SHORT COMMUNICATION

FUSARIUM SPECIES CAUSING POKKAH BOENG AND THEIR EFFECT ON *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

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Abstract

Pokkah boeng is a fungal disease that can cause serious yield losses in susceptible varieties. Pokkah boeng is caused by *Fusarium*, but there is some debate regarding the species involved. Previous research has revealed that some *Fusarium* species isolated from sugarcane could benefit the development of the stalk borer, *Eldana saccharina*, a serious pest of sugarcane in South Africa. The aim of this research was to isolate and identify the causal organism of pokkah boeng and to determine the effect of the isolates on the development of *E. saccharina*. Eight representative isolates were inoculated into pokkah boeng susceptible varieties N34 and N41. Typical pokkah boeng symptoms were evident after three weeks. Isolates inoculated into the pot trial and the isolates obtained from inoculated, symptomatic plants were identified as *F. sacchari*, *F. proliferatum* and *F. andiyazi*. The isolates were included in *E. saccharina* diet and olfactory choice assays. When compared to controls in the dietary inclusion assays, three isolates were considered to be antagonistic (two identified as *F. andiyazi* and one as *F. proliferatum*) to *E. saccharina*, with the mean mass of surviving larvae being significantly lower than the controls. In this study, no isolates resulted in significantly greater larval mass than the controls. Results from the olfactory choice assays suggested that all isolates were attractive to *E. saccharina*, with significantly more larvae feeding on inoculated maize kernels than the uninoculated controls.

Keywords: sugarcane disease, stalk borer, disease control, pokkah boeng, *Eldana saccharina*

Introduction

Pokkah boeng is present in most sugarcane producing areas of the world and symptoms of the disease tend to develop during periods of rapid crop growth when rainfall is high (Martin *et al*., 1961). The result is a malformed or damaged top and stalk. Malformation or distortion of the young leaves is usually accompanied by a distinct wrinkling, twisting and shortening of the leaves. The most serious and advanced stage of pokkah boeng is top rot, where the growing point is killed and the entire top of the plant dies. Pokkah boeng is a fungal disease caused by *Fusarium* but there is some debate regarding the species involved.

The African sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) causes substantial yield losses in the South African sugar industry. The pest is most problematic during drought and plant stress situations (Downing *et al.,* 2000). A number of different species of *Fusarium* have been isolated from sugarcane in South Africa (McFarlane and Rutherford, 2005, McFarlane *et al*., 2009). Some were associated with pokkah boeng

symptoms while others were endophytic, showing no disease symptoms. In dietary inclusion assays, some isolates resulted in increased survival and mean larval mass, while others adversely affected larval development. Results also suggested that some isolates were attractive to neonate larvae while others were repellent. The presence of *Fusarium* in sugarcane may therefore influence the severity of *E. saccharina* damage. This study was conducted to identify the *Fusarium* species causing pokkah boeng in sugarcane and to assess the effects of the isolates on the development of *E. saccharina*.

Materials and Methods

Isolations were made from different varieties of sugarcane showing pokkah boeng symptoms. The plant tissue sampled was surface sterilised and plated onto Nash and Snyder Agar, a semi-selective medium for *Fusarium* (Nash and Snyder, 1962). Morphological features and Inter-Simple Sequence Repeats (ISSRs) were used to group the isolates obtained. A pot trial using pokkah boeng susceptible varieties N41 and N34 was undertaken to confirm Koch's Postulates. The *Fusarium* isolates that were used to inoculate the pot trial, as well as the isolates obtained from symptomatic plants were identified by sequencing the translation elongation factor-α1 region. Dietary inclusion and olfactory choice assays were conducted according to the procedures described by McFarlane *et al*. (2009) to assess the effects of the isolates on *E. saccharina*.

