SHORT COMMUNICATION

THE PRESENCE OF *WOLBACHIA* IN *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE): IMPLICATIONS FOR BIOLOGICAL CONTROL

SWEBY DL[1], MARTIN LA[1], GOVENDER S[1], CONLONG DE[1,2] AND RUTHERFORD RS[1]

[1]*South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa*
[2]*School of Biological and Conservation Sciences, Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg Campus, P/Bag X01, Scottsville, 3610, South Africa*
deborah.sweby@sugar.org.za lauren.martin@sugar.org.za shivani.govender@sugar.org.za
des.conlong@sugar.org.za stuart.rutherford@sugar.org.za

Abstract

Wolbachia is a maternally-inherited endoparasitic bacterium infecting many different insect species. It causes phenotypic changes resulting in mating disruptions in its host insect populations. There is thus much interest in the potential of *Wolbachia* as a biological control agent. *Eldana saccharina* (eldana) is well known for its damaging effects on South African sugarcane. It has been sampled from various African countries (Tanzania, Uganda, Kenya, Ethiopia, Benin, Mozambique) and different locations within South Africa (KZN Midlands, Transkei, Limpopo, Richards Bay, Mount Edgecombe). At its putative centre of origin in Central/East Africa and in south-eastern Africa, eldana has not reached pest status in its natural host plant species. The presence or absence of *Wolbachia* in these different eldana populations has been established by PCR amplification of *Wolbachia*-specific DNA sequences. *Wolbachia* was found to be present in eldana from Uganda, Tanzania and Kenya only. Eldana colonies from Tanzania and Kenya have been established at SASRI. Test matings conducted between *Wolbachia*-positive males from Tanzania and *Wolbachia*-negative females (SASRI lab colony) revealed that the fertility of the mating was almost half that of *Wolbachia*-positive females (Tanzania) and *Wolbachia*-negative males (SASRI lab culture). These preliminary results suggest that *Wolbachia* causes cytoplasmic incompatibility in infected males, thereby reducing the number of viable progeny produced. DNA sequence analysis of *Wolbachia*-positive individuals established that all eldana populations tested were infected with the same *Wolbachia* genotype, supergroup A.

Keywords: Wolbachia, Eldana saccharina, biocontrol, PCR, cytoplasmic incompatibility

Introduction

Wolbachia are obligate intracellular bacteria infecting many diverse arthropod species. They manipulate host reproduction by causing several phenotypes that benefit their own survival and transmission through host populations (Saiful Islam, 2007). *Wolbachia* are maternally inherited, therefore spread of infection in host populations is achieved either by producing more infected female hosts, or by negatively affecting fitness of non-transmitting, uninfected individuals. The consequent alteration in sex ratio of the host species results in mating disruptions that can lead to reductions in population numbers. This characteristic of

Wolbachia infection has lead to much interest in its potential as a biological control agent to suppress natural populations of arthropod pests (Floate *et al.*, 2006).

One of the most damaging insect pests of sugarcane in the South African sugar industry is the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (eldana). Indigenous to Africa, it occurs in numerous wetland sedges. The area with greatest genetic diversity between populations was shown to be in Central/East Africa (Assefa *et al.*, 2006a). Eldana has however, adopted as new hosts several graminaceous crop species where it has become a pest. Previous research has revealed that, although natural predators are found in indigenous habitats of eldana, none have been found during surveys in South African sugarcane fields (Conlong 2001). It is possible that biological control agents present in indigenous host species are not present in new hosts.

No information is available in the published literature on the *Wolbachia* status of eldana, both from indigenous hosts and new hosts. Moreover, it is not known what impact *Wolbachia* infection has on eldana populations in their indigenous hosts.

A molecular approach using PCR analysis of eldana DNA with *Wolbachia*-specific DNA primers was used to establish whether eldana populations in Africa are infected with *Wolbachia.* Collections were made from wild host plants and sugarcane in Central, East, West and Southern Africa. Cross-mating between *Wolbachia*-infected and uninfected eldana was also conducted to assess the effect of *Wolbachia* infection on fertility. This is the first report of *Wolbachia* incidence in eldana and provides valuable information that can be used for future biological control studies.

Materials and Methods

Eldana collections
Eldana was collected from both indigenous host plants and sugarcane. Living colonies were established from eldana obtained from Uganda, Kenya and Tanzania. They were initially reared at the Government Quarantine Facility in Pretoria and then transferred to SASRI.

Dead moths were obtained from collections from Democratic Republic of Congo, Uganda, Ethiopia, Kenya, Mozambique, Tanzania and Benin. Sampling was also completed at several South African locations: KZN Midlands, Transkei, Limpopo, Richards Bay and Mount Edgecombe. Moths were preserved in 95% Ethanol for molecular analyses.

