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# Impact of push–pull cropping system on pest management and occurrence of ear rots and mycotoxin contamination of maize in western Kenya

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## Abstract

Push–pull involves intercropping of cereals with *Desmodium* as a "push" crop and planting Napier grass/*Brachiaria* as the "pull" crop at the border. The technology has been reported to effectively control stemborers, striga weed, and fall armyworm (FAW), and to improve soil nutrition, resulting in increased grain yield. This study evaluated the impact of stemborer and FAW management using this technology on incidence of maize ear rots and preharvest contamination of grains with aflatoxin and fumonisin in western Kenya. The study was conducted during three cropping seasons on maize grown under the push–pull system and as a monocrop. Incidence of stemborer and FAW damage was determined as percentage of damaged plants, while incidence of ear rots was determined as percentage of ears with symptoms. At harvest, fungi were isolated from kernels and aflatoxin and fumonisin were quantified using enzyme-linked immunosorbent assay. Stemborer and FAW damage was significantly ($p = .001$) reduced by over 50% under the push–pull system. There was also a significant ($p < .001$) reduction in the incidence of *Fusarium verticillioides* (60%) and *Aspergillus flavus* (86%), which was reflected in a reduced incidence of ear rots (50%) with the push–pull system ($p = .001$). Fumonisin in maize from push-pull farms was significantly ($p = .048$) reduced (39%) but the technology had no significant ($p > .05$) effect on aflatoxin. The study showed that push–pull is an effective strategy for managing maize ear rots and fumonisins, and therefore could play a role in improving food safety among smallholder maize farmers in the region.

**KEYWORDS**
aflatoxin, ear rots, fall armyworm, fumonisin, push–pull cropping system, stemborer

## 1 | INTRODUCTION

The complex interactions of climate factors, insect infestation, and pre- and postharvest handling have been associated with

fungal infection of maize, *Zea mays* (Miller, 2008; Vacher *et al.*, 2008; Fountain *et al.*, 2014). Climate change greatly influences the levels of mycotoxins as a result of erratic rainfall or recurrent drought, which in turn influences temperature, moisture, and relative humidity,

which are some of the main ecological factors that influence mycotoxin contamination in grains (Miller, 2008; Fountain *et al.*, 2014). *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp. are the commonly isolated fungal genera in maize (Mukanga *et al.*, 2010). Among the *Fusarium* spp., *Fusarium verticillioides* is the most commonly isolated species from maize worldwide (Mukanga *et al.*, 2010; Nyangi, 2016).

Infection of maize ears by *F. verticillioides* at any growth stage can cause ear rot and has been associated with fumonisin contamination of maize (Bigirwa *et al.*, 2007; Mukanga *et al.*, 2010). Fumonisins, mainly produced by *F. verticillioides*, and to a lesser extent by *F. proliferatum*, are a group of economically important mycotoxins produced by *Fusarium* spp. (Leslie and Summerell, 2006) that are associated with human oesophageal and liver cancer (Sun *et al.*, 2007; Burger *et al.*, 2017).

In contrast, symptoms of ear rot caused by *Aspergillus flavus* are not always severe in the field, but the fungus can still be present at levels that can contaminate maize with aflatoxins (Mukanga *et al.*, 2010). However, infections caused by *A. flavus* are mostly invisible and therefore show no visible spore masses on the surface of the kernels (Schoeman, 2012). Indeed, visually healthy maize can be highly infected by the fungus. Infection of kernels with aflatoxin-producing *A. flavus* and *A. parasiticus* may lead to contamination with aflatoxins, which are hepatoxic to humans and animals (Mutegi *et al.*, 2018). Consumption of food products that have high levels of aflatoxins can be lethal (Lewis *et al.*, 2005), while chronic exposure exacerbates the epidemics of many diseases including malaria, tuberculosis, and human immunodeficiency virus (HIV/AIDs) through suppression of the immune system and interference with nutrition (Williams *et al.*, 2005). Therefore, chronic exposure to aflatoxins contributes to the disease burden in countries in sub-Saharan Africa (Mutegi *et al.*, 2018).