Results and Discussion

Eight groups of isolates were obtained; one isolate was selected from each group for the pot trial as well as the dietary inclusion and olfactory choice assays. Symptoms of pokkah boeng were first observed in the pot trial three weeks after inoculation (Figures 1a and b).

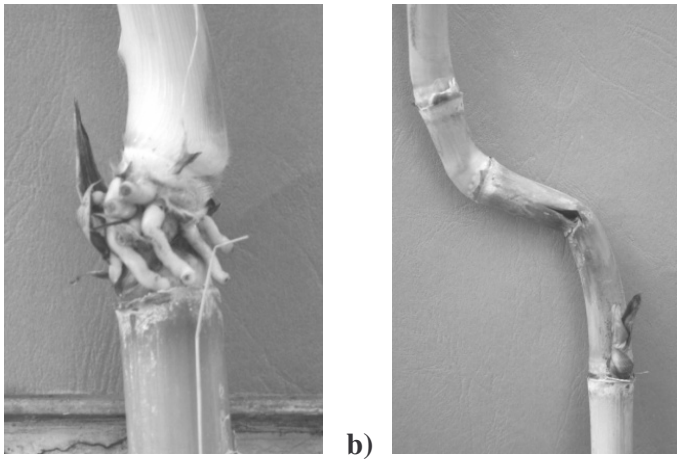
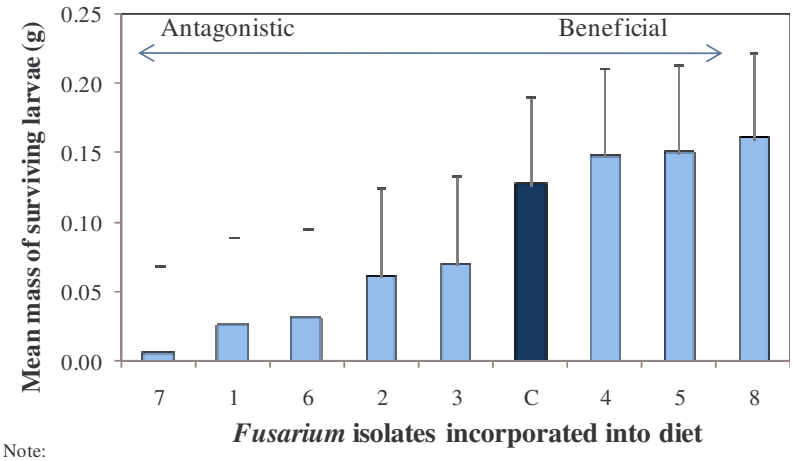


Figure 1. Pokkah boeng symptoms caused by (a) isolate 3 (*F. andiyazi*), showing unusual growth at the internode, and (b) isolate 8 (*F. sacchari*), showing a conspicuous bend in the stalk.

Four of the eight *Fusarium* isolates produced typical pokkah boeng symptoms. These were isolates 3, 4 and 6 (all *F. andiyazi*) and isolate 8 (*F. sacchari*). Sequencing results confirmed that the isolates obtained from the symptomatic plants in the pot trial matched those that were inoculated into the plants, fulfilling Koch's postulates. *F. andiyazi*, *F. sacchari* and *F.*

proliferatum were identified, with *F. andiyazi* being the predominant species. This species has previously only been recorded in sorghum and is closely related to *F. verticillioides* (Leslie and Summerell, 2006), a species commonly reported to be associated with pokkah boeng infections.

The difference in the mass of surviving larvae raised on *E. saccharina* diet incorporating different *Fusarium* isolates was significant (Figure 2, P<0.01). Three isolates, *F. andiyazi* (two isolates) and *F. proliferatum* produced larvae that weighed significantly less than the two controls that were included in the assay and were considered to be antagonistic to *E. saccharina*. It is possible that these isolates produce insecticidal toxins that have a detrimental effect on the growth of *E. saccharina*. This is currently being investigated. One of these antagonistic isolates (*F. andiyazi*: isolate 6) produced typical pokkah boeng symptoms. *Fusarium sacchari* (isolate 8) was the most beneficial isolate, although the mean larval mass was not significantly different to the controls. This isolate produced striking pokkah boeng symptoms when inoculated into variety N41 (Figure 1b).



