

Determining the efficacy of push-pull for management of Eldana saccharina (Walker (Lepidoptera: Pyralidae) in sugarcane through on-farm field trials

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An area-wide integrated pest management (AW-IPM) programme was developed to improve management of Eldana saccharina Walker (Lepidoptera: Pyralidae), a major pest of South African sugarcane. Push-pull technology is an important component of this AW-IPM approach. The sugarcane push-pull programme uses plants which are both repellent (Melinis minutiflora P. Beauv. (Cyperales: Poaceae)) and attractive (Cyperus dives Delile and Cyperus papyrus L. (both Cyperales: Cyperaceae) to E. saccharina. Previous research demonstrated the efficacy of push-pull in the Midlands North region of KwaZulu-Natal (KZN), South Africa. To date, little research has been conducted in coastal sugarcane growing areas. The aim of this study was to assess the feasibility of using push-pull for management of E. saccharina in coastal KZN, using large-scale on-farm field trials conducted on five model farms. On each farm, wetland habitats were rehabilitated with pull plants (C. dives and C. papyrus) and fields were intercropped with the repellent grass M. minutiflora. Eldana saccharina damage and infestation levels were recorded to assess the efficacy of push-pull, using a multiple before-after-control-impact (mBACI) design. Push-pull treatment sites showed a significant reduction in mean percentage stalk damage and E. saccharina abundance relative to control sites. Furthermore, stemborer surveys in wetland habitats revealed higher numbers of E. saccharina within Cyperus spp. stands. Pull plants were therefore effective at attracting E. saccharina away from sugarcane. The success of the push-pull trials in this study indicates that it is a useful tool for managing E. saccharina populations in coastal sugarcane, especially in conjunction with other management practices.

INTRODUCTION

Eldana saccharina Walker (Lepidoptera: Pyralidae) is the most damaging stem boring pest of sugarcane in South Africa, causing yield losses in excess of \$60 million per year (Zhou and Mokwele 2016). In response to E. saccharina related losses, the South African Sugarcane Research Institute (SASRI) developed an area-wide integrated pest management (AW-IPM) programme for the control of the stemborer (Rutherford and Conlong 2010; Rutherford 2015). The programme includes information on varietal resistance, soil management practices, crop nutrition recommendations and insecticide use. The development and implementation of a pushpull strategy for the control of E. saccharina also forms an important component of this AW-IPM approach (Rutherford 2015).

Push-pull technology is a habitat management strategy that seeks to manipulate the distribution of insect pest populations within an agroecosystem (Conlong and Rutherford 2009). The push-pull programme in South African sugarcane is based on research that was conducted in East Africa for the control of cereal stemborers, such as Chilo partellus Swinhoe (Lepidoptera: Crambidae) and Busseola fusca Fuller (Lepidoptera: Noctuidae) (Khan et al. 1997a; Midega et al. 2005). This method of push-pull uses plants that both repel and attract targeted pests in a manner that decreases crop infestations (Kasl 2004; Barker et al. 2006).

In sugarcane, Melinis minutiflora P. Beauv (Cyperales: Poaceae) is used against E. saccharina as a 'push' or repellent plant (Kasl 2004; Barker 2008; Cockburn 2013). Melinis minutiflora produces volatile plant defence chemicals (Khan et al. 2000) that repel the egg-laying adults of *E. saccharina*, whilst simultaneously attracting beneficial natural enemies of the pest (Khan et al. 1997b; Kasl 2004; Barker 2008). Since E. saccharina is native to southern Africa, indigenous hosts can be used as 'pull' plants to attract the pest away from sugarcane (Conlong 2001). Kasl (2004) demonstrated that E. saccharina moths have a significant ovipositional preference for Cyperus papyrus L. and Cyperus dives Delile (Cyperales: Cyperaceae). Additionally, Conlong (1990) demonstrated the controlling impact of the indigenous parasitoids present within these sedge habitats, on E. saccharina populations therein. These sedge species were selected as the most effective 'pull' plants for the push-pull programme in sugarcane. Gravid moths also showed a strong ovipositional preference for conventional and Bt-maize (Keeping et al. 2007). Maize is therefore used as an alternative 'pull' plant in areas where sedges cannot be planted (Keeping et al. 2007). Furthermore, Bt-maize is considered a dead-end trap crop within the push-pull system, as the insecticidal cry proteins incorporated into Bt-maize kill lepidopteran stem boring pests that feed on it (Khan et al. 2000; Keeping et al. 2007).

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Initial research into the development of a push-pull programme for sugarcane was completed at SASRI, where conditions could be readily managed (Kasl 2004; Barker et al. 2006; Barker 2008). Kasl (2004) established large-scale field trials at the SASRI Research Farm in Gingindlovu (29°01'46.4" S; 31°36'42.5" E), and on private farms in Pongola (27°22' E; 31°38' E) in the northern parts of the KwaZulu-Natal (KZN) province, South Africa. Based on Kasl's (2004) results, Barker (2008) further established large-scale field trials in the Midlands North sugarcane growing region of KZN (29°35' S; 30°30' E), and at a private grower in Emoyeni (28°57' S; 31°39' E) in northern KZN. Following these successful trials, the research has progressed to include more large-scale on-farm field trials in the Midlands North sugarcane growing region KZN (Cockburn 2013). Kasl (2004), Barker et al. (2006), Barker (2008), and Cockburn (2013) all demonstrated that push-pull is effective on large-scale farms, even where variables such as soil type, sugarcane variety, sugarcane age and water availability cannot be controlled. Push-pull has since been adopted by many large-scale sugarcane growers in the Midlands North region. The large-scale growers have been implementing, planting, and maintaining their own push-pull systems with help from the local pest, disease and variety control committee (Cockburn et al. 2012; Conlong et al. 2016). Khan et al. (2008) demonstrated similar increases in the implementation of pushpull following the introduction of grower-managed on-farm field trials in Kenya. In Kenya, farmer-farmer technology dissemination allowed early adopting farmers to convey the benefits of new practices, thereby influencing other growers to adopt innovations such as push-pull (Amudavi et al. 2009).