Molecular analysis
Genomic DNA was extracted from eldana abdomens using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA quality was checked by PCR amplification of the mitochondrial cytochrome oxidase subunit I gene (COI). *Wolbachia* was detected by PCR analysis using the following *Wolbachia*-specific DNA primers: WSP, GLTA, FTSZ and 16S. PCR products were separated on a 1% agarose gel and results visualised after staining with GelRed (Anatech). Eldana samples were recorded as positive for *Wolbachia* infection when PCR products of the correct size were obtained with all four *Wolbachia*-specific DNA primers.

DNA sequences were generated by dye terminator cycle sequencing using COI, WSP and FTSZ primers. The BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems) was

used, and cycle sequencing was performed in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems). Sequence analysis was performed using an ABI Prism 310 Genetic Analyser (Applied Biosystems).

Phylogenetic analysis
DNA sequence results were analysed and formatted using the programmes Geneious (Biomatters), BioEDIT (Tom Hall, Ibis Therapeutics) and ClustalX (Larkin *et al*., 2007). A phylogenetic tree was constructed using MEGA (Kumar *et al*., 2008) and TreeView (GubaSoft).

Results and Discussion

There were 269 dead moths tested for *Wolbachia* infection, 87 from South Africa and 182 from other African countries. Of the South African eldana tested (sampled both from wild hosts and sugarcane), none were infected with *Wolbachia*. Only eldana sampled from Uganda, Kenya and Tanzania were infected (Figure 1). For Kenya and Tanzania more than 60% of eldana tested were positive for *Wolbachia* infection. For Uganda, only 22% of eldana tested were *Wolbachia*-positive. These eldana had been sampled from the indigenous host *Cyperus papyrus*. The remaining *Wolbachia*-negative eldana were obtained from sugarcane grown adjacent to *C. papyrus*.

Wolbachia-positive eldana colonies from Uganda, Kenya and Tanzania were established at the SASRI Insect Rearing Unit. Cross-mating studies were performed between *Wolbachia*-positive and negative eldana to assess the effect of the *Wolbachia* infection on fertility. *Wolbachia* infection was lost from the Uganda population kept at SASRI, thus cross-mating studies were conducted only on the Kenya and Tanzania populations. For these studies, *Wolbachia*-positive male eldana moths were mated with *Wolbachia*-negative female moths (obtained from the SASRI Insect Rearing Unit), and vice versa. Results indicated that the fertility of the cross between a *Wolbachia*-positive male and a *Wolbachia*-negative female was approximately half of that obtained from the reciprocal cross. Furthermore, the progeny of the former cross all tested negative for *Wolbachia*, whilst the converse occurred when a *Wolbachia*-positive female was cross-mated with a *Wolbachia*-negative male. *Wolbachia* infection in arthropods induces various phenotypes that favour the spread of infection. These are feminisation (male offspring are turned into functional females), male killing (male offspring from infected females die), parthenogenesis induction (females develop from unfertilised eggs through chromosome duplication) and cytoplasmic incompatibility (embryo mortality occurs when infected males mate with uninfected females) (Charlat and Mercot, 2000). The cross-mating results obtained in this study between infected eldana males and uninfected females suggest that cytoplasmic incompatibility (CI) is the most likely phenotype induced by the *Wolbachia* infection. The CI phenotype is the most common phenotype imposed by *Wolbachia* on their hosts (Floate *et al*., 2006), and there are various reports where CI has been used to disrupt population growth in several arthropod species (Zabalou *et al*., 2004; Floate *et al*., 2006; Bourtzis, 2007).

COI sequence analysis was conducted on DNA isolated from both *Wolbachia*-positive and negative eldana. Consensus sequences generated from the COI sequences from each African country were used to construct a phylogenetic tree that differentiated between those countries that had both *Wolbachia*-positive and negative eldana (Figure 1). Other eldana COI sequences available from the NCBI GenBank database were included in the tree and a COI sequence from *Galleria mellonella* was used to root as an outgroup.