ISOLATE	IDENTITY
1 - 98F6	*Fusarium proliferatum*
2 - 98F1	*Fusarium andiyazi*
3 - N25 9	*Fusarium andiyazi*
4 - 06U 5	*Fusarium andiyazi*
5 - 98F 8	*Fusarium andiyazi*
6 - 06U 7	*Fusarium andiyazi*
7 - 06U6	*Fusarium andiyazi*
8 - N25 5	*Fusarium sacchari*

Note:
C = Control (diet with cane and rice, no *Fusarium*)

Figure 2. Mean mass of surviving *Eldana saccharina* larvae in dietary inclusion assays. Restricted (or residual) maximum likelihood (REML) analysis bars represent Holm-Sidak (5%), P<0.01. Identities of isolates 1 to 8 obtained from sequencing are shown in the adjoining table.

All isolates appeared to be attractive to *E. saccharina* in the olfactory choice assay, with more larvae feeding on the *Fusarium* inoculated maize kernels than the uninoculated controls (P<0.001). By being attractive to insects, the probability of the fungus being spread by the insect is likely to be increased. A screening programme for varietal resistance to *Fusarium* species has potential in reducing the incidence of pokkah boeng in the industry and could also result in lower levels of *E. saccharina* damage by excluding potentially beneficial *Fusarium* strains such as those identified in the previous study by McFarlane *et al*. (2009). Those isolates that are attractive but detrimental to *E. saccharina* development could be inoculated into trap crops to act as lures in the field.

Conclusion

Fusarium andiyazi and *F. sacchari* resulted in the development of typical pokkah boeng symptoms. The predominant species, *F. andiyazi*, has not been associated with pokkah boeng

symptoms previously and has not been reported in sugarcane. While all eight isolates selected for this study were attractive to *E. saccharina* in olfactory choice assays, three had a detrimental effect on larval development in dietary inclusion assays. Only one of these antagonistic isolates, *F. andiyazi* (isolate 6) produced pokkah boeng symptoms when inoculated into sugarcane. No isolate significantly improved larval development in this study but the most beneficial of the eight isolates, *F. sacchari,* resulted in pokkah boeng symptoms.

REFERENCES

Downing JK, Leslie G and Thomson AJ (2000). Biocontrol of the sugarcane borer *Eldana saccharina* by expression of *Bacillus thuringiensis cry1Ac7* and *Serratia marcescens chiA* genes in sugarcane-associated bacteria. *Appl Environ Micro* 66: 2804-2810.

Leslie FJ and Summerell AB (2006). Media-Recipes and Preparation, pp 7-8 In: *The Fusarium Laboratory Manual*. Blackwell publishing Ltd. Australia.

Martin JP, Han Loie Hong and Wismer CA (1961). Pokkah boeng, pp 247-257 In: Martin JP, Abbott EV and Hughes CG (Eds) *Sugarcane Diseases of the World*. Vol. 1, Elsevier Publishing Company.

McFarlane SA and Rutherford RS (2005). *Fusarium* species isolated from sugarcane in KwaZulu-Natal and their effect on *Eldana saccharina* (Lepidoptera: Pyralidae) development *in vitro*. *S Afr Sug Technol Ass* 79: 120-123.

McFarlane SA, Govender P and Rutherford RS (2009). Interactions between *Fusarium* species from sugarcane and the stalk borer, *Eldana saccharina* (Lepidoptera: Pyralidae). *Ann Appl Biol* 155: 349-359.

Nash SN and Snyder WC (1962). Quantitative estimations by plate counts of propagules of the bean rot *Fusarium* in field soils. *Phytopath* 73: 458-462.