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EVALUATION OF STEM BORER SPECIES DIVERSITY AND IDENTIFICATION OF THE FALL ARMYWORM STRAIN IN CEREAL CROPS AND SUGARCANE FIELDS OF SWAZILAND

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ABSTRACT

Lepidopterous stem borers constitute one of the major constraints to cereal and sugarcane production in Swaziland and the invasive fall armyworm is also becoming a major threat to maize production in the country. Valid identification of these pests is vital for the development of effective and sustainable pest management programmes. In this study geographical populations of Spodoptera frugiperda (JE Smith) (Lepidoptera: Noctuidae) and cereal stem borers collected during surveys conducted in the year 2017 were identified using sequences of the mitochondrial cytochrome-c oxidase I (COI) gene. Phylogenetic analyses and percentage sequence divergence of the collected specimens revealed that cereals and sugarcane in the country are attacked by four species of stem borers [(Busseola fusca Fuller (Lepidoptera:Noctuidae), Sesamia calamistis Hampson (Lepidoptera:Noctuidae) Chilo partellus Swinhoe (Lepidoptera: Crambidae) and Eldana saccharina Walker (Lepidoptera: Pyralidae)] and a single strain (Rice Strain) of fall armyworm. These information could be used in future studies on migration pattern, host range and natural enemy of the pests that are vital for developing sound and sustainable fall armyworm and/or stem borer management programs.

INTRODUCTION

Graminaceous crops such as maize, sorghum, millet and sugarcane in Africa suffer from yield loss by several species of indigenous noctuid, pyralid and crambid stem borers (Polaszek, 1998) and very recently from the invasive Fall Armyworm, Spodoptera frugiperda (JE Smith) (Lepidoptera: Noctuidae), (Jeger et al., 2017). The potential yield loss in cereal grains and sugarcane due to stem borers in Sub-Saharan Africa is estimated to be between 20-90% (Nyukuri et al., 2014) and fall armyworm is predicted to cause a yield loss of more than $3 billion over 2017-2018 seasons (Abrahams et al., 2017). The widespread damage to cereals and sugarcane by stem borers and the unprecedented invasion by fall armyworm are challenges threatening the livelihoods of Swazi farmers. Hence, it is fundamental to enhance capacity at country and regional level, to prevent, detect and respond rapidly to the threats these pests pose to agriculture.

Understanding the species and genetic diversity of stem borers and fall armyworm is the first and fundamental step in the development of sound pest management strategies. Many attempts of redistributing natural enemies for the management of stem borers in the past ended up in failure due to lack of knowledge on the ecology, population structure and phylogenetic relationships between populations of the pests and their natural enemies (Conlong, 2001). The invasive fall armyworm populations consist of morphologically indistinguishable corn and rice strains (Prowell et al., 2004) that are reliably distinguishable by molecular methods (Nagoshi et al., 2007; Cock et al., 2017). Studies suggest that the behavioural and physiological variations between the strains may influence

population monitoring studies, pest control strategies and host plant resistance breeding programs (Lu and Adang, 1996).

Undertaking extensive surveys and identification of cereal stem borers and fall armyworm in maize, sorghum and sugarcane fields of the country is of paramount importance as it provides initial baseline data on the genetic diversity of these pests that will be used for development of appropriate pest management strategy. This study analyses the genetic diversity in populations of stem borers and fall armyworm from maize, sorghum and sugarcane in Swaziland using sequences of the cytochrome-c oxidase I (COI) gene of mitochondrial DNA. Results of this study are useful to prevent, detect and respond rapidly respond to fall armyworm invasion and assist in designing effective stem borer and fall armyworm management programmes in the country.

PROBLEM STATEMENT

Cereals, especially maize and sorghum, are the most important field crops grown by resource-poor small-scale farmers in Swaziland. Sugarcane is, also, an important cash crop grown in many parts of the country. However, stem borers pose a threat to cultivated cereals and sugarcane production. Stem borer larvae are responsible for up to 88% cereal yield losses in Swaziland and the invasive fall armyworm is also becoming a major threat to maize production in the country. The country currently spends over E100 million per annum in maize imports from other countries. Despite the importance of detailed knowledge on these pests, information on the diversity of stem borers in the country is scarce and the strain of fall armyworm that invaded maize and sorghum fields of the country is not known. Thus a study on the diversity of stem borers and identification of the strain of fall armyworm in the country was a prerequisite for an informed and well planned stem borer and fall armyworm management strategy.

OBJECTIVES

General objective:

To provide information on the strain of fall army worm that invaded Swaziland and determine the distribution, species diversity and abundance of cereal and sugarcane stem in the country.

