SHORT COMMUNICATION

FUSARIUM SPECIES ISOLATED FROM SUGARCANE IN KWAZULU-NATAL AND THEIR EFFECT ON *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE) DEVELOPMENT *IN VITRO*

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Abstract

Recent evidence suggests that *Fusarium* can affect the development and fecundity of *Eldana saccharina*. Studies in West African maize showed that survival of larvae, growth rate and the extent of stalk damage were significantly greater when *Fusarium verticillioides* was present. There is also evidence to suggest that moth oviposition is positively affected by epiphytic and endophytic symptomless colonisation by *F. verticillioides*. A similar study was initiated in KwaZulu-Natal using *Fusarium* species isolated from sugarcane stalks with and without *E. saccharina* damage. The results of the first part of this study are described in this communication. More than 400 stalks were collected, representing 14 varieties and 11 extension areas. A total of 223 isolates were obtained. Of these, 117 were isolated directly from borings, while 65 were isolated from undamaged cane and were considered to be endophytic. The remaining 41 were isolated from cane showing symptoms of pokkah boeng, a disease caused by *F. verticillioides* and *F. subglutinans*. Attenuated isolates were incorporated into *E. saccharina* diet, and those isolates that were most beneficial or antagonistic to the development of *E. saccharina* were selected and identified.

Keywords: sugarcane, *Eldana saccharina, Fusarium*

Introduction

A recent study showed that maize infected with *Fusarium verticillioides* was more attractive to ovipositing female *Eldana saccharina* Walker (Lepidoptera: Pyralidae) moths, resulting in a four-fold increase in the number of eggs laid (Ako *et al.,* 2003). This in turn resulted in a higher survival rate of *E. saccharina* larvae and increased damage in infected stalks. This increase in damage in *F. verticillioides*-infected maize confirmed previous findings of Schulthess *et al.* (2001); a study that lead to this project being initiated. Conversely, certain *Fusarium* species are known to produce varying amounts of compounds such as beauvericin that can be toxic to insects (Shephard *et al.*, 1999) and these could be of benefit in the control of *E. saccharina*.

Materials and Methods

Isolations
Sugarcane stalks that were collected for this study were either damaged by *E. saccharina*, did not show any signs of damage or were infected with pokkah boeng, a disease caused by *F. verticillioides* and *F. subglutinans*. Isolations were made onto Nash-Snyder agar, a semi-selective medium for *Fusarium* (Nash and Snyder, 1962).

Fusarium-E. saccharina *assays*
Maize kernels were inoculated with different *Fusarium* isolates, incubated at room temperature for three weeks, dried and ground to a fine powder. An equal quantity of the

resulting powder and dried, crushed sugarcane were added to *E. saccharina* diet (Gillespie, 1993) and dispensed into 32-well trays (one tray per isolate). One first instar *E. saccharina* larva was transferred to each well and the trays were incubated at 27ºC. The resulting larvae were weighed after three weeks.

Molecular analysis

DNA was isolated from mycelium grown on low dextrose PDA as described previously (Rehmany *et al.,* 2000). Polymerase chain reaction (PCR) was performed using two different primer sets to amplify fragments from genes coding for the translation elongation factor-1 and β-tubulin proteins according to the method described by O'Donnell *et al.* (1998). The isolates were grouped and assigned a preliminary identification by restriction analysis (RFLP) of the resulting PCR products. The elongation factor fragment was restricted with *Rsa*1 and *Pvu*1 and the β-tubulin fragment was restricted with *Rsa*1 and *Tru*1.

Results and discussion

More than 400 sugarcane stalks were collected, representing 11 extension areas and 14 varieties. A total of 223 isolates were obtained. Of these, 117 were isolated directly from *E. saccharina* borings, while 65 were isolated from undamaged cane and were considered to be endophytic. The remaining 41 were isolated from cane showing symptoms of pokkah boeng. The mass of the larvae was highest when fed on diet containing maize rotted by fusaria isolated from stalks of varieties N34 and 94H0570 that exhibited symptoms of pokkah boeng, and lowest on isolates from N29, a variety that has some resistance to *E. saccharina* damage. Isolates from the Zululand North extension area appeared to be most antagonistic, but there was little difference in the frequencies of antagonistic and beneficial isolates from the other areas. This was, however, not a random survey and sample sizes representing the various areas and varieties differed markedly.

