SHORT, NON-REFEREED PAPER

EFFECTS OF *WOLBACHIA* INFECTION ON THE INTERACTIONS BETWEEN *ELDANA SACCHARINA* AND ITS INSECT PARASITOIDS

PILLAY P[1,3], SWEBY D L[1], MARTIN LA[1], ZULU M[1], CONLONG D E[1,2] AND RUTHERFORD R S[1]

[1]*South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300, South Africa*
[2]*School of Biological and Conservation Sciences, Faculty of Science and Agriculture, University of KwaZulu-Natal, P/Bag X01, Scottsville, 3610, South Africa*
[3]*School of Biological and Conservation Sciences, Faculty of Science and Agriculture, University of KwaZulu-Natal, P/Bag X54001, Durban, 4000, South Africa*
prathiva.pillay@sugar.org.za *deborah.sweby@sugar.org.za*
lauren.martin@sugar.org.za *maphilisi.zulu@sugar.org.za*
des.conlong@sugar.org.za *stuart.rutherford@sugar.org.za*

Abstract

Wolbachia are maternally inherited obligate intracellular bacteria that infect approximately 70% of all insect species. *Wolbachia* causes phenotypic changes in its host which lead to reproductive disruptions. This characteristic makes *Wolbachia* a potential biological control agent of insect pests. *Eldana saccharina* is a pyralid stemborer indigenous to Africa and is a destructive pest in the South African sugarcane industry. Previous SASRI research has established that local *E. saccharina* populations are not infected with *Wolbachia*, whereas certain populations from East Africa are. To assess whether *Wolbachia* infection could cause mating disruptions in local *E. saccharina* populations, a suitable method for stable transmission of the bacteria into *E.saccharina* is required. A possible method involves horizontal transmission between parasitoids and their insect hosts. As a first step towards testing for horizontal transfer, host and parasitoid colonies must be established and their *Wolbachia* status determined. Two commonly occurring parasitoids of noctuid stemborers in South African sugarcane (*Pediobius furvus* and *Cotesia sesamiae),* have been collected. Both have tested positive for *Wolbachia* infection. Parasitism studies were conducted using *Wolbachia*-negative (Wol-) *E. saccharina*, *Chilo partellus* (a crambid) and *Sesamia calamistis* (a noctuid) as hosts. Results revealed that *S. calamistis* was parasitised by *C. sesamiae* and *E. saccharina* by *P. furvus* (only after the pupal case was removed). *C. sesamiae* (Wol+) was encapsulated by *E. saccharina* (Wol-) and this scenario provides a good model to test for horizontal transfer of *Wolbachia* from parasitoid to host. This is the case as it will be observed whether the encapsulated Wol+ parasitoid (*C. sesamiae*) eggs are able to successfully transfer the *Wolbachia* infection to its host (*E. saccharina*).

Keywords: Wolbachia, encapsulation, host, parasitoid

Introduction

Wolbachia are obligate intracellular bacteria which reside in the reproductive tissues of their hosts (Werren, 1997; Floate *et al*., 2006). They are maternally inherited and act to increase females in the population to selfishly increase its spread. *Wolbachia* alters the reproductive capability of its host in one of four ways; cytoplasmic incompatibility, parthenogenesis, male-killing and feminisation of males into functional females (Werren, 1997). This results in mating disruptions that can lead to a reduction in population numbers of the host (Floate *et al*., 2006). This characteristic of *Wolbachia* has attracted interest because of its potential as a biological control agent of insect pests.

Eldana saccharina Walker (Lepidoptera: Pyralidae) is one of the most destructive and economically important pests of sugarcane in South Africa (Dick, 1945; Conlong, 1994). It is a stemborer indigenous to Africa, naturally found in wetland sedges and indigenous grasses (Assefa *et al*., 2008). It has, however, adopted various graminaceous crops as host plants in which it has attained pest status (Conlong, 1994).