Specific objectives:

1. **Assess the diversity of stem borers in cereal crops and sugarcane in Swaziland**
2. **Determine the strain of fall armyworm that invaded Swaziland**

METHODOLOGY

Surveys

Field surveys were conducted from January to March 2017 in cereal and sugarcane fields in all the four regions (Highveld, Middleveld, Lowveld and Lubombo) of Swaziland. A total of 112 localities in the four regions of Swaziland were visited to evaluate the diversity and distribution of stem borer pests and fall armyworm in the country.

Molecular studies

Tissue samples of the representative stem borer and fall armyworm specimens collected in the surveys were sent to Inqaba Biotechnical Industries in Pretoria, South Africa for DNA extraction, Polymerase Chain Reaction (PCR) amplification, and sequencing. DNA sequence chromatograms from Inqaba Biotech were edited and assembled using the Staden package (Staden, 1996). Individual sequences were compared with sequences of identified stem borers and fall armyworm in our collection for species level identification. The identified sequences of fall armyworm and stem borers were then aligned with sequences from our collection and from the GenBank. Phylogenetic tree reconstruction and calculation of genetic distances were performed as indicated in Assefa et al. (2017).

RESULTS

Diversity of lepidopteran stem borers and fall armyworm on cereals and sugarcane in Swaziland

A total of 318 larvae belonging to the families of Noctuidae, Pyralidae and Crambidae were collected. Molecular analyses on the representative sample specimens confirmed that the specimens collected on maize, sorghum and sugarcane were Busseola fusca Fuller (Lepidoptera:Noctuidae), Sesamia calamistis Hampson (Lepidoptera:Noctuidae), Spodoptera frugiperda (J.E. Smith) (Lepidoptera:Noctuidae) Chilo partellus Swinhoe (Lepidoptera: Crambidae) and Eldana saccharina Walker (Lepidoptera: Pyralidae). Larvae belonging to the family Noctuidae were the dominant pests of cereals and they were the most widely distributed groups. They constitute 77.5% of the larvae recovered and were found in all the three host crops and all the agro-ecologies included in the survey (Table 1). The Pyralid, E. saccharina was recovered only from sugarcane and was found to be the primary pest of sugarcane in all the areas the crop was sampled. Spodoptera frugiperda was not detected on sugarcane but it was the main pest of maize in all the sited visited.

Table 1. Localities from where specimens of stem borers and fall armyworm were collected.

AEZ	No of localities	Host Plant	No of specimens	Percentage species composition				
				B. fusca	*S. calamistis*	*C. partellus*	*E. saccharina*	*S. frugiperda*
HV	40	Maize	86	39.1	2.3	13.8	0	44.8
MV	22	Maize	118	23.7	2.5	12.7	0	61
	3	Sorghum	3	100	0	0	0	0
	1	Sugarcane	1	0	0	0	100	0
LV	15	Maize	35	14.3	5.7	22.9	0	57.1
	7	Sorghum	37	0	0	40.5	0	59.5
	18	Sugarcane	19	5.3	10.5	0	84.2	0
LB	6	Maize	18	11.1	0	27.8	0	61.1
Total	112		318	23	2.8	17.4	5	51.7

AEZ=Agro-Ecological Zones, HV= Highveld, MV= Middleveld, LV= Lowveld and LB = Lubombo.

Phylogenetic analysis

Maximum parsimony (MP) analyses generated ten equiparsimonious trees (length 202, CI = 0.85000, RI = 0.95083) is shown in Fig. 1. The final dataset consisted of 18 aligned sequences of 584 bp. The phylogenetic tree analysis clearly separated the analysed sequences in to five distinct clades each representing a species (Fig. 1). The first clade included sequences from maize and sugarcane in the Highveld, Middleveld and Lowveld and it was identified as B. fusca. The second clade that was identified as S. calamistis was composed of a sequence from maize in the Highveld and a sequence from sugarcane in the Lowveld. Three sequences from maize in the Highveld, Lowveld and Lubombo region were included in the third clade which was identified to be C. partellus. The fourth clade was formed by S. frugiperda sequences recovered from maize in the Highveld and Lowveld. Most of the specimens collected from sugarcane in the Lowveld were identified as E. saccharina and formed the fifth clade (Fig 1).

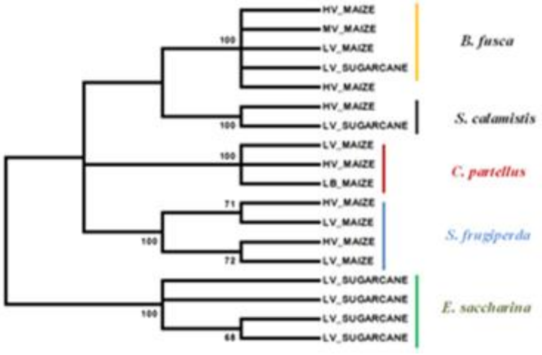

Figure 1. A Maximum Parsimony tree showing the genetic relationship between the armyworm and stem borer species recovered in the study. HV, MV, LV and LB stands for Highveld, Middleveld, Lowveld and Lubombo Agro-Ecological Zones of Swaziland, respectively. Numbers below branches are Bootstrap values.