Despite successful research being conducted in many KZN locations, and despite increasing adoption in the Midlands North, implementation of AW-IPM and push-pull in other sugarcane growing regions of KZN has been poor (Kasl 2004; Barker et al. 2006; Barker 2008; Cockburn 2013). While the Midlands North provided a good base for the development of push-pull technology, E. saccharina levels in this area were typically low when compared to other sugarcane growing regions of KZN (Assefa et al. 2008; Cockburn et al. 2012). Sugarcane farmers in the coastal belt of KZN experience much higher levels of E. saccharina, resulting in greater yield losses (Assefa et al. 2008). Coastal growers are forced to harvest their sugarcane earlier than the recommended 18-24 months, even with careful varietal control (Assefa et al. 2008; Barker 2008; Rutherford 2015). The increased use of insecticides has, in these regions, been used to reduce E. saccharina infestations, allowing farmers to increase yields and the age of their sugarcane before harvest (Rutherford 2015). However, this is expensive, and insecticides do not provide a long-term solution due to the potential for non-target effects and the development of insecticide resistance (Whalon 2008; Leslie 2009; Ramburan et al. 2009).

Farmers are typically unwilling to risk their profits and livelihoods by implementing new, or unknown practices (Pannell 2003), especially in regions where pest levels are historically high (Rodriguez et al. 2008). Farmers want 'proof' of the efficacy of innovations, such as push-pull, before they are prepared to invest time and money implementing them (Pannell 2003; Rodriguez et al. 2008; Cockburn et al. 2014). The poor adoption of push-pull technology amongst coastal sugarcane farmers is thought to be a result of the lack of push-pull research being conducted in the area.

This study aimed to assess the efficacy of push-pull technology, as well as its ease of implementation, in the coastal regions of KZN using on-farm field trials. The field trials were conducted on selected model farms and were used to assess the impact of push-pull on infestation levels of *E. saccharina*. The working model for the implementation of push-pull in the Midlands North region, as developed by Cockburn (2013), was reviewed and improved to suit the management activities of coastal sugarcane farmers. Furthermore, the information gathered in this study, will be used to help local farmers make more informed decisions, and offset any concerns regarding the perceived risk of adopting AW-IPM technologies with a push-pull component.

MATERIALS AND METHODS

Five farms from two coastal sugarcane growing regions were selected for on-farm push-pull trials. Two were located in the North Coast and three in the South Coast region of KZN (Table 1). The farms were selected for high levels of *E. saccharina*, and were in different homogenous climate zones, or ecozones (Figure 1). Ecozones are characterised by similar soil substrata, annual rainfall, altitude, and proximity to the coast. Thus, the results reflected the efficacy of push-pull (as a component of AW-IPM) on a wide range of coastal farms.

The push-pull sites on each farm were selected for suitability of topography. Trial fields needed appropriate contour banks for the planting of *M. minutiflora*, as the 'push' component in the push-pull trials. Contours had to run parallel to a wetland area, which could be rehabilitated into a 'pull site' through the planting of wetland sedges, *C. papyrus* and *C. dives* (Figure 2 A–E). All model farms had historically high *E. saccharina* levels (> 10 larvae per 100 stalks) or were at risk of developing high *E. saccharina* infestations. It was also important to select farms whose owners/managers were committed to implementing pushpull. This ensured the study was conducted smoothly and that trials were correctly maintained. Farmers willingly provided labour and equipment to prepare and manage the sites. They also ensured that all push-pull components were planted, watered, and supplied with any necessary fertilisers or herbicides.

Experimental design

A multiple before-after-control-impact (mBACI) experimental design was used for this study (Downes et al. 2002). Before-aftercontrol-impact designs are an effective method for evaluating environmental changes caused by natural and human-induced disturbances (Conner et al. 2016). They are typically used in large-scale ecological studies when treatment sites cannot be randomly chosen (Conner et al. 2016), as they can isolate the effect of the impact from natural variability, especially if the timing and location of the impact are known and adequate predata are collected (Smokorowski and Randall 2017). According to Conner et al. (2016), mBACI is particularly beneficial if impacted and control sites are treated as fixed effects, with

Table 1. Characteristics of farms and fields chosen for push-pull trial sites in the North and South Coast of KZN

	Kahlamba Estate	Evelyn Park	Glen Rosa	Sezela MCP	Ellingham Estate
GPS co-ordinates	29°16′16.4″ S, 31°19′34.3″ E	29°24′32.5″ S, 31°02′22.7″ E	30°17′53.5″ S, 30°30′08.2″ E	30°24′51.6″ S, 30°39′21.8″ E	30°19′33.6″ S, 30°42′20.3″ E
Region	North Coast	North Coast	South Coast	South Coast	South Coast
Fields used in study	B21, B23	CE15, CE13, CE11	2, 7A	41, 42	28, 53
Push-pull field size	13.8 ha	14.6 ha	10 ha	10.4 ha	12.8 ha
Soil types	Natal-Group Sandstone	Oakleaf, Swartland	Glen Rosa, Clovelly	Glen Rosa	Glen Rosa, Cartref, Granite
Sugarcane varieties	N29, N12	N37	N12	N12	N12, N21, N39