Local *E. saccharina* populations are not infected with *Wolbachia* (Sweby *et al*., 2010). To assess whether *Wolbachia* could be transmitted to these local populations to cause mating disruptions, a method to introduce *Wolbachia* into local *E. saccharina* needs to be established. One such method is horizontal transfer of these bacteria between species (Vavre *et al*., 1999). Horizontal transfer can occur in several ways, with a common method being between parasitoids and their insect hosts (Vavre *et al*., 1999; Huigens *et al*., 2000). Various studies (Heath *et al*., 1999; Vavre *et al*., 1999; Batista and Keddie, 2010) have shown transmission of the bacteria from host to parasitoid during parasitism. However, there is no information in the published literature regarding transmission from parasitoid to host.

In this study, parasitoid and potential host colonies were established and their *Wolbachia* status determined. The results provide a first step towards assessing whether parasitoids are stable vectors for transmission of *Wolbachia* into Wol- *E. saccharina* populations.

Materials and Methods

Parasitoid and host collections

Parasitoids (*Cotesia sesamiae* (Hymenoptera: Braconidae) and *Pediobius furvus* (Hymenoptera: Eulophidae)) were collected from different sugarcane growing regions in South Africa. *Cotesia sesamiae* (host: *Sesamia calamistis* (Lepidoptera: Noctuidae); host plant: *Coix lacryma-jobi* L) were obtained from Sezela while *P. furvus* (host: *Sesamia calamistis*; host plant: *Saccharum* spp L) were obtained from Tinley Manor.

The hosts used to rear *C. sesamiae* were third instar *S. calamistis* and *Chilo partellus* (Lepidoptera: Crambidae) larvae, whilst *P. furvus* was reared on pupae of *E. saccharina* and *C. partellus*. The host insects were obtained from laboratory colonies maintained at the South African Sugarcane Research Institute (SASRI) Insect Rearing Unit.

Routine SASRI field collections provided *Busseola fusca*, *Xanthopimpla stemmator* and *Chilo partellus* from Potchefstroom, Mauritius and Komatipoort, respectively.

Parasitoid colony establishment
Cotesia sesamiae parasitises larger instar *S. calamistis* larvae. Parasitoid cocoons obtained from field collections (Sezela) were placed in plastic vials (25 mL), sealed with a screw top and maintained in the Quarantine laboratory at SASRI (28 °C and 65% humidity) until emergence. When *C. sesamiae* adults emerged, they were presented to *S. calamistis* larvae for parasitism. *Sesamia calamistis* larvae were prepared by removing them from artificial diet and placing them in crushed sugarcane for two hours prior to parasitism. Three to five larvae were placed in the *C. sesamiae*-containing vial and observed to establish that the larvae were stung by the wasps. Once larvae had been stung, they were removed and placed back into the artificial diet to allow parasitoid development.

Larvae were checked for cocoons 10 to 15 days later. When found, they were removed and placed in a clean empty vial. Upon adult parasitoid emergence, larval introduction was repeated as described above.

Once dead, *C. sesamiae* adults and *S. calamistis* larvae were stored in 99% ethanol for molecular analysis. A lab colony of *Pediobius fervus* was established at the SASRI Insect Rearing Unit; however, this colony has not been maintained.

Molecular analyses
DNA from the host insects was extracted using the DNeasy Blood and Tissue Kit (Qiagen) as per the manufacturer's instructions. Parasitoid DNA was extracted using the method of Rugman-Jones *et al*. (2006).

The *Wolbachia* status of the insects were assessed using four *Wolbachia*-specific primers on the DNA obtained from the insects; WSP, FTSZ, 16 S and GLTA according to the protocols described in Sweby *et al*. (2010). DNA sequence analysis was conducted using WSP primers according to the methods described in Sweby *et al*. (2010). Sequences obtained were compared to other known DNA sequences on the NCBI database using BLAST to establish which strain(s) of *Wolbachia* infected the insects.

Results and Discussion

Parasitoid acceptance of different hosts
Before examining whether *Wolbachia* can be transferred from Wol+ parasitoids to Wol- hosts it was necessary to establish whether the parasitoids successfully attacked and parasitised the hosts available (Table 1).

