Effect of Metarhizium anisopliae var anisopliae Metschnikoff (Sorokin) (Deuteromycotina: Hyphomycetes) on fecundity of the Sugarcane Froghopper, Aeneolamia varia saccharina distant (Homoptera: Cercopidae) in Trinidad.

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Abstract

The entomopathogenic fungus, *Metarhizium anisopliae* Metsch. (Sorokin) (Deuteromycotina: Hyphomycetes) is a component of the Integrated Pest Management programme for the management of the sugarcane froghopper, *Aeneolamia varia saccharina* Distant (Homoptera: Cercopidae), a serious wet season pest of sugarcane in Trinidad and Tobago. The laboratory studies conducted to determine the effect *M. anisopliae* on the fecundity of *A. varia saccharina* indicated that there was a significant effect ($p<0.005$) of soil inoculum on ovipositing females. In addition, there was a significant difference ($p<0.001$) in the total mean number of eggs oviposited between the treated and untreated females with the average oviposition being 66.28 eggs and 32.66 eggs respectively for the untreated and treated females of *A. varia saccharina*.

EFFECT OF METARHIZIUM ANISOPLIAE VAR ANISOPLIAE METSCHNIKOFF (SOROKIN) (DEUTEROMYCOTINA: HYPHOMYCETES) ON FECUNDITY OF THE SUGARCANE FROGHOPPER, AENEOLAMIA VARIA SACCHARINA DISTANT (HOMOPTERA: CERCOPIDAE) IN TRINIDAD.

INTRODUCTION

The sugarcane froghopper, *Aeneolamia varia saccharina* Distant (Homoptera: Cercopidae) is a serious wet season pest of sugarcane in Trinidad and Tobago. A biopesticide component is being developed as part to the Integrated Pest Management strategy for management of *A. varia saccharina*. The research studies included testing the fundamental dose-response relationship of the entomopathogenic fungus, *Metarhizium anisopliae* var *anisopliae* Metsch. (Sorokin) against various stages of *A. varia saccharina* to obtain stage-specific dose-mortality data and understand the impact of the entomopathogen on the host.

According to Feng *et al.* (1985) these responses can provide an initial experimental base which can lead to the selection of an optimal entomopathogenic fungal isolate which is crucial to successful control of pests (Hajek *et al.*, 1994). This report summarizes the effect of *M. anisopliae* on the fecundity and eggs of *A. varia saccharina.*

Materials and methods

Rice seedlings ('Oryzica 1') were sown in growth chambers comprising of petri-dishes (10-cm) with moist cotton wool covered by unhardened paper. The seeds were incubated at 28.0^0C $\pm$ 2.0^0C in complete darkness. After 1 week the

germinated seeds were removed and the surface rooted seedlings used.

Effect of varying soil inocula of M. anisopliae on ovipositing A. varia saccharina

M. anisopliae was cultured on Sabouraud Dextrose Agar (SDA) and incubated in darkness for 14 days at 28.0^0C ± 2.0°C and 80± 5% relative humidity after which the conidia were harvested under sterile conditions using sterile distilled water containing 0.1% Tween 80^R. The relevant dilutions were made with sterile distilled water containing 0.1% Tween 80^R. All conidia counts were carried out on an improved Neubauer haemocytometer whilst viability tests were conducted on all the suspensions using the agar slide technique (Hall, 1976).

Suspensions of 1 x 10^7, 2.5 x 10^6, 6.2 x 10^5, 1.5 x 10^5, 3.9 x 10^4 conidia/ml were added to 100 g of soil at a rate of 1 ml of suspension /g of soil. 5 adult *A. varia saccharina* were placed in each of 10 containers containing the treated soil. Pieces of sugarcane leaves were placed in the container to provide food. Mortality was recorded over a 7-day period.

Effect of infection on fecundity of *A. varia saccharina*

25 newly emerged adult female *A. varia saccharina* were treated with a 5 x 10^6 conidia/ml suspension. The treated insects were air-dried and placed in oviposition chambers with newly emerged adult male *A. varia saccharina* (1 pair per chamber). Oviposition was monitored daily until death of the female. Dead males were replaced until death of the females to ensure availability of mating

partners. 25 female *A. varia saccharina* treated with water + 0.1% Tween 80 was used as a control. Eggs were removed daily, recorded and incubated whilst egg development was monitored on a daily basis until hatching to determine the effect of froghopper infection.

In all experiments dead insects were removed and transferred to petri-dishes lined with moist paper to stimulate emergence of the fungal mycelia and confirm fungus-induced mortality.

The median lethal time (MLT), the average survival time and its standard deviation (SD) were used to describe the rate of kill.

Multi dose bioassay against eggs of the *A. varia saccharina*

100 newly oviposited eggs of *A. varia saccharina* were treated with 1 x 10^8, 5 x 10^7, 1 x 10^6 and 5 x 10^6,conidia/ml and placed on moist paper in petri dishes. Egg development was monitored over a 3-week period until hatching occurred.

Results

The white mycelium of *M. anisopliae* emerged within the 24-hour period after insect death. The entomopathogen emerged from the spiracles and covered the outer surface of the abdominal sternites, the joint between the head and thorax, thorax and abdomen. . A pale green tinge on the white mycelia was visible 36- 48 hours after insect death indicating that sporulation had been initiated and

continued to turn dark green indicating continued sporulation.