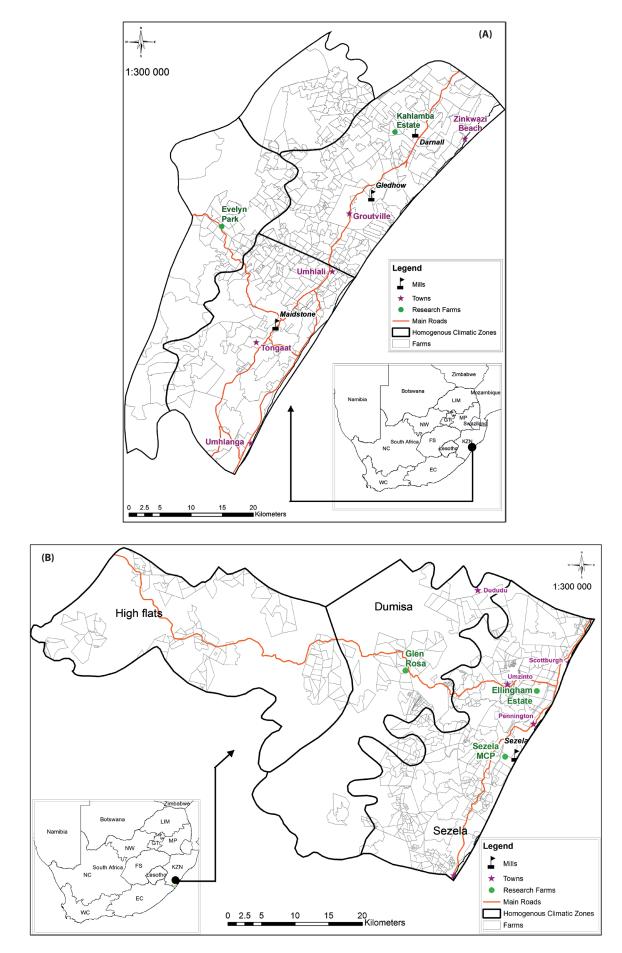


Figure 1. Map of the North Coast (A) and South Coast (B) sugarcane growing regions of KZN, with push-pull trial/research farms indicated in green: Evelyn Park and Kahlamba Estate on the North Coast; Glen Rosa, Sezela MCP and Ellingham Estate on the South Coast.

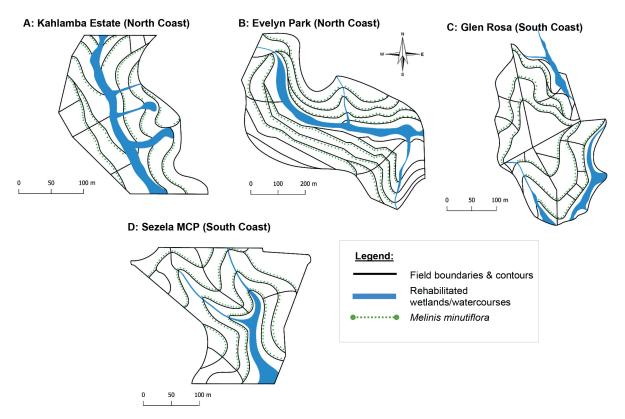


Figure 2. Maps showing the spatial arrangement of push-pull treatment areas on trial farms.

sampling being conducted at simultaneous (paired) time periods at each site, both before, and after disturbance. Unfortunately, a lack of replication in treatment and control areas, and a high degree of natural variability in environmental conditions, can hinder the detection of responses in typical BACI designs (Underwood 1994; Loughin et al. 2018). The inclusion of multiple sites and survey times can be used to improve upon unsuitable spatial and temporal replication (Roberts et al. 2007). Thus, the replicated mBACI design allowed us to separate treatment effects from natural processes with relatively high confidence (Downes et al. 2002).

Layout and preparation of push-pull treatment sites

To ensure a strong mBACI design, both the treatment and control sites on each farm contained sugarcane of a similar age, variety, and ratoon cycle. Efforts were made to ensure that the areas were located along the same water course, that they had similar topographical characteristics (such as slope and aspect) and that they were approximately the same size. The Evelyn Park site had an additional push-pull treatment site, so that six treatment sites, and five control sites were surveyed in total. The data from each of the sites were combined and means calculated accordingly, with each site serving as a replicate in compliance with the mBACI design.

In June, July and August 2014 *M. minutiflora* seedlings were delivered to the farmers for planting at treatment sites. Areas where *M. minutiflora* was to be planted had been mapped out prior to delivery. Seedlings were planted approximately 1 m apart in the centre of the contours. This arrangement was based on recommendations from the Midlands North region (Cockburn 2013). The central placement of *M. minutiflora* seedlings allowed farmers to access their contours for transport without damaging young plants. Planting began during the dry winter season of June–September 2014. *Melinis minutiflora* was planted with an absorbing agent (Grovida AQUA-STOR KM[~]), which facilitated water retention around the seedlings until the summer rains began. The grass at three push-pull treatment sites (Ellingham Estate, Evelyn Park and Kahlamba Estate) had to be gap-planted

(planting of additional seedlings to improve stand count/cover abundance) later in the season to account for seedling mortality.

Wetlands and water courses at each of the push-pull sites were rehabilitated by removing sugarcane and invasive plants growing there. Some farms (Evelyn Park, Kahlamba Estates and Glen Rosa) already had sedges growing on other parts of the property. Sections of these sedges were transplanted to wetlands at the push-pull sites. Additional pull plants, namely *C. papyrus* and *C. dives*, were transported from the Midlands North area and planted at the remaining two treatment sites (Sezela MCP and Ellingham Estate). Residual plants were distributed between Evelyn Park, Kahlamba Estates and Glen Rosa, thereby augmenting existing sedge populations.

Three out of five farmers (Kahlamba Estate, Evelyn Park and Ellingham Estate) opted not to use *Bt*-maize at their treatment sites as it was expensive and management intensive. Further, it is also prone to destruction by wild bush pigs, *Potamochoerus larvatus* Cuvier (Artiodactyla: Suidae), which are prevalent on farms in KZN (Ramesh and Downs 2015). The goal of this study was to tailor push-pull to suite the farmers wants and needs, as such we decided to forgo the use of *Bt*-maize on these farms. Two farms in the south coast region (Glen Rosa and Sezela MCP) already had *Bt*-maize growing near treatment sites. The maize may have acted as an additional attractant to *E. saccharina*, but it was not specifically maintained as part of the push-pull trials.

Assessment of Eldana saccharina infestation and damage

Surveys for *E. saccharina* infestation and damage were completed at each treatment and control site, with the help of biosecurity teams from SASRI. Due to high levels of *E. saccharina*, sugarcane in the coastal regions is typically harvested at 12–15 months instead of the recommended 18–24 months (Rostron 1972). Therefore, each control and treatment site were only surveyed twice, in a manner that accounted for the age of the sugarcane, time of moth peaks and harvesting schedules. In accordance with the mBACI design, both the control and pushpull sites were subject to a pre-trial (before) and post-trial (after) assessment/survey. Thus, the first survey was conducted at each of the treatment and control sites before push-pull had been implemented. This survey was completed in September, October and November 2014, after which the fields were harvested. Pushpull trials were then established, and the harvested sugarcane was allowed to ratoon and grow as per the farmers' schedule. The fields were sampled a second time, in Nov/Dec 2015. These dates ensured that the push-pull trial had been running for more than a year so that the sugarcane was mature and was at the right age for sampling and harvesting.