After repeated *Fusarium-E. saccharina* assays, 10 isolates that were consistently most beneficial to the development of *E. saccharina* and 10 that were most antagonistic were selected for further study. The difference in the mass of the larvae that developed on diet containing maize rotted by the most beneficial isolates and the most antagonistic isolates was highly significant (Figure 1).

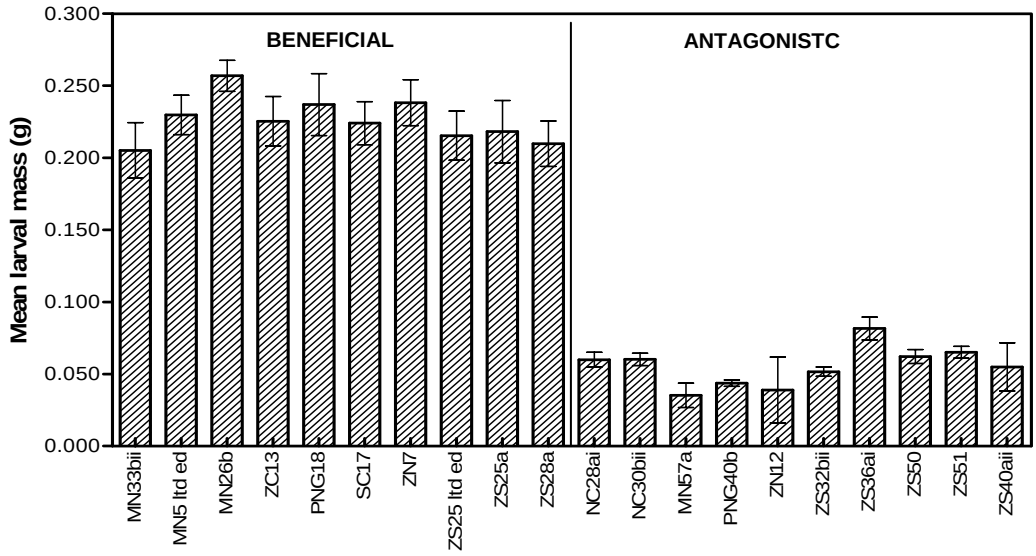

Figure 1. The effect of 20 *Fusarium* isolates on the development of *Eldana saccharina* (P<0.0001).

Four groups of fusaria were obtained using RFLP anaylsis and could be tentatively identified based on their banding patterns. Most common was *F. sacchari,* but *F. proliferatum*, *F. verticillioides* and *F. subglutinans* were also identified (Table 1). *F. napiforme* was also identified but was not ranked amongst the 20 selected isolates.

Table 1. Fusarium species isolated from sugarcane and their effect on *Eldana saccharina* development.

***Fusarium* species**	**Effect on *E. saccharina* development**	
	Beneficial	Antagonistic
F. sacchari	4	8
F. proliferatum	3	1
F. verticillioides	1	1
F. subglutinans	2	0

F. sacchari, F. proliferatum and *F. subglutinans* would be expected to be antagonistic to the development of *E. saccharina* since they have been shown to produce insecticidal toxins (e.g. fusaproliferin and beauvericin). Different strains within *Fusarium* species have however, been shown to produce such toxins in varying amounts, which could explain the fact that two of these species contained both beneficial and antagonistic strains. Both *F. verticillioides* and *F. proliferatum* produce fumonisins, secondary metabolites that may be linked to the increased borer damage seen in maize. The proportion of the different metabolites produced could be critical to the effect that various *Fusarium* species and strains have on *E. saccharina* development.

The identity of these *Fusarium* isolates will be confirmed by sequencing and they will be characterised further using inter-simple sequence repeats. Their effect on the development of *E. saccharina in vivo* will be assessed in a pot trial, and their ability to endophytically colonise undamaged sugarcane will be assessed.

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