Geographical Distribution of Sugarcane Longhorn Stem Borer, *Dorysthenes Buqueti* Guerin (Coleoptera: Cerambycidae) and Virulence Bioassay of *Metarhizium Anisopliae* (Metchnikoff) Sorokin Isolates

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Submission: August 02, 2018; Published: August 21, 2018

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Abstract

*Dorysthenes buqueti* Guerin (Coleoptera: Cerambycidae) is an economically important pest of sugarcane in Thailand, which causes great reduction in sugarcane yields. Survey of the of its geographical distribution during 2014 to 2015 revealed that *D. buqueti* was found in Central, East, Lower North and Northeastern regions of Thailand. The percentage of infestation by *D. buqueti* larvae varied from 0 to 57.6%. Factors influencing infestation intensity were determined by considering spatial autocorrelation. Akaike's Information Criteria (AIC) were compared by changing cluster size of survey plots, which was included as a random effect in GLMM using a logistic regression. One-km spatial autocorrelation was found, which are likely to be related to dispersals ability of *D. buqueti* adults. The results of the GLMM also indicated that *D. buqueti* was less abundant in heavy clay soil type whilst it commonly found in clay loam with heavy infestation. The percentage of Sugarcane area (SPC) inside a circle with a 10-km radius showed the smallest AIC, with a significantly negative coefficient of the SPC, indicating that the infestation decreased with a percentage of sugarcane area within the circle with 10-km radius. It suggests a possibility that *D. buqueti* is regulated by specific natural enemies to *D. buqueti* that can disperse as far as 10 km. The Green Muscadine Fungus (*Metarhizium anisopliae*) Sorokin has been reported that GMF infects and kills the Sugarcane Longhorn Stem Borer (*SLSB*). *Dorysthenes buqueti*/Guerin so that the GMF is a possible biological control agent of *SLSB*. This study revealed that an isolate from Khon Kaen (KK) showed the highest virulence to instars 5th-9th of *SLSB*. In biological control, aqueous suspension containing 10^8 conidia/ml of KK isolate is best from a viewpoint of an economical cost/benefit trade-offs between mass production cost and consequent mortality after application.

Keywords: Spatial autocorrelation; % Sugarcane area; Generalized linear mixed model; Geological information system; Biological control; Conidial suspension; Plant; Rhizosphere stage; Environment; Hymenoptera; lepidoptera; *M. Anisopliae* species; Sugarcane; Production; *Callosobruchus maculatus*; Infestation; Fungus; Information criterion

Abbreviations: AIC: Akaike’s Information Criteria; GMF: Green Muscadine Fungus; SLSB: Sugarcane Longhorn Stem Borer; KK: Khon Kaen; NBCRC: National Biological Control Research Center; AIC: Akaike Information Criterion

Introduction

Sugarcane stem boring grub; *Dorysthenes buqueti* Guerin (Coleoptera: Cerambycidae) recently become a serious insect pest of sugarcane. The severe injury by *D. buqueti* has been extended to sugarcane growing areas i.e.; East and Northeastern region of Thailand -. It can cause more than 70% yield loss in the outbreak areas. This insect pest attack sugarcane plant by the larval instar bores into the ratoon or the base of stalk and feed sugarcane tissue inside; which results the whole plant turned brown and die. Moreover; it can attack cassava stem and tuber that found in Kanchanaburi province. The concern of extensive insecticide application has been enforced explorations of natural enemies to control *D. buqueti*. *Metarhizium anisopliae* (Metchnikoff) Sorokin is a soil borne entomopathogenic fungus had been reported to exploiting to control *D. buqueti* in sugarcane fields. *Metarhizium* spp. are always designated as soil saprophytes that were observed in associations with plant roots in the rhizosphere stage and survive well in that environment over the longtime [1]. The Green
Study on geographical distribution of D. buqueti  

216 survey points were arbitrarily selected from sugarcane plantations within 50 km radius from a sugar mill. The GPS map 60CSx (Garmin; USA) was used for recording latitude; longitude; and elevation data of the survey points. These survey points were put on the sugarcane plantation map: data source from the Office of The Cane and Sugar Board.

Materials and Methods

Study on geographical distribution of D. buqueti

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Study on factors influencing D. buqueti distribution and infestation

30 stumps were arbitrarily selected at each survey point. Numbers of infested/uninfested stalks in each stump were recorded. The model selection to determine the effects of soil types were calculated by GLMM. Soil type of each survey point was obtained from LDD and Sugarcane plantation map: data source from the Office of The Cane and Sugar Board.