Previous studies reported a significant increase in infection of maize, both with and without symptoms, by mycotoxin-producing fungi with increased infestation of the maize by insect pests such as stemborers (Opoku *et al.*, 2019). Several approaches have therefore been devised to reduce ravages caused by insect pests on maize. Some of the methods include genetic engineering for resistance to European corn borer, *Ostrinia nubilalis*, breeding for resistance to causative fungi, especially *Fusarium* spp., and cultural practices such as crop rotation, tillage, planting date, and management of fertilization (Munkvold *et al.*, 1997; Munkvold, 2003; Mesterházy *et al.*, 2012). However, cultural practices have had very little effect on infection of maize with ear rot fungi and the practices are not readily adopted by resource-constrained smallholder farmers. Although genetic engineering and breeding are viable options, there is no legislation on usage of genetically modified crops in Kenya and many other countries in sub-Saharan Africa (SSA). This calls for development and evaluation of integrated, cost-effective, and smallholder farmer-friendly methods of managing maize ear rots and their associated mycotoxins.

A companion cropping system, "push–pull", has been shown to effectively control stemborers in maize production (Khan *et al.*, 2000). The technology involves intercropping cereals, mainly maize or sorghum, with insect-repellent forage legumes in the genus *Desmodium* (commonly known as desmodium) and planting around the intercrop Napier grass (*Pennisetum purpureum*), or *Brachiaria* 'Mulato II' (Khan *et al.*, 2000, 2011; Midega *et al.*, 2018). Desmodium emits repugnant semiochemicals that push away the stemborer moths that are simultaneously attracted to the Napier grass or *Brachiaria* trap plants, which emit attractive volatile organic compounds (Khan *et al.*, 2011; Midega *et al.*, 2018). However, the grass does not support significant survival of the emerging larvae, thus ensuring that the grass is not destroyed by them (Khan *et al.*, 2011). The technology also effectively controls fall armyworm (FAW, *Spodoptera frugiperda*), an invasive pest of maize and other crops that recently invaded Africa (Midega *et al.*, 2018). Desmodium also suppresses the parasitic weed striga through allelopathy, and contributes to soil health improvement via nitrogen fixation, improved organic matter content, and conservation of soil moisture, resulting in improved grain yield (Khan and Pickett, 2004). Moreover, desmodium grows extensively, both during the rainy and dry seasons, thus acting as live mulch (Khan *et al.*, 2011).

These companion plants are nutritious livestock fodder, thus facilitating crop–livestock integration (Khan *et al.*, 2008). This farming system has been widely adopted in eastern Africa as it fits well with traditional mixed farming systems in the region. Therefore, integration of the technology in management of mycotoxin contamination in maize would add an additional benefit to the farmers. The objectives of the current study were to determine: (a) the incidence of stemborer and FAW damage in maize under push–pull and maize monocrop; (b) occurrence of maize ear rots in physiologically mature maize under push–pull and maize monocrop; (c) contamination of physiologically mature maize grown under push–pull and monocrop with *Aspergillus* spp., *Fusarium* spp. and associated mycotoxins; and (d) the association among insect damage, ear rots, ear rot fungi, and mycotoxin contamination of maize in three counties over three cropping seasons. The results of this study established the impact and effectiveness of reduction of stemborer and FAW damage under a push–pull cropping system on occurrence of ear rots and contamination of maize with aflatoxin and fumonisin. This study also provides valuable information for integrated management of insect pests, ear rot fungi, and mycotoxins through modification of cropping practices.

