Biological Control 66 (2013) 56–64

Contents lists available at SciVerse ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Enhanced susceptibility of *Tibraca limbativentris* (Heteroptera: Pentatomidae) to *Metarhizium anisopliae* with sublethal doses of chemical insecticides

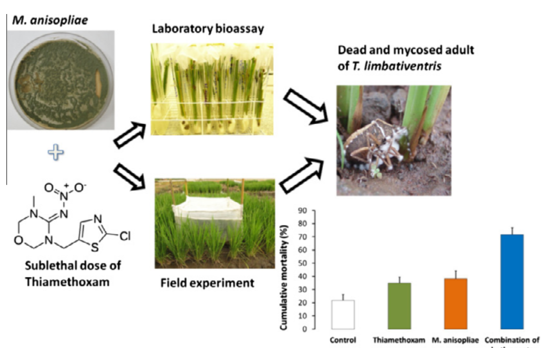
Eliane Dias Quintela [a,*], Gabriel Moura Mascarin [a], Rodrigo Alves da Silva [a], José Alexandre Freitas Barrigossi [a], José Francisco da Silva Martins [b]

[a] EMBRAPA Arroz e Feijão, Rodovia GO 462, km 12, Zona Rural, C.P. 179, 75375-000 Santo Antônio de Goiás, GO, Brazil
[b] EMBRAPA Clima Temperado, Rodovia BR 392, km 78, Monte Bonito, 96001-970 Pelotas, RS, Brasil

HIGHLIGHTS

- Adults of *Tibraca limbativentris* are more susceptible to thiamethoxam than to lambda-cyhalothrin.
- *T. Limbativentris* exhibited a natural resistance to *Metarhizium anisopliae*.
- Subdoses of chemical insecticides enhanced susceptibility of *T. limbativentris* to *M. anisopliae*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 August 2012
Accepted 24 March 2013
Available online 8 April 2013

Keywords:
Biocontrol
Entomopathogenic fungi
Rice stalk stink bug
Neonicotinoid
Pyrethroid
Synergism

ABSTRACT

This study investigated the interaction of the fungus *Metarhizium anisopliae* (Metsch.) Sorok. with sublethal doses of synthetic chemical insecticides thiamethoxam and lambda-cyhalothrin for the control of *Tibraca limbativentris* adults under laboratory and field conditions. Median lethal time (LT_{50}) was reduced significantly when *M. anisopliae* (5×10^6–5×10^8 conidia/mL) was combined with a sublethal dose (0.77 ppm AI) of thiamethoxam compared with fungus only. A similar response on host mortality was observed for *M. anisopliae* at 5×10^7 conidia/mL in combination with sublethal dose of lambda-cyhalothrin at 9.33 ppm (AI). Additionally, the thiamethoxam-fungus combination increased overall mortality and percent mycosed insects in comparison to their counterparts alone. Increasing fungus concentration did not increase insect susceptibility when combined with thiamethoxam either at 0.77 or 0.38 ppm (AI). In a field experiment, the combination of *M. anisopliae* at 1×10^{12} viable conidia/ha with thiamethoxam at 12.5 g (AI)/ha (¼ full dose) synergistically increased mortality and mycosis of adults of *T. limbativentris*. Therefore, enhanced *T. limbativentris* control could potentially be achieved within label rates of fungus (5×10^6 conidia/mL) and sublethal thiamethoxam (0.77 ppm). The strategy of using sublethal doses of chemical insecticides in combination with entomopathogenic fungi is a promising approach to battle the rice stalk stink bug in rice fields.

1. Introduction

The rice stalk stink bug, *Tibraca limbativentris* Stal. (Heteroptera: Pentatomidae), is a major pest of rice-growing areas (*Oryza sativa* L.) in Brazil and other countries in South America (Pantoja, 1997). This insect, particularly the adult stage, can reduce rice yield by 10–80% (Costa and Link, 1992; Ferreira et al., 1997). Nymphs and adults feed on the developing stalks following beginning of vegetative tillering, but the main damage takes place during preflowering and panicle formation (Costa and Link, 1992). Direct damages are divided into early attacks, which cause dead hearts,

* Corresponding author. Fax: +55 62 35332100.
E-mail address: eliane.quintela@embrapa.br (E.D. Quintela).