For the surveys, 200 stalks were randomly selected per treatment and control site on each farm. Additional surveys were done at Evelyn Park to account for the added push-pull treatment site. Stalks were randomly chosen by walking along the contour banks and selecting 10 stalks every 50 m. Five stalks were selected amongst the first three rows and another five were selected from the centre of the field/panel. This ensured that most of the field was surveyed and eliminated edge effect as a confounding factor. A total of 4400 stalks were sampled throughout the sampling period.

Sugarcane stalks were split along their length and inspected for stemborer damage. The total number of internodes, as well as the number of damaged internodes, were counted and recorded per stalk selected (Barker et al. 2006; Cockburn 2013). The damage patterns of E. saccharina and Sesamia calamistis Hampson (Lepidoptera: Noctuidae) (a minor pest of sugarcane) are similar. The larvae of both species bore into sugarcane stalks and feed on the internal tissues, creating tunnels within the stem that are typically filled with frass and are discoloured due to secondary fungal infections. (Girling 1971; Carnegie 1974 Schulthess et al. 2002; Way and Goebel 2003). Due to the similarities, all damage typical of these two species was recorded broadly as stemborer damage. This is reflected in the results. As a minor pest of sugarcane, S. calamistis damage is far less frequent, extensive, and destructive than E. saccharina (Rutherford 2015) and thus would have had little impact on the overall distribution of the data. Any larvae found were placed in labelled 30 ml plastic vials with gauze lids. The vials contained 8 ml of artificial diet (Gillespie 1993). The collected larvae were transported to the SASRI insect rearing unit and placed in a quarantine room with a controlled temperature of 28 °C and relative humidity of 75%. The larvae were monitored until the moths and/or parasitoids emerged. Moths were identified and any parasitoids that emerged from the larvae or pupae were preserved for identification at SASRI.

Assessment of *Melinis minutiflora* edge effect and biomass effect

To determine whether the repellent properties of *M. minutiflora* decreased with increasing distance from the grass, an edge effect analysis was completed in Nov/Dec 2015. *Eldana saccharina* damage and infestation data were collected in a similar manner as discussed above, but only on Kahlamba Estate, Evelyn Park, and Ellingham Estate. The efficacy of *M. minutiflora*, as a repellent, was determined by comparing stemborer damage and infestation levels from sugarcane rows on the edge of the field (where *M. minutiflora* was planted), to rows in the centre

of the field (where there was no *M. minutiflora*). Twenty stalks were sampled per panel of sugarcane selected. Ten stalks were sampled from the edge of the field, where sugarcane grew alongside *M. minutiflora* contours. A further ten stalks were taken from the centre of the field. This was repeated five times at random points for each push-pull site, with a total of 100 stalks sampled per site. The samples were taken approximately two weeks after the final *E. saccharina* assessments were completed.

Percentage seedling establishment of M. minutiflora was recorded to determine whether biomass had any effect on stemborer damage. This was done by assessing how many planted seedlings established successfully and by calculating the plant cover abundance of M. minutiflora stands (Mueller-Dombois and Ellenberg 1974; Cockburn 2013). These results were then tested against stemborer damage and infestation levels. Percentage seedling establishment was recorded in December 2015 by walking the length of the contour banks containing M. minutiflora and counting the number of plants established per running metre. Cover abundance was estimated using the Braun-Blanquet method for five contour banks planted to M. minutiflora per farm. In the Braun-Blanquet method, the cover abundance of species (in one-metre quadrats) is estimated by a single assessor using a scale of classes (Table 2) (Mueller-Dombois and Ellenberg 1974; Cockburn 2013). Twenty quadrats were assessed using the Braun-Blanquet method per contour on five contour banks per farm. The mean cover abundance was calculated by averaging the Braun-Blanquet scores for M. minutiflora across all quadrats.

Determining the efficacy of wetland sedges to attract *Eldana saccharina*

To verify whether 'pull' plants were successfully attracting gravid E. saccharina moths, surveys were conducted in rehabilitated wetlands at each farm. Sedges were sampled in Nov/Dec 2015 while E. saccharina surveys were done in adjacent sugarcane. To avoid undermining rehabilitated wetlands through destructive sampling, only 50 randomly selected plants from each 'pull' species (C. dives and C. papyrus) were sampled at push-pull sites. The plants' umbels, stalks and rhizomes were assessed for presence of stemborers, and/or parasitoids. The number of damaged plants per sample was recorded as well as stemborer presence. Stemborers and parasitoids were collected and transported to the SASRI quarantine facility. The insects were reared to determine species and any parasitoids found were preserved for identification. Levels of E. saccharina infestation and damage in wetlands were compared to the levels found in sugarcane to determine whether the wetlands were successfully attracting gravid E. saccharina moths.

Statistical analysis

The percentage of stalks damaged, mean percentage of internodes damaged, and the number of *E. saccharina* found per 100 stalks (E/100 stalks) were calculated (Leslie 2009; Rutherford 2015). Before the analyses were conducted, the data were assessed for normality using the Shapiro-Wilks test and were found to be normal. Due to the paired nature of the before-after data that

Table 2. The Braun-Blanquet scale as used to estimate the cover abundance of Melinis minutiflora along contour banks at push-pull trial sites

Braun-Blanquet Class	Range of plant cover in quadratic area (%)	
5	75–100 %	
4	50-75 %	
3	25–50 %	
2	5–25 %	
1	1–5 %	
r	< 1 %	
Note: For the purpose of this analysis, r was ignored and co	onsidered insignificant (Mueller-Dombois and Ellenberg 1974).	

were collected, a paired *t*-test was performed on the percentage of stalks damaged at push-pull treatment and control sites to test for significant differences (p < 0.05). This was also done for mean number of E. saccharina found per 100 stalks at each of the sites, as well as the mean percentage of internodes bored. The average treatment effects were also calculated for each of these variables. Treatment effects for the mBACI design were estimated by calculating mean difference (MD) between treatment and control sites after treatment, minus the mean difference between treatment and control sites before treatment (MD_{treat-} control(after) - MD_{treat-control(before)}) (Stewart-Oaten et al. 1986; Bence et al. 1996). A Spearman's rank-order correlation assessed the relationship between M. minutiflora cover abundance, percentage plant establishment, and the percentage of internodes damaged by stemborers. Graphs and statistical analyses were generated using Microsoft Excel and RStudio v. 3.6.