Effect of varying soil inocula of M. anisopliae on ovipositing A. varia saccharina

There was a significant effect ($p<0.005$) of soil inoculum on ovipositing females. Mortality due to infection at 4 days post-treatment was visible in those insects exposed to a soil inoculum of 10^8 conidia/g soil. At the end of the experimental period, mortality due to exposure to inoculated soil was seen at 10^6, 10^7 and 10^8 conidia/g soil. The LD_{50} for day 8 was 3.07×10^7 conidia/g (± 0.08). As expected a decrease in LT_{50} and LT_{90} required increasing soil inoculum (Table 1).

Table 1 Mortality data for multi-dose soil inoculations against the *A. varia saccharina*

Day	LD_{50}	LD_{90}
Day 4	$8.8x10^{10}$ (±0.34)	$8.22x10^{12}$ (±0.34)
Day 5	$6.63x10^{10}$ (±0.13)	$6.19x10^{12}$ (±0.08)
Day 6	$3.27x10^{8}$ (±0.10)	$3.27x10^{8}$ (±0.10)
Day 7	$3.07x10^{8}$ (±0.08)	$1.34x10^{8}$ (±0.08)
Day 8	$3.07x10^{7}$ (±0.08)	$2.12x10^{8}$(±0.09)

Effect of infection on fecundity

After adult froghopper emergence there was an initial 24-hour pre-oviposition period during which no eggs were laid although pairing did occur. Pairing was observed to have occurred on more than one occasion. The total number of eggs per female ranged from 77 to 183. Most of the eggs were produced between Day 3-6 (18.39, 22.24, 21.16 and 17.58 respectively. The daily oviposition decreased thereafter.

There was a significant effect ($p<0.001$) of infection on the fecundity of female froghoppers. No eggs were laid the first day after emergence. In addition there were no significant differences ($p>0.05$) in the number of eggs laid by treated and untreated females during the second and third day post-treatment (mean= 6.5 eggs (±0.33) and 20.0 eggs (±0.37) respectively. However the differences became apparent from the

fourth day post-treatment when the number of eggs laid by the treated and untreated females were significantly different ($p<0.005$) as follows in Table 2.

Table 2 Comparison of fecundity between treated and untreated *A. varia saccharina* females

Day	Average number of eggs per female	
	Treated females	**Untreated females**
2	6.9 (±0.44)	6.2 (±0.22)
3	19 (±0.21)	21 (±0.53)
4	14.8 (±0.20)	25.8 (±0.42)
5	6.7 (±0.15)	20.6 (±0.52)
6	1.9 (±0.11)	16.6 (±0.33)
7	0.1 (±0.11)	11.7 (±0.21)

All infected females had died by day 8 post-treatment. The untreated females continued to oviposit until day 13 post-treatment. The total mean number of eggs laid by the 50 treated and untreated females was significantly different ($p<0.001$) being a total of 3314 eggs and 1633 eggs for the treated and untreated females respectively. The average oviposition was 66.28 eggs and 32.66 eggs respectively for the untreated and treated female *A. varia saccharina*.

Multi dose bioassay against eggs of the *A. varia saccharina*

There was no apparent effect of any of the tested doses of *M. anisopliae* on egg development which was easily visible under the binocular microscope. The eggs became swollen and a black hatching line became visible followed by the splitting of the chorion along the hatching line that exposed the black hatching lid. An orange coloured pigment spot was visible on the fourth day in a central position on the egg. It was observed that the orange pigment spot was in different positions in the egg during the development period. The splitting of the chorion was visible as well as a pair of lateral spots in the abdomen of the embryo. Two eyespots later appeared at the anterior pole of the egg.

All eggs hatched within 21 days and first instar nymphs exhibited no signs of infection, attaching themselves to the roots and enveloping themselves in the characteristic spittle masses where they continued to moult.

Discussion

Although Steinhaus (1965) stressed the importance of investigating the effect of pathogenic fungi on eggs of susceptible insects, these studies indicated that eggs of *A. varia saccharina* were not susceptible to infection by the range of concentrations of *M. anisopliae* tested. Although this is in contrast to what was found by Arango *et al.* (1994) who found that eggs of *Aeneolamia* spp. were as equally susceptible as nymphs and adults to *M. anisopliae* treatments, it supports the results by Fransen (1987) and Fransen *et al* (1987) who found that eggs of *T. vaporariorum* were not affected by *Aschersonia aleyrodis* despite using very high dosages. In whiteflies, Fransen (1987) suggested that

this might be due to structural differences between the insect cuticle and the chorion (egg-shell).

Although no information has been found in the literature on the specific effect of soil inoculum on insect fecundity, there have been numerous reports of soil inoculum resulting in high infection rates of insects and good pest control. With respect to the higher levels of inoculum needed to cause infection that was observed in these studies, Jackson and O'Callaghan (1997) identified that inundative microbial control of soil-dwelling pests is a promising concept although few products have been developed. Once in the soil the agents must compete with the existing soil microflora to become established and available as infective propagules to initiate disease in the target organism.

The effect of soil inocula on ovipositing *A. varia saccharina* and subsequent infection on fecundity indicated a potential that can be explored with respect to soil inoculations for use in the management strategy for the *A. varia saccharina*. Infection of *A. varia saccharina* females can result in as much as 50% decrease in fecundity which directly impacts on the *A. varia saccharina* 'egg bank' that resides within the soil and gives rise to subsequent generations of sugarcane froghopper. It should also be noted that each infected and sporulating *A. varia saccharina* also contributes to the reservoir of *M. anisopliae* surviving in the soil.

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