1049-9644/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.biocontrol.2013.03.018

and late attacks, which produce white heads where panicles become empty with unfilled grains (Ferreira et al., 1997).

The irrigated environment where nymphs and adults of the rice stalk stink bug are located are conducive for the development of entomopathogenic fungi due to the high humidity conditions (Martins et al., 2004). In the offseason, the rice stalk stink bug hibernates at the base of different plant species along the soil surface where there is high moisture (Link et al., 1996). When the adults migrate to new rice fields, they initially infest the base of plants, among the stalks, where there is generally no standing water (Botton et al., 1996). We have observed epizootics of *Metarhizium anisopliae* (Metsch.) Sorok. (Hypocreales: Clavicipitaceae) complex on populations of *T. limbativentris* under greenhouse conditions and also under irrigated rice field in states of Goiás and Mato Grosso, Brazil. Although environmental conditions are suitable for the use of fungi, many studies have shown that *M. anisopliae* has controlled only 50–60% of this insect (Martins and Lima, 1994; Martins et al., 1997, 2004). Low rate of infection by entomopathogenic hypocrealean fungi on related pentatomid species infesting soybeans has also been observed for *M. anisopliae* and *Beauveria bassiana* (Sosa-Gómez et al., 1993). Subsequently, it was determined that chemical compounds in the *Nezara viridula* (L.) cuticle were able to reduce adhesion, germination and were also fungistatic to conidia of *M. anisopliae* (Borges et al., 1993a; Sosa-Gómez et al., 1997).

One promising approach to overcome host inherent resistance of *T. limbativentris* to entomopathogenic fungi involves exploiting potential synergistic interactions with chemical insecticides, especially the 4A class (neonicotinoids), which can be used to target this pest (Anderson et al., 1989; Boucias et al., 1996; Hiromori and Nishigaki, 2001; Kaakeh et al., 1997; Pachamuthu and Kamble, 2000; Quintela and McCoy, 1998a, 1998b, 1997; Russel et al., 2010). Prior works have demonstrated that sublethal doses of insecticides can increase stress and compromise the immune system or otherwise alter the insect behavior that leads to improved performance of the fungal pathogen (Boucias et al., 1996; Hiromori and Nishigaki, 2001; Quintela and McCoy, 1998a). Integrating sublethal doses of insecticides with fungal entomopathogens can increase pest mortality as well as reduce the time to kill in comparison with either agents alone (Pachamuthu and Kamble, 2000; Paula et al., 2011). However, studies on combinations of entomopathogenic fungi and insecticides for the control of pentatomid stink bugs are lacking.

We hypothesized that the combination of pathogen and chemical insecticides might overcome this natural cuticle-chemical barrier of the insect to the fungus. Our specific objectives were: (1) determine the sublethal concentrations of thiamethoxam and lambda-cyhalothrin to adults; (2) compare the virulence of two strains and an emulsifiable oil formulation of *M. anisopliae* toward adults; (3) evaluate the susceptibility of adults to different rates of the fungus *M. anisopliae* (strain CG168) alone or combined with low rates of thiamethoxam and lambda-cyhalothrin; (4) assess the efficacy of the combined treatment of *M. anisopliae* with subdose of thiamethoxam under flooded rice field conditions.

2. Materials and methods

2.1. Insect colony

Adults of *T. limbativentris* were obtained from a greenhouse colony at the National Rice and Beans Research Center of the Brazilian Agricultural Research Corporation (EMBRAPA Rice and Beans) located at 16°28′00" S, 49°17′00" W and 823 m a.s.l. Insects were fed on potted rice plants (*Oryza sativa* L.) cv. BR-IRGA 409. The insect colony was derived from a field population originally collected in Santo Antônio de Goiás, state of Goiás, Brazil. Adults with specific age after emergence were collected from this stock colony and used in all experiments.