## 2 | MATERIALS AND METHODS

### 2.1 | Description of the study sites

The study was conducted over a period of three cropping seasons between March 2017 and August 2018 in three agroecologically different counties of Siaya, Vihiga, and Migori in western Kenya. These counties are characterized by a bimodal rainfall pattern, with the main cropping season running from March to August and the short cropping season from October to January. Siaya County lies at 1,200–1,500 m a.s.l., experiences rainfall of 1,450–1,900 mm and temperatures between 20.9 and 22.3°C (Jaetzold *et al.*, 2009; 2010). The altitude, rainfall, and temperature parameters of Migori County are 1,300–1,550 m a.s.l., 1,300–1,800 mm, and 20.4–21.7°C,

respectively; while the corresponding parameters in Vihiga County are 1,300–1,900 m a.s.l., 1,650 to >2,000 mm, and 20.1–22.2°C, respectively.

These counties are representative of major maize growing and utilization regions in Kenya where the majority of the farmers practice smallholder mixed farming. The counties also represent regions in western Kenya where the push–pull cropping system has been widely adopted by smallholder maize farmers for pest management. The technology has been adopted by over 207,000 smallholder farmers in eastern and central Africa (www.push-pull.net). One hundred and twenty fields were sampled each season; 20 push–pull and 20 maize monocrops from each of the three sites. Push–pull involved intercropping of maize with the fodder legume desmodium and planting Napier/*Brachiaria* grass at the border of the intercrop. Maize monocropping involved planting pure stands of maize. The same farms were maintained in each county across the three cropping seasons and same maize variety was planted in both push-pull and maize monocrop plots in a season. The farms were approximately 0.04–0.10 ha in size, and the push-pull and its monocrop control were either side by side or up to 100 m apart.

## 2.2 | Determination of pest damage and ear rots in maize

Damage of maize by stemborers and FAW was assessed at milk growth stage of the crop (R2–R4; https://www.pioneer.com/us/agronomy/staging_corn_growth.html#). The incidence of foliage and ear damage was determined as the number of damaged plants out of 100 arbitrarily selected maize plants in each farm. Stemborer damage on leaves was characterized by pin holes and window-paning marks on the leaves, while damage on the stem was determined as the number of entry and exit holes and tunnel length caused by feeding stemborer larvae. Ears infested by stemborer had sawdust-like faeces. FAW damage on the leaves was characterized as skeletonized leaves and windowed whorls loaded with larval frass, while ear damage was characterized by holes filled with larval frass.

At harvest (R6), 100 maize ears were randomly selected in each push–pull and monocrop farm, dehusked, and assessed for symptoms of ear rots. The incidence of common types of ear rots was determined as the number of infected ears out of the 100 randomly harvested maize ears as described by Mutitu (2003). The type of ear rot was identified based on causal fungi as illustrated in a compendium of maize diseases (CIMMYT Maize Program, 2004). There were no visual symptoms of *Aspergillus* ear rots, which are usually characterized by greenish-yellow mouldiness of the maize ear. *Fusarium* ear rot-infected ears showed whitish-pinkish to violet mouldy kernels scattered among healthy-looking kernels. *Diplodia* ear rot was characterized by white mouldiness over and between maize kernels, starting from the bottom of the ear that left the kernels lightweight and greyish brown, especially in early infection. *Gibberella* ear rot was observed as white to pink mould covering the tip to the upper half of the maize ear, while *Penicillium* ear rot developed as blue-green to

green mould at the tips of damaged maize ears. For ears with more than one type of ear rot, the incidence of each type was recorded separately. About 10–20 maize ears were randomly sampled from the 100 ears assessed for ear rots in each push–pull and monocrop farm to make one sample per farm, which was sun dried for 1 week, manually shelled, and finely ground (Bunn-O-Matic Corporation Coffee Mill, G3-000). The grinding machine was cleaned between samples by first removing residues using a vacuum cleaner (LG VC6820NHAY) and a paint brush, then wiping with cotton wool wet with 70% ethanol and grinding a little bit of the next sample , which was trashed before collecting the rest of the sample. The flour was subdivided into two subsamples: one for fungal isolation and the other for aflatoxin and fumonisin analysis. The flour was then packed in Khaki bags and stored at 4°C until analyses of mycological and mycotoxin contamination as described in subsequent sections.