Cotesia sesamiae accepts *C. partellus* and *S. calamistis* larvae as hosts (Table 1). These hosts were used to maintain the laboratory colony of *C. sesamiae*. In contrast, *Eldana saccharina* was apparently not parasitised (Table 1). According to Potting *et al*. (1999), *E. saccharina* encapsulates the eggs of *C. sesamiae* rendering it incapable of any further development in *E. saccharina*. *E. saccharina* can thus be regarded as a Wol- host, and used to assess whether *Wolbachia* can be transmitted from Wol+ *C. sesamiae* to Wol- *E. saccharina*, with the parasitoid as the vector.

Under lab conditions, *Pediobius furvus* was shown to parasitise *E. saccharina* (however, this only occurred after the host's silk cocoon was removed) and *C. partellus* pupae

(Table 1). *P. furvus* could therefore, in theory, have been a potential candidate for horizontal transfer of *Wolbachia* to *E. saccharina* populations.

Table 1. Host response to parasitism by *Cotesia sesamiae* and *Pediobius furvus.*

Parasitoids	Hosts	No. of hosts presented	No. of hosts successfully parasitised	Non-parasitised hosts	
				Died*	Pupated*
Cotesia sesamiae	*Sesamia calamistis* larvae	8 8 9 10	1 3# # 4#	3 # # #	4 1 # #
	Chilo partellus larvae	10 4	2# 0	# 0	# 4
	Eldana saccharina larvae	10 15	0 0	0 0	10 15
Pediobius furvus	*Chilo partellus* pupae	5 3 4	4 0 4	1 3 0	
	Eldana saccharina pupae	10 6 12 25	6 3 4 8	4 3 8 17	

*applies only to *Cotesia sesamiae* (# = experiment still in progress).

Wolbachia status and DNA sequence analyses of insects

All host species tested negative for *Wolbachia* infection except for *C. partellus* collected from maize fields at SASRI and at Komatipoort (Table 2). However, *Chilo partellus* reared in the lab tested negative for *Wolbachia*. This could suggest a loss of *Wolbachia* infection in *C. partellus*. Loss of infection is known to occur, often as a result of different selection pressures present in new environments (Reuter *et al*., 2005). More research is required to explain these differences in *Wolbachia* status between the *C. partellus* collections.

All three species of parasitoids tested positive for *Wolbachia* infection (Table 2). However, it is noteworthy that whilst the *Xanthopimpla stemmator* obtained from Mauritius was Wol+ the SASRI lab reared colony was negative.

DNA sequence analyses and BLAST homology searches were conducted on the parasitoids to establish which strain of *Wolbachia* was present. It is necessary to establish that there are not multiple *Wolbachia* strains infecting the parasitoid populations as this could have implications for the success of horizontal transfer. All insects tested were infected with *Wolbachia* supergroup A (Table 2), the most common strain infecting arthropods (Casiraghi *et al*., 2005).

Work in progress

Testing for horizontal transfer of *Wolbachia* with *C. sesamiae* as the vector and *E. saccharina* as the target, is underway. Eggs obtained from exposed *E. saccharina* adults will be tested for *Wolbachia* infection. In addition, a method is being developed using

tetracycline to cure *C. sesamiae* of *Wolbachia*. The Wol- *C. sesamiae* will be used to parasitise *E. saccharina* to assess whether *Wolbachia* influences the process of encapsulation.

Information gained from these studies will provide valuable insight towards establishing mechanisms for introducing *Wolbachia* into *E. saccharina* populations.

Table 2 *Wolbachia* status, as determined from DNA sequences of hosts and parasitoids obtained from different locations.