RESULTS

Percentage of stalks damaged

The percentage of damaged sugarcane stalks across all treatment sites decreased significantly (p = 0.035) after push-pull was implemented (Figure 3A). In comparison to treatment sites, the percentage of stalks damaged before and after the trials were conducted did not differ significantly at control sites (p = 0.669) (Figure 3A). The overall average treatment effect of the push-pull treatment on percentage stalk damage was negative (-25.1% stalks damaged). This demonstrates that push-pull was able to decrease the percentage of sugarcane stalks damaged by *E. saccharina* relative to control sites.

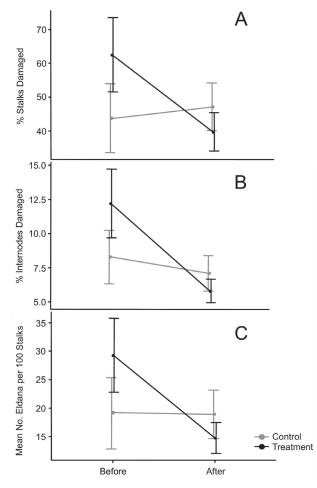


Figure 3. mBACI analyses showing change in percentage of stalks damaged (A), mean percentage of internodes damaged (B), and *Eldana saccharina* infestation levels per 100 stalks (C), in push-pull treatment and control sites, over the full study period, across all model farms. Error bars represent standard error (SE).

Mean percentage of internodes damaged per sugarcane stalk

The mean percentage of internodes damaged per sugarcane stalk across all treatment sites also decreased significantly (p = 0.022) after push-pull was implemented (Figure 3B). At the control sites, there were no differences recorded between the mean percentage of internodes damaged per sugarcane stalk before and after the trials were conducted (p = 0.468) (Figure 3B). The overall average treatment effect of the push-pull treatment on mean percentage internodes damaged per sugarcane stalk across all model farms was again negative (-5.194% internodes damaged). Data indicate that push-pull decreased the mean percentage of internodes damaged per sugarcane stalk.

Eldana saccharina infestation levels

Significant reductions in *E. saccharina* larvae found per 100 stalks occurred in push-pull treated sites (p = 0.018) (Figure 3C). On average, *E. saccharina* infestations across all the model farms decreased by more than 35% at sites where push-pull was implemented (Figure 3C). Control sites showed little to no difference in *E. saccharina* numbers after the trials had been completed (p = 0.958) (Figure 3C). Similar to the other measures used above (percentage stalk damage and mean percentage of internodes damaged) to determine stemborer infestations, the overall average treatment effect of the push-pull treatment on number of E/100 stalks was negative (-14.3 E/100 stalks). Thus, push-pull treatments reduced the average population of *E. saccharina* larvae infesting sugarcane stalks across all model farms.

Effect of *Melinis minutiflora* on *Eldana saccharina* damage and infestation levels

Stalks growing closest to contours containing M. minutiflora had fewer damaged internodes than those growing further away (Figure 4). At Kahlamba Estate and Evelyn Park, the inner row of sugarcane (where no M. minutiflora was planted) had significantly more damaged internodes than the outer row (where M. minutiflora was planted) (Figure 4A and 4B). These differences were not significant at Ellingham Estate (Figure 4C). Melinis minutiflora at this farm had a lower overall cover abundance than at the other two farms (2.95 on the Braun-Blanquet scale), which could explain discrepancies (Figure 5). Mean percentage seedling establishment of the grass at Ellingham Estate (79.8%), while high, was also lower than the other farms (Figure 5). The mean percentage establishment of the M. minutiflora in the contours at Kahlamba Estate and Evelyn Park was 90.1% and 81.7%, respectively (Figure 5). The number of E/100 stalks similarly increased from the outer rows to the inner rows of sugarcane, although overall these differences were not significant (t = 1.84; p = 0.076) (Figure 6). Overall, increasing distance from M. minutiflora was associated with an increase in E. saccharina damage and infestation levels.

Although extension personnel and farmers use the first three measurements to inform their threshold-based decisions, mean percentage of internodes damaged is considered a more reliable estimation of *E. saccharina* infestations than percentage stalks damaged, or number of larvae found per 100 stalks (Leslie 2008). As such, this measurement was selected to test the relationship between *E. saccharina* infestation and *M. minutiflora* biomass. The Spearman's rank order correlation, testing the relationship between *M. minutiflora* plant establishment and the percentage of sugarcane internodes damaged, was negative at all three model farms sampled (Table 3). As *M. minutiflora* establishment increased, the percentage of internodes damaged decreased at all push-pull sites (Table 3). However, the relationship was weak at Kahlamba Estate and Evelyn Park, and not significant

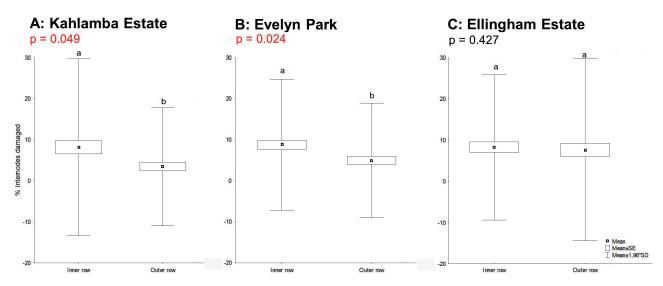
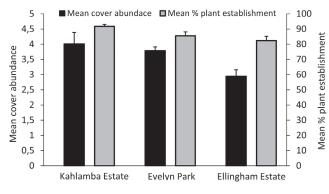


Figure 4. Box and whisker diagram comparing the percentage of internodes damaged in the outer and inner rows of push-pull sugarcane fields, planted with *Melinis minutiflora*, on three model farms: Kahlamba Estate (A), Evelyn Park (B) and Ellingham Estate (C). Box represents SE, error bars indicate standard deviation (SD) and letters indicate significant differences between means (Mann-Whitney U, p < 0.05).