## 2.3 | Quantification and identification of *Aspergillus* spp. and *Fusarium* spp. in maize

One gram of the ground maize samples was suspended in 9 ml sterile distilled water, vortexed for 30 s, and then serially diluted to $10^{-2}$ dilution factor in sterile distilled water. One hundred microlitre aliquots of the suspension were spread on half-strength potato dextrose agar (PDA 17 g, $KH_2PO_4$ 1 g, $KNO_3$ 1 g, $MgSO_4$ 0.5 g, agar 10 g, 1 L distilled water) amended with 50 mg each of tetracycline, streptomycin, chloramphenicol, and pentachloronitrobenzene (PCNB). PCNB was added before autoclaving, while the antibiotics were added after autoclaving, and the medium was cooled to about 45°C. Each sample was plated in three plates and the plates were incubated at 25°C for 3 days. After incubation, the number of colonies of each fungus was counted in each plate. The number of colony-forming units per gram (cfu/g) of ground maize was calculated using the formula:

$$cfu/g = no. \ of \ colonies/(amount \ plated \ in \ \mu l \times dilution).$$

The frequency of different fungal genera was calculated as:

$$Frequency \ (\%) = 100 \times no. \ of \ isolates \ of \ a \ genus \ within \ a \ sample \ / \ total \ number \ of \ isolates \ of \ all \ genera \ within \ a \ sample \ .$$

Characteristic colonies of each fungus were subcultured on PDA and incubated at 25°C for 7–14 days. Colonies of *Fusarium* spp. were also subcultured on synthetic nutrient agar (SNA; $KH_2PO_4$ 1 g, $KNO_3$ 1 g, $MgSO_4$ 0.5 g, KCl 0.5 g, glucose 0.2 g, agar 20 g/L) and incubated for 14–21 days under near-UV light to enhance sporulation (Nirenberg, 1981). Colonies of *Aspergillus* spp. were subcultured on Czapek Dox agar and incubated for 5–7 days at 25°C.

Fungal genera were identified based on cultural and microscopic (400×) morphological characteristics using the manual by Humber (1997). *Fusarium* spp. were identified using the manual by Leslie and Summerell (2006), while *Aspergillus* spp. were identified using the manual by Klich (2007). Some of the morphological features used for identification of *Fusarium* spp. included growth pattern, colour

of aerial mycelia, reverse colony colour, macroconidia presence and morphology, microconidia presence or absence and morphology, type of conidiophore, type of phialides and chlamydospores. *Aspergillus* spp. were identified based on colony colour on Czapek Dox agar, colony diameter, reverse colony colour, sclerotia formation, seriation, size and shape of vesicles, and conidia colour, size, and surface texture. In addition, there were five fungal genera that were unidentified and therefore recorded as "other".

## 2.4 | Detection and quantification of aflatoxin and fumonisin in maize

Twenty grams of finely ground maize was extracted with 100 ml of 70% methanol for aflatoxin, and 40 ml of 90% methanol for fumonisin. The samples were mixed by shaking in sealed containers for 2 min for aflatoxin and 1 min for fumonisin. After particulate matter was allowed to settle, the extracts were filtered through Whatman no. 1 filter paper. The sample extracts for fumonisin analysis were diluted with distilled water in the ratio of 1:20 (sample: distilled water). Aflatoxin and fumonisin levels were quantified by direct competitive enzyme-linked immunosorbent assay (ELISA) using Helica Biosystems Inc. kits following the manufacturer's instructions. The lower and the upper limits of detection for the aflatoxin kits were 1 and 20 μg/kg, respectively, while the corresponding limits for fumonisin kits were 100 and 6,000 μg/kg, respectively. A standard curve for each test kit was plotted and used to compare the absorbances of the samples with those of the kit standards to determine the levels of mycotoxins in the test samples. Samples with mycotoxin levels above the limit of detection were diluted and the toxin levels quantified again, with consideration of the additional dilution factor in the interpretation of the results.