Parasitoids	Number of individuals	Location	Host plant and insect	*Wolbachia* status	Strain (WSP)*
Xanthopimpla stemmator (Hym: Ichneumonidae)	10	Mauritius	Sugarcane, *Chilo sacchariphagus*	Wol+	
X. stemmator	8	SASRI Lab colony		Wol -	
Cotesia sesamiae	15	Tinley Manor	Unknown plant, *Sesamiae calamistis*	Wol+	A
	20	Lab reared	*Sesamiae calamistis*	Wol+	A
	14	Sezela	*Coix lacryma-jobi* L, *Sesamiae calamistis*	Wol+	A
	22	Midlands	Sugarcane, *Sesamiae calamistis*	Wol +	A
Pediobius furvus	13	Tinley Manor	Sugarcane, *Sesamiae calamistis*	Wol+	A
	20	Lab reared	*Eldana saccharina*	Wol+	
Hosts					
Chilo partellus	17	Fields outside SASRI	Maize	Wol+	
Chilo partellus	15	Komatipoort	Maize	Wol+	
Chilo partellus	30	Lab colony (colony initiated from SASRI fields)		Wol-	
Busseola fusca (Lep: Noctuidae)	10	Potchefstroom	Maize	Wol-	
Sesamia calamistis	22	Lab colony		Wol-	

*Strain refers to the *Wolbachia* supergroup. This is established using primers that are specific to the *Wolbachia* cell surface protein (WSP).

REFERENCES

Assefa Y, Conlong DE, van den Berg J and Le Ru BP (2008). The wider distribution of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa and its potential risk to maize production. *Proc S Afr Sug Technol Ass* 81: 290-297.

Batista PD and Keddie BA (2010). Phylogenetic placement and evidence for horizontal transfer of *Wolbachia* in *Plutella xylostella* (Lepidoptera: Plutellidae) and its parasitiods, *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Ent Soc Canada* 142: 57-64.

Casiraghi M, Bordenstein SR, Baldo L, Lo N, Beninati T, Wernegreen JJ, Werren JH, Bandi C (2005). Phylogeny of *Wolbachia pipientis* based on *gltA*, *groEL* and *ftsZ* gene sequences: Clustering of arthropod and nematode symbionts in the F supergroup and evidence for further diversity in the *Wolbachia* tree. *Microbiology* 151: 4015-4022.

Conlong DE (1994). A review and perspectives for the biological control of the African sugarcane stalkborer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Agric Ecosys Environ* 48: 9-17.

Dick J (1945). Some data on the biology of the sugarcane borer (*Eldana saccharina* Walker). *Proc S Afr Sug Technol Ass* 19: 75-79.

Floate KD, Kyei-Poku GK and Coghlin PC (2006). Overview and relevance of *Wolbachia* bacteria in biocontrol research. *Biocontrol Science and Technology* 16(8): 767-788.

Heath BD, Butcher RDJ, Whitfield WGF and Hubbard SF (1999). Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. *Current Biology* 9: 313-316.

Huigens ME, Luck RF, Klaassen RHG, Maas MFPM, Timmermans MJTN, Stouthamer R (2000). Infectious parthenogenesis. *Nature* 405: 178-179.

Potting, RPJ, Vermeulen, NE and Conlong, DE (1999). Active defence of herbivorous hosts against parasitism: Adult parasitoid mortality risk involved in attacking a concealed stemboring host. *Entomologia Experimentalis et Applicata* 91: 143-148.

Reuter M, Pedersen JS and Keller L (2005). Loss of *Wolbachia* infection during colonisation in the invasive Argentine ant *Linepithema humile. Heredity* (94): 364-369.

Rugman-Jones PF, Hoddle MS, Mound LA, Stouthamer R (2006). Molecular identification key for pest species of *Scirtothrips* (Thysanoptera: Thripidae). *Molecular Entomology* 6: 1813-1819.

Sweby DL, Martin L, Govender S, Conlong DE and Rutherford RS (2010). The presence of *Wolbachia* in *Eldana saccharina* Walker (Lepidoptera: Pyralidae): implications for biological control. *Proc S Afr Sug Technol Ass* 83: 257-261.

Vavre F, Fleury F, Lepetit D, Fouillet P, Bouletreau M (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in Host-Parasitoid associations. *Molecular Biology and Entomology* 16(12): 1711-1723.

Werren JH (1997). Biology of *Wolbachia*. *Ann Rev Ent* 42: 587-609.