Uncorrected pairwise genetic distances for the dataset ranged from 0.00 to 22.5% and from 0.00 to 0.05% within clades (Table 2). The highest values were recorded between clades E saccharina and S. frugiperda, while the lowest genetic distances were found between clades of S. calamistis and B. fusca (Table 2). The within-clade sequence variation was very low and there is a clear barcode gap.

Table 2. Uncorrected sequence divergence between haplotypes of fall armyworm and stem borers.

	B. fusca	*S. calamistis*	*C. partellus*	*S. frugiperda*	*E. saccharina*
B. fusca	0.00				
S. calamistis	12.3	0.00			
C. partellus	14.8	12.2	0.00		
S. frugiperda	17.8	18.5	16.3-16.5	0.02	
E. saccharina	18.9-19.6	20.3-21.0	18.7-19.3	22.2-22.5	0.02-0.05

Identification of fall armyworm strain in Swaziland

Fall army was recorded in all regions of the country causing a serious damage to maize. We included sequences of fall armyworm found on maize in Highveld, Middleveld, Lowveld and Lubombo regions of the country. Evaluations of the COI gene sequences from armyworm populations collected during the survey revealed that subsistence maize fields in all regions of Swaziland are attacked by the rice strain. We constructed a phylogenetic tree and Neighbour-joining tree correctly grouped the fall armyworm sequences into their respective strain with 84 and 91% bootstrap support (Figure 2).

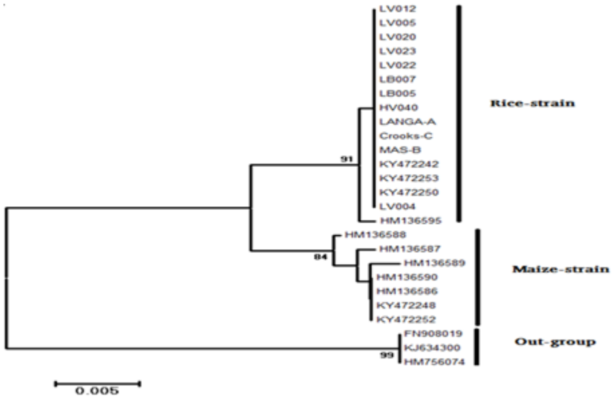

Figure 2. NJ tree showing the relationships among the fall armyworm strains.

Bootstrap support is indicated on the branches. Specimen codes for samples from Swaziland start with abriviations of Agro-Ecological Zones indicated in Table 1, except for Langa-A, Crooks-C and Mas-E which were collected from the Lowveld. The remaining codes are GenBank accession numbers of sequences used for identification and analyses of genetic distances. This tree includes selected barcodes of African samples deposited by Cock et al. (2017) and American samples deposited by Nagoshi et al. (2011) to show the relationship between the two strains of fall armyworm. *Spodoptera littoralis* was used as an outgroup in the analysis

Uncorrected pairwise sequence distances within this clade ranged from 0 to 0.18% (Table 3).

Table 3. Uncorrected sequence divergence (%) within and between fall armyworm strains

	Rice strain	Maize strain	*Spodoptera littoralis*
(Rice strain	0.00-0.18		
Maize strain	1.11-1.67	0.00-0.37	
Spodoptera littoralis	4.54-4.74	4.34-4.74	0.00

DISCUSSION

Accurate identification and understanding of the genetic diversity of an insect pest are essential to develop and improve monitoring and biological control strategies. Recent investigations indicate that the identified species of noctuid borers in Africa are far fewer than the unknowns (Assefa et al. , 2017) mainly because of the acute shortage of taxonomists and very little attention to research on the diversity of these groups in the natural habitats in the continent. This made the use of molecular phylogenetic approaches in identification of new indigenous borers invading crop fields difficult. Fortunately, identification of the strain of fall armyworm and evaluating the genetic relationship of stem borer populations in this study was possible because the availability of COI sequences of the positively identified specimens. Results of this study are of importance to answering stem borer and fall armyworm management questions in cereal and sugarcane fields.

Surprisingly the maize strain of fall armyworm has not been recorded in this study. Further studies are underway to determine whether maize strain is in the country or not. Molecular identification of the rice strain in the country will have a significant contribution in exploiting available information from its original range to prevent, detect and respond rapidly to the threats this pest pose to agriculture until more is known about it.

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