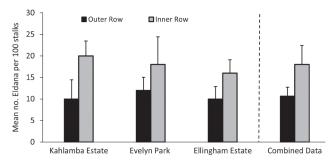


Figure 5. The Braun-Blanquet mean cover abundance levels (left y-axis) and % seedling establishment (right y-axis) of *Melinis minutiflora* at 3 of the model farms; Kahlamba Estate, Evelyn Park and Ellingham Estate. Error bars represent SE.

Figure 6. A comparison of the mean number of *Eldana saccharina* larvae found per 100 stalks in the outer and inner rows of push-pull sugarcane fields, planted with *Melinis minutiflora*, located at three of the model farms: Kahlamba Estate, Evelyn Park and Ellingham Estate. Error bars represent SE.

Table 3. Spearman's rank order correlation showing relationships between mean % internodes damaged and % plant establishment and mean cover abundance of *Melinis minutiflora*. (Significant correlations are indicated in bold font).

Sample:	Spearman's R _s	P-value
Model farm A: Kahlamba Estate:		
Mean % internodes damaged and % <i>M. minutiflora</i> plant establishment	-0.175	0.083
Mean % internodes damaged and <i>M. minutiflora</i> cover abundance	-0.382	<0.001
Model Farm B: Evelyn Park:		
Mean % internodes damaged and % <i>M. minutiflora</i> plant establishment	-0.079	0.436
Mean % internodes damaged and <i>M. minutiflora</i> cover abundance	-0.296	0.003
Model Farm E: Ellingham Estate:		
Mean % internodes damaged and % <i>M. minutiflora</i> plant establishment	-0.289	0.004
Mean % internodes damaged and <i>M. minutiflora</i> cover abundance	-0.235	0.019

(p > 0.05) (Table 3). At Ellingham Estate, *M. minutiflora* plant establishment had a more meaningful effect on the mean percentage of internodes damaged (Rs = -0.289 and p < 0.05) (Table 3). The relationship between *M. minutiflora* cover abundance and percentage of damaged internodes was also monotonic. Damage decreased significantly as cover abundance of the grass increased at all three sites.

Determining the efficacy of wetland sedges to attract *Eldana saccharina*

Sedges were sampled in Nov/Dec 2015 and recorded as damaged if either the umbel, stem or rhizome had evidence of stemborer feeding. The *C. dives* stand at Ellingham Estate had the highest percentage of damaged sedges (Figure 7) and the

highest levels of E/100 stalks (Figure 8). On average, more than one larva was found per plant at this farm. Glen Rosa had the highest percentage of plants damaged amongst the *C. papyrus* stands (Figure 7). The incidence of damage and the number of *E. saccharina* larvae found in *C. dives* plants was also high at Glen Rosa (Figures 7 and 8). All farms experienced high levels of plant damage and high levels of *E. saccharina* in both *C. dives* and *C. papyrus* stands. The pull plant, *C. dives*, had greater levels of damage and infestation than its relative *C. papyrus*. Only two farms had stands of *C. papyrus* that had higher levels of stemborer damage than adjacent *C. dives* plants. These were located at Sezela MCP and Evelyn Park. However, higher numbers of *E. saccharina* larvae were found in *C. dives* plants than in *C. papyrus* plants at all push-pull sites.

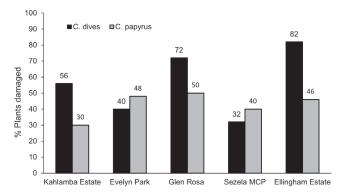


Figure 7. Comparison of percentage stalks damaged in push-pull treated fields and in *Cyperus dives* and *Cyperus papyrus* fields in corresponding rehabilitated wetland areas, at each of the model farms.

Eight different *E. saccharina* parasitoid species emerged from larvae collected in the rehabilitated water courses on model farms. Three were recorded at Glen Rosa, two from Evelyn Park, two from Ellingham Estate, and one from Kahlamba Estate.

DISCUSSION

Using mBACI analysis to determine the efficacy of using push-pull interventions against *Eldana saccharina*

The influence of push-pull on E. saccharina populations at each of the model farms in the current study was variable. Different fields and farms have different soils, topography, water and nutrient availability, climatic factors, and ratoon cycles, all of which can affect E. saccharina numbers and damage within sugarcane (Nuss et al. 1986). Farm management can also affect populations of E. saccharina, with varietal choice, sanitation practices and sugarcane age all playing a role in pest infestation (Cockburn 2013). Because of the high level of variability within and between farms, it was necessary to compare the before and aftereffects of push-pull management at the treatment sites, and to compare these results to carefully monitored control sites, where no E. saccharina management was being implemented. A multiple before-after-control-impact (mBACI) analysis is a useful tool for assessing the implementation of AW-IPM strategies, across different sites, because it controls for spatial-temporal variability and can thus determine the effects of an impact/treatment more accurately (Underwood 1992; Conner et al. 2016)

Using the mBACI approach, we demonstrated that push-pull treated sugarcane sites experienced a significant reduction in *E. saccharina* damage and population levels. This increases the evidence that farmers can successfully manage infestations of *E. saccharina* using push-pull (as part of an AW-IPM approach), as shown by Kasl (2004), Barker et al. (2006) and Cockburn (2013). This study also demonstrates that in addition to Pongola (Kasl 2004) and the Midlands North region (Barker 2008; Cockburn 2013), the technology is also applicable to the North and South Coast sugarcane growing regions of KZN.