2.2. Fungi and insecticides

Strain CG168 (=ARSEF1883, USDA, Ithaca, NY) of *M. anisopliae* was originally isolated from *T. limbativentris* during a greenhouse epizootic at EMBRAPA Rice and Beans in 1985. This strain is preserved at −80 °C in the Laboratory of Invertebrate Mycology, at EMBRAPA Genetic Resources and Biotechnology (Brasília, Brazil). This fungal strain was identified through the multigene sequencing approach described by Bischoff et al. (2009). A commercial strain ESALQ-1037 of *M. anisopliae* sensu lato (Metarril, Itaforte BioProdutos Ltda., Itapetininga, SP, Brazil) isolated in 1992 from *Solenopsis invicta* in Porto Alegre, state of Rio Grande do Sul, Brazil, was also used. The fungi were cultured on PDA (200 g potato infusion +5 g dextrose +15 g agar with 0.2 g tetracycline per liter) and incubated for 10–15 d at 26 ± 1 °C, 70 ± 10% relative humidity (r.h.), 12:12 (L:D) h photoperiod. Conidial viability was determined by counting germ tubes produced on PDA plates after 20 h of incubation using a phase contrast microscope at 400× magnification. Conidia viability was >90% in all cases.

The synthetic chemical insecticides used in the experiments were thiamethoxam (Actara™ 250 WG [dispersible granules], 25% [AI], technical grade 3-(2-cloro-tiazol-5-ilmetil)-5-metil-[1,3,5] × adiazinan-4-ilideno-N-nitroamina) and lambda-cyhalothrin (Karate Zeon™ 50 CS [suspension of microcapsules], 5% [AI], technical grade (S)-a-cyano-3-phenoxybenzyl(Z)-(1R,3R)-3-(2-chloro-3,3,3-rifluoro prop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (R)-a-cyano-3-phenoxybenzyl(Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate (Syngenta Crop Protection, 2010). These products are currently registered for the control of *T. limbativentris* in rice crops in Brazil (Agrofit, 2012).

2.3. Determination of sublethal concentration of chemical insecticides to adults of T. limbativentris

In test 1, a topical bioassay with thiamethoxam diluted in sterile distilled water (dH_2O) to concentrations of 6.25, 3.1, 1.6, 0.77, 0.38, and 0.19 ppm (AI) was used to determine the sublethal concentration (LC_{30}) to adults of *T. limbativentris*. Adults 15 days old were anesthetized with CO_2 for 10 s and treated dorsally with 10 μL of each chemical concentration placed on the adult's pronotum using a micropipette. The control group was treated with sterile dH_2O. Treated insects were placed in groups of five into glass tube vial (20 × 2.5 cm) and then fed with three rice stalks (cv. BR-IRGA 409). The rice stalks was previously surface sterilized with bleach solution (containing 2–2.5% of sodium hypochlorite) at 1% v/v and rinsed twice with distilled water. The base of the rice stalk was surrounded by moist cotton. The tubes were sealed with cheesecloth (30-μm mesh size) and fixed by a rubber band. There were six replicates (tubes) containing five insects each (30 insects per treatment). The tubes were incubated at 26 ± 1 °C, 70 ± 10% r.h. and 12:12 (L:D) h photoperiod inside a growth chamber. Adult mortality was monitored daily over 14 days.

In test 2, formulated lambda-cyhalothrin (Karate Zeon™ 50 SC, Syngenta, Greensboro, NC, USA) was tested at concentrations of 2.5, 5.0, 7.5, 10, and 12.5 ppm (AI). The experimental protocol was the same as for test 1. Tests 1 and 2 were repeated twice on different occasions.