Virulence bioassays

The bioassay was carried out at the National Biological Control Research Center (NBCRC); Central Regional Center (CRC); Kasetsart University; Kamphaeng Saen Campus; Nakhon Pathom. The larvae of SLSB that were collected from sugarcane fields in Lao Khwan district; Kanchanaburi and Kamphaeng Saen district; Nakhon Pathom were reared individually in plastic cups with artificial diet for the sugarcane borer; Diatraea saccharalis; (Southland Product Inc; AR; USA) for 30 days to ensure they have not been parasitized by any parasitoids. The healthy larvae were used for the virulence bioassays. The 5th to 9th starls of SLSB were evaluated against four fungal isolates (KK; LKKB; BBCB and DMTKB) among the six. Fifty micro liters of a conidial suspension of three concentration levels; 103; 104 and 105 conidia/ml (conidia mixed with sterile distilled H2O 80 ml and Triton X 0.05%) were dropped on larvae cuticle. Larvae that were treated by 50 μl of distilled water mixed with Triton X 0.05% were equally used as control.

Results and Discussion

Study on geographical distribution of D. buqueti

Survey of the of its geographical distribution during 2014 to 2015 revealed that D. buqueti was found in Central; East; Lower North and Northeastern regions of Thailand (Figure 1).

Study on factors influencing D. buqueti distribution and infestation

Heavy day depressed the damage on sugarcane stalks by D. buqueti (Table 1). The percentage of infestation by D. buqueti larvae varied from 0 to 57.6%. The damage tended to be severe in 2014 than 2015. Two different scales of mechanisms acted to %damage by D. buqueti via land use found that a positive effect (increase in damage with %sugarcane fields) at a small scale with 0.5 km; and a negative effect (decrease in damage with %sugarcane fields) at a larger scale with 20 km. (Table 2).
Virulence bioassays

By inoculation experiment; mortality tended to increase with concentration of conidial suspension (Figure 2). No mortality was observed in the control (data were not shown in the graph) and at $10^3$ conidia/ml. A delta AIC between the full model and a model with two explanatory variables among the three was greater than 2.57 for all the three explanatory variables indicating that effects of the three factors were all significant ($p < 0.01$) (Table 3). In a result of the full model; a coefficient of concentration was positive and significantly differed from zero ($p < 0.01$) indicating the mortality increased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1. A coefficient of instar was negative and significantly differed from zero ($p < 0.01$) indicating that the mortality decreased with concentration in Figure 1.
was shown by Cherry, et al. [2]; which revealed that aqueous suspension containing $10^8$ conidia/ml showed high virulence to Callosobruchus maculatus in stored cowpea. Mishra, et al. [6] also reported that suspension containing $4.1 \times 10^8$ conidia/ml acted as an effective larvicide to Musca domestica. Benserradj and Mihoubi [1] reported that suspension containing $10^9$ conidia/ml showed the highest mortality to the 4th instar of Culex pipiens. These supports that our conclusion that GMF containing $10^8$ conidia/ml was likely the most suitable concentration to control D. buqueti larvae from a viewpoint of an economical cost-benefit trade-offs.

**Figure 2:** The percentage mortality of D. buqueti larvae by conidial suspension of four green muscadine fungus isolates. Instars: 5th-9th, Conidial concentrations: 103, 108 and 1013 conidia/ml. No mortality was found in a control

| Model          | AIC    | ⊥AIC |
|----------------|--------|------|
| Full model     | 666.5  |      |
| - Isolate      | 677.5  | 11   |
| - Instar       | 844.7  | 178.2|
| - Concentration| 1557   | 890.5|

| Model          | Estimate | SE      | z value | p-value |
|----------------|----------|---------|---------|---------|
| Intercept      | 0.44292  | 0.41803 | 1.06    | 0.2893  |
| Isolate (BBCB) | -0.37403 | 0.22111 | -1.692  | 0.0907  |
| Isolate (DMTKB)| -0.44678 | 0.22139 | -2.018  | 0.0436 *|
| Isolate (LKKB) | -0.90906 | 0.22429 | -4.053  | 5.06e-05 ***|
| Instar         | -0.76833 | 0.06453 | -11.91  |< 2e-16 ***|
| Concentration  | 0.5834   | 0.02879 | 20.267  |< 2e-16 ***|

**Conclusion**

Heavy clay depressed the damage on sugarcane stalks by D. buqueti. %damage by D. buqueti via land use found that a positive effect (increase in damage with %sugarcane fields) at a small scale with 0.5 km; and a negative effect (decrease in damage with %sugarcane fields) at a larger scale with 20 km. A positive effect at a small scale is likely to relate to movement of D. buqueti. A negative effect at a larger scale is possibly to be related to distribution or movement of natural enemies. This study revealed that KK isolate which was collected from Khon Kaen showed the highest virulence to L5 - L9 of D. buqueti. In biological control; aqueous suspension containing $10^8$ conidia/ml of KK isolates is best from a viewpoint of an economical cost/benefit trade-offs between mass production cost and consequent mortality after application.

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Time to cite this article: Nichanun K, Sopon U, Naoto K. Geographical Distribution of Sugarcane Longhorn Stem Borer, *Dorysthenes buqueti* Guerin (Coleoptera: Cerambycidae) and Virulence Bioassay of *Metarhizium anisopliae* (Metschnikoff) Sorokin Isolates. *Adv Biotech & Micro* 2018; 10(2): 555782. DOI: 10.19080/AIBM.2018.10.555782.

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