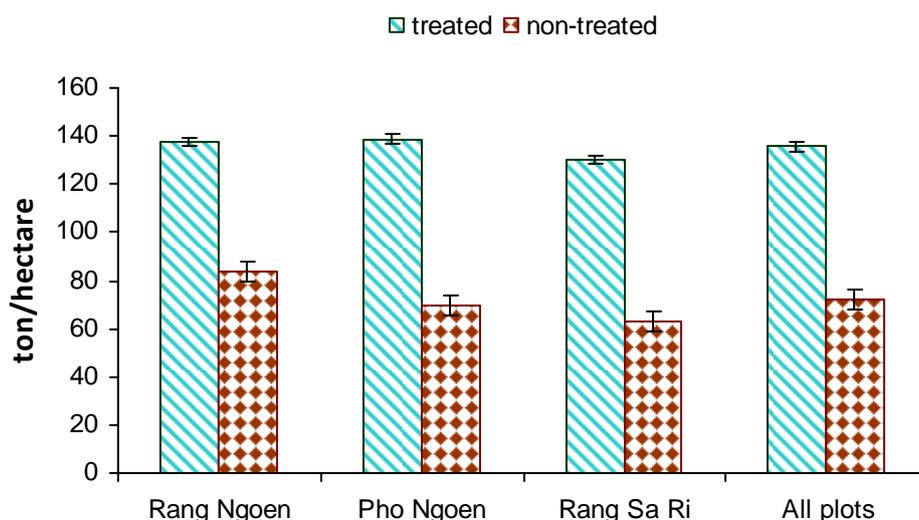


Fig. 5—Yield of sugarcane in treated and non-treated plots at Rang Ngoen, Pho Ngoen and Rang Sa Ri districts and average yield in all treated and all non-treated plots in every location in 2008.

## Conclusions

The application of 80 kg fresh culture of the green muscardine, *M. anisopliae*, mixed with 1000 kg of biofertiliser per hectare during planting of sugarcane will provide effective control of the sugarcane longhorn stem borer, *D. buqueti*. The high numbers of infected larvae of *D. buqueti* and the higher yields of sugarcane in treated plots than in non-treated plots show the efficiency of *M. anisopliae* in controlling *D. buqueti* in Thailand sugarcane fields.

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**UTILISATION DE LA MUSCARDINE VERTE, *METARHIZIUM ANISOPLIAE*, DANS LA LUTTE CONTRE LE LONGICORNE FOREUR DE TIGE DE LA CANNE À SUCRE *DORYSTHENES BUQUETI* GUERIN (COLEOPTERA: CERAMBYCIDAE)**

Par

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**MOTS-CLÉS: Lutte Biologique, *Metarhizium anisopliae*, Entomopathogène, Longicorne Foreur De Tige.**

**Résumé**

LA MUSCARDINE verte, *Metarhizium anisopliae* est utilisée dans la lutte contre le longicorne foreur de tige, *Dorystenes buqueti*, dans les zones de canne à sucre infestées en Thaïlande. Les champs ont été traités en dispersant une culture de *M. anisopliae* ( $2.7 \times 10^8$  spores/g) nouvellement préparée sur le riz cuit. Une seule application de *M. anisopliae* au taux de 80 kg/ha a été faite sur trois sites à Suphanburi et dans la province de Kanchanaburi en 2008. L'effet de la muscardine verte sur les larves du longicorne foreur de tige et sur le rendement de canne a été évalué. Les résultats ont montré que l'application du champignon tuait en moyenne  $35.09 \pm 8.68\%$  des larves du foreur dans toutes les parcelles traitées et  $4.49 \pm 1.64\%$  dans toutes les parcelles non-traitées. La moyenne la plus élevée de larves infestées était de  $41.67 \pm 36.03\%$  au district de Rang Sa Ri, dans la province de Kanchanaburi. Le rendement de canne était en moyenne de  $135.61 \pm 4.67$  t/ha dans les parcelles traitées, alors que celui des parcelles non-traitées était en moyenne de  $72.11 \pm 10.51$  t/ha. Cette étude démontre que *M. anisopliae* est un agent efficace de lutte biologique contre le longicorne foreur de tige en Thaïlande.

**UTILIZACIÓN DE LA MUSCARDINA VERDE, *Metarhizium anisopliae*, EN EL CONTROL DEL BARRENADOR DE ANTENAS LARGAS DEL TALLO DE LA CAÑA DE AZÚCAR, *Dorysthenes buqueti* GUERIN (COLEOPTERA: CERAMBYCIDAE)**

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**PALABRAS CLAVE: Control Biológico, *Metarhizium anisopliae*, Hongos Entomopatógenos, Barrenador del Tallo de Antenas Largas de la Caña de Azúcar.**

**Resumen**

LA MUSCARDINA verde, *Metarhizium anisopliae* se utiliza en el control del barrenador de antenas largas del tallo, *Dorysthenes buqueti*, en áreas infestadas de cultivos de caña de azúcar en Tailandia. Los campos se trataron asperjando cultivos frescos de *M. anisopliae* cultivado en un sustrato de arroz cocido ( $2.7 \times 10^8$  esporas/g). Una sola aplicación de *M. anisopliae* en la dosis de 80 kg/ha se hizo en tres localidades en las Provincias de Suphanburi y Kanchanaburi en 2008. Se evaluó el efecto del hongo sobre las larvas del barrenador, así como la producción de caña de azúcar. Los resultados mostraron que, después de la aplicación del hongo, este mató en promedio  $35.09 \pm 8.68\%$  de las larvas del barrenador en todas las parcelas tratadas, observándose una mortalidad de  $4.49 \pm 1.64\%$  en las parcelas no tratadas. El porcentaje más alto de infestación por las larvas fue de  $41.67 \pm 36.03\%$ , en el distrito de Rang a Ri, de la Provincia Kanchanaburi. El promedio de producción de la caña de azúcar fue de  $135.61 \pm 4.67$  t/ha en las parcelas tratadas, mientras que en las parcelas no tratadas, la producción promedio fue  $72.11 \pm 10.51$  t/ha. Estos resultados indican que *M. anisopliae* fue un controlador biológico efectivo en el control de este barrenador de la caña de azúcar en Tailandia.