Impact of push-pull on *Eldana saccharina* damage in sugarcane

Four of the model farms were located in areas where high levels of stemborer damage and E/100 stalks are common. The mean percentage of stalks damaged in treatment sites at the commencement of the study was 8.5% higher than the economic injury level (EIL) of 54% stalk damage (Goebel et al. 2005; Leslie 2009). Almost all the fields sampled at the beginning of the study had damage and infestation levels that exceeded recommended thresholds. Fields that did not exceed the threshold level were at a high risk of doing so if the sugarcane had not been harvested. The only exceptions being sites located at Glen Rosa. These had low numbers of *E. saccharina* and the damage was well below the

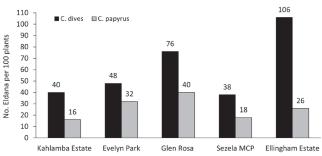


Figure 8. Mean *Eldana saccharina* infestation levels per 100 plants sampled in sedges from rehabilitated wetlands areas and in sugarcane from adjacent push-pull sites, at each of the model farms.

EIL (Mulcahy 2018). Whilst Glen Rosa falls under the South Coast sugarcane growing region, it is located more inland, where altitude, lower winter temperatures, better soil conditions and improved water availability (Le Roux 1993) help to keep the *E. saccharina* numbers low (Dick 1945; Way 1994; Rutherford 2015).

Push-pull was able to significantly reduce the number of stalks damaged across treatment sites. In fact, the percentage of stalks damaged at treatment sites decreased to below the EIL. The potential of push-pull to reduce damage to below economic threshold levels means that farmers should be able to grow their sugarcane for longer periods of time before harvesting (to improve sucrose yields) (Rostron 1972; Rutherford 2015). This has beneficial cost implications for many coastal sugarcane farmers, who are financially constrained by having to harvest their sugarcane at 12-15 months, instead of the recommended 18-24 months (Bezuidenhout et al. 2002; Inman-Bamber 1991; Ramburan 2015). This is especially important during drought years when sugarcane becomes water-stressed and more susceptible to stemborer infestations (Girling 1978; Way and Goebel 2003; Gounou and Schulthess 2004). Poor rainfall in the 2015 season meant that coastal areas of KZN were experiencing a drought at the time of the study (Singels et al. 2016). Push-pull not only reduced stalk damage but may have prevented E. saccharina from taking advantage of water-stressed sugarcane plants.

Percentage stalk length red (SLR) is closely related to the percentage of internodes damaged per stalk. It provides an accurate measure of the history of *E. saccharina* within a sugarcane field (Leslie 2008). Damage done by stemborers (and subsequent tissue discolouration caused by secondary fungal infections) is still evident within the stalk even after larvae have pupated and left the plant (Leslie 2008). Push-pull treatment sites showed a significant decrease in the number of internodes damaged per stalk sampled. Once the study had been completed, the percentage of damaged internodes decreased to below the EIL of 7% SLR (Leslie 2009). This again signifies that push-pull can be used as a management tool for *E. saccharina* in the KZN coastal regions.

Impact of push-pull on *Eldana saccharina* populations in sugarcane

Most sites sampled exceeded the *E. saccharina* economic threshold level of 10 E/100 stalks (Leslie 2009) at the commencement of the study. Such numbers are severely damaging to sugarcane and can also pose a risk to nearby fields (Atkinson 1981). Treatment sites saw a dramatic decrease in the mean number of *E. saccharina* found per 100 stalks after push-pull was implemented, while control sites experienced almost no reduction in the number of larvae found. Previous work showed similar reductions in *E. saccharina* populations in push-pull treated plots (Kasl 2004; Barker et al. 2006; Barker 2008; Cockburn 2013). It can therefore be concluded that push-pull did have a meaningful impact on populations of the stemborer within treated fields.

Melinis minutiflora impacts on Eldana saccharina infestation levels and damage in adjacent sugarcane

Our results indicate that E. saccharina damage and infestation levels increase with increasing distance from M. minutiflora. At Kahlamba Estate and Evelyn Park there was a significant increase in the percentage of internodes damaged in sugarcane growing further away from M. minutiflora plantings. Although more studies need to be conducted to confirm our findings, this study indicates that M. minutiflora may be repelling the pests away from the sugarcane growing closest to the grass. Whilst this demonstrates the efficacy of M. minutiflora, farmers could also benefit by planting more of the grass in other areas of their farms. Barker et al. (2006) suggested that, in addition to using contours to implement push-pull, farmers could replace every 20th row of sugarcane with a strip of M. minutiflora. Increasing in-field abundance of M. minutiflora would multiply the effects of beneficial, deterrent semio-chemicals within the agroecosystem (Barker et al. 2006). This would provide field-wide protection, so that all sugarcane is under push-pull management. Melinis minutiflora does not compete with sugarcane and such actions would not be detrimental to the crop (Barker et al. 2006). The economic benefits of planting extra rows of M. minutiflora, and the additional weed suppressing capabilities of the grass (Conlong and Campbell 2010), would make-up for loss of income resulting from the removal of single rows of sugarcane (Barker et al. 2006).

In this study, biomass of M. minutiflora within push-pull trials was high. The plant establishment and cover abundance of the grass was good at all three of the farms sampled. Even Ellingham Estates, with the lowest overall biomass, had better establishment and cover abundance of M. minutiflora than most of the push-pull farms in the study by Cockburn (2013). Weekly watering of the grass at the beginning of the trial ensured that plants established and grew, despite the region experiencing drought conditions. Thus, watering at the seedling stage is recommended to farmers wishing to plant this grass as part of a push-pull programme in the coastal sugarcane belt. At all the push-pull sites sampled, an increase in M. minutiflora aboveground biomass was correlated with a significant decrease of E. saccharina damage in sugarcane. Although the correlation was weak, the negative relationship between M. minutiflora cover abundance and percentage of internodes damaged was significant, especially with good seedling establishment.