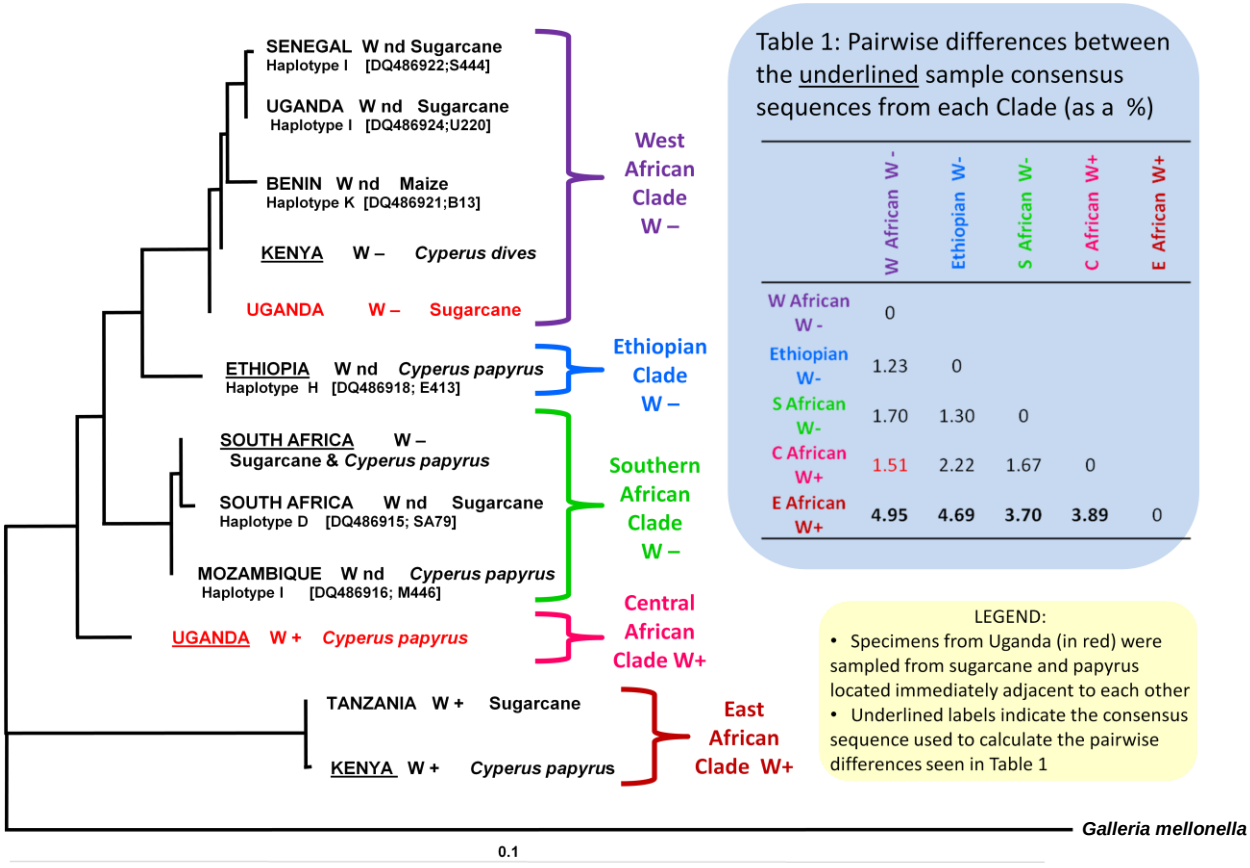

Figure 1: Phylogenetic tree representing relationships of *Eldana saccharina* from eight African countries based on COI gene sequences. *Wolbachia* status is indicated as W+ (positive for *Wolbachia* infection), W- (negative for *Wolbachia* infection) or W nd (not determined: sequence obtained from GenBank). The host plant from which the eldana was collected is also displayed.

COI sequences clustered into five distinct clades based on geographical distribution. These results confirm those generated by Assefa *et al.* (2006a). The *Wolbachia*-positive eldana clustered together into two different clades, the Central African and East African, whilst the remaining three clades were all *Wolbachia*-negative (Figure 1). For eldana collected from Uganda a noteworthy observation was made. The *Wolbachia*-positive eldana collected in *C. papyrus* formed the Central African clade whilst the *Wolbachia*-negative eldana collected from sugarcane growing adjacent to the *C. papyrus* clustered into the West African clade. DNA sequence analysis of the *Wolbachia* WSP and FTSZ genes established that all *Wolbachia* detected in this study belonged to supergroup A (data not shown). Gene phylogenies have revealed six major *Wolbachia* clades, A–F (termed supergroups) (Casiraghi *et al*., 2005). Most of the *Wolbachia* found in arthropods fall into either supergroup A or B (Werren *et al*., 1995). The difference in *Wolbachia* status between the two Ugandan collections can therefore not be directly attributed to infection by diverse *Wolbachia* supergroups. Further research is required to establish the basis for this difference. Also of interest was the *Wolbachia*-positive eldana collected from sugarcane in Tanzania. This is the only record from the current study of *Wolbachia* infecting eldana in sugarcane.

Detection of *Wolbachia* in Central and East African eldana and not in Southern African eldana is the first step towards assessing the role of *Wolbachia* in eldana population control.

Further research is in progress to investigate mechanisms for *Wolbachia* infection of South African eldana and whether this leads to any mating disruptions.

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