Impact of indigenous host plants in rehabilitated wetlands on *Eldana saccharina* populations and damage in adjacent sugarcane

Pull plants are integral to push-pull systems and are planted within the agroecosystem to attract pests away from the crop (Khan et al. 2000). Eldana saccharina is native to wetland habitats, therefore, its indigenous host plants C. papyrus and C. dives were used as pull plants and planted in rehabilitated water courses on model farms (Conlong 2001; Kasl 2004). At all the farms sampled, levels of damage and infestation in sedges were consistently high. This confirms the attractiveness of these plants to E. saccharina, as demonstrated by Kasl (2004). In the current study damage levels were generally higher in C. dives, and at all treatment sites more larvae were found in C. dives than in C. papyrus. In the Midlands North region, the majority of E. saccharina were also found in C. dives (Cockburn 2013). This indicates that C. dives is the preferential host plant of the stemborer in these regions. However, C. dives only flowers in the summer, whilst C. papyrus flowers throughout the year (Carruthers 1997). Since E. saccharina usually feeds on the umbels of these plants (Conlong 1990), C. papyrus likely provides a consistent year-round food source for the pest. Thus, C. papyrus remains an important pull plant, and farmers are encouraged to plant both sedges as part of a push-pull strategy.

Some farmers were hesitant to plant sedges on their farms, for fear of creating a refuge for E. saccharina, which may result in future infestations. The results gathered here suggest that sedges act as a sink, not a refuge. Data from Glen Rosa show that E. saccharina numbers in both C. dives and C. papyrus were high. This contrasts with data gathered from sugarcane at Glen Rosa, which had comparatively low levels of E. saccharina infestation and damage (Mulcahy 2018). If wetland sedges acted as a reservoir for E. saccharina, pest levels within the sugarcane would be much higher. Previous studies showed that there is a high degree of E. saccharina parasitism in indigenous hosts (Conlong 1990). In comparison to this, natural enemy abundance, and levels of parasitism in sugarcane are very low (Conlong and Kasl 2001). Conlong (1990, 2000) revealed that a complex of parasitoids and other natural enemies (pathogens, predators, fungi, and nematodes) readily attack populations of E. saccharina within indigenous host plants in South Africa, and other African countries. This helps to control and maintain pest levels in wetlands and prevents them from re-infesting nearby sugarcane (Assefa et al. 2006), a very valuable ecological service.

Eight different parasitoid species emerged from E. saccharina larvae collected during the study. This serves as confirmation that natural enemies are present and active in indigenous host plants at these push-pull sites. This builds on the work of Conlong (1990), who showed that E. saccharina was targeted by nine indigenous parasitoid species in a C. papyrus dominated wetland in northern KZN. Additionally, M. minutiflora inter-cropping not only decreases stemborer infestations in cereal crops, but also increases larval parasitism (Khan et al. 1997b). Volatiles emitted by the grass, which repel gravid moths, contain components that simultaneously attract parasitoids, thereby increasing parasitism within the surrounding area (Khan et al. 1997b). Conlong and Kasl (2001) found that parasi-tism of E. saccharina by the parasitoid Xanthopimpla stemmator Thunberg (Hymenoptera: Ichneumonidae), increased in sugarcane when M. minutiflora was present. Parasitoid numbers could potentially increase and impact E. saccharina populations in sugarcane, if push-pull technology is maintained and increased in the area.

Both conventional and Bt-maize is attractive to E. saccharina and can be used as a pull plant in areas without adequate water courses to plant sedges (Keeping et al. 2007). However, the model farmers at Kahlamba Estate, Evelyn Park and Ellingham Estates decided that Bt-maize was too costly and time consuming to be used as a trap crop in push-pull trials. The efficacy of Bt-maize in push-pull systems is also short lived. It must be replanted for it to be effective over more than one moth peak (Cockburn 2013). The viability of growing maize as part of a push-pull system was also questioned by other large-scale farmers in the coastal sugarcane growing regions (Mulcahy 2018). Although there is not enough evidence here to rule out the usefulness of Bt-maize, the results indicate that push-pull can function effectively with or without maize, if a strong 'pull' factor is developed through rehabilitation of wetlands. Bt-maize is still a tool for farmers who want to implement pushpull, but who do not have suitable wet areas for C. papyrus and C. dives plants (Cockburn 2013). Furthermore, the Bt-maize at Sezela MCP was destroyed by bush-pigs, which are known pests of maize in KZN (Ehler-Smith 2016). Farmers using Bt-maize for push-pull may have to employ additional pest management strategies to safeguard the plantings.

CONCLUSION

The inclusion of push-pull as a component of a well implemented AW-IPM programme is essential, as push-pull technology is knowledge intensive. The technology, for example, must be timed properly so that it can impact gravid moths, and so that vulnerable ageing sugarcane is protected correctly (Kasl 2004;

Barker 2008; Cockburn 2013). Farmers need to be aware of E. saccharina biology, so that they can successfully coordinate the planting of push-pull plants. Push-pull plants need time to grow and mature before E. saccharina moth peaks occur in April and November (Carnegie and Leslie 1990; Cockburn 2013). This linked to good agronomic and sustainable control practices (Conlong and Rutherford 2009; Rutherford 2015), will substantially increase the control of E. saccharina in southern African sugarcane fields. Evidence from the model farms shows that, when implemented correctly, push-pull technology is an effective tool for the management of E. saccharina in coastal sugarcane. The model farms experienced a marked decrease in the levels of E. saccharina infestation and damage at pushpull treatment sites. The results also show that M. minutiflora, C. papyrus and C. dives are successful push-pull plants, both in repelling and attracting E. saccharina away from protected sugarcane fields. The discovery of parasitoids in E. saccharina larvae within wetland sedges, demonstrates the potential benefits that push-pull has on the recruitment of natural enemies. However, more can be done to conserve and augment populations of these parasitoids in the sugarcane agroecosystem, to improve pest management through biological control. More rigorous studies are required to test the repellent capabilities of M. minutiflora on large-scale sugarcane farms and multi-year trials are needed to assess the long-term impacts of push-pull systems on pest populations and sugarcane yield.

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AUTHOR CONTRIBUTIONS

Des Conlong and Megan Mulcahy conceived and developed the original idea as well as the methodology for the study. Des Conlong provided contacts for collaborators and other stakeholders. Martin Hill was responsible for funding acquisition and supervised the project. Megan Mulcahy and Des Conlong conducted experiments, collected data and carried out labotratory work. Megan Mulcahy curated and analysed data and wrote the manuscript with input from the other authors, who reviewed and edited the final draft. All authors provided critical feedback and helped shape the research, analysis and manuscript.

CONFLICTS OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

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