## 2.5 | Data analysis

Data on insect damage, ear rots, and fungal species under push–pull and monocrop systems were analysed using linear mixed models fitted by REML at 5% significance level in R studio software. Data for each season were analysed separately and then combined to compare the differences among the seasons. Data found to have higher residual than null deviance were analysed with generalized linear mixed models fitted by REML. Cropping system (push–pull vs. monocrop), county, season, and their interactions were used as fixed factors, while name of farmer from which data and samples of a pair of treatments (a push–pull and monocrop farm) were collected was set as a random effect in the model. Means of foliage and ear damage by stemborer and FAW larvae were compared using paired *t* test. The cross tabulation procedure of SPSS v. 22 (IBM Corp.) was used to categorize aflatoxin and fumonisin data into three levels: (a) samples below the limit of detection of the kits; (b) samples contaminated with toxin levels below the regulatory threshold; and (c) samples with toxin levels above the regulatory threshold set by Kenya Bureau of Standards (KEBS) for aflatoxin and European Commission (EC), which Kenya adopts, for fumonisin (Gong *et al.*, 2015). The three aflatoxin and fumonisin categories were grouped under the cropping systems (push–pull and monocrop). A chi-square test of association between the aflatoxin and fumonisin levels with the cropping system was performed in SPSS. Nonparametric correlation was performed in SPSS to establish the relationship among insect damage, ear rots, ear rot fungi, and their respective mycotoxins. The data used were combined over seasons, counties, and cropping systems to facilitate interpretation of the results. However, no tests of heterogeneity in error variance were conducted prior to combining data across seasons, counties, and cropping systems.

## 3 | RESULTS

### 3.1 | Incidence of stemborer and FAW infestation in maize

Overall, incidence of both stemborer and FAW damage on foliar parts and ears were significantly lower ($p < .001$) in maize grown under the push–pull cropping system than in maize grown as a monocrop across the counties and seasons (Table 1). The difference was also significant within counties during individual seasons ($p = .005$), except for FAW foliage damage that did not significantly ($p = .589$) differ across the seasons. Stemborer and FAW damage on maize foliage and ears within cropping systems was also significantly ($p < .05$) influenced by season and county, except FAW ear damage that was not significantly ($p = .399$) influenced by season. The incidence of stemborer and FAW damage within individual counties was also significantly ($p < .05$) influenced by season. Incidence of foliage and ear damage by stemborer was significantly ($p < .05$) less than the incidence of FAW damage across seasons. Results of the *t* test analysis showed that both insects caused significantly ($p < .001$) less damage on the ears than on the foliage.

### 3.2 | Incidence of common types of maize ear rots

The most commonly recorded types of maize ear rots in order of decreasing incidence were those caused by *Fusarium*, *Penicillium*, and *Diplodia* spp. *Gibberella* ear rot was also recorded but the incidence was less than 1%. Prevalence of *Fusarium* ear rots was highest during the 2018 long rain cropping season. The incidence of *Fusarium* ear rot and total ear rots were significantly lower ($p < .001$) in maize grown under the push–pull cropping system during the 2017 short rain and 2018 long rain cropping seasons than in maize grown as a monocrop (Table 2). Occurrence of *Fusarium* and total ear rots varied significantly ($p = .007$) among seasons. Incidence of *Fusarium* ear rot within counties was significantly ($p = .001$) influenced by season. Although *Penicillium* ear rot was not significantly reduced under the push–pull cropping system, incidence was significantly ($p < .001$) influenced by seasons.

**TABLE 1** Incidence (%) of stemborer and fall armyworm damage on foliage and ears of maize grown under push–pull and monocrop systems in three counties during three cropping seasons in western Kenya

| Insect | Type of damage | County | Long rain 2017 | | Short rain 2017 | | Long rain 2018 | |
|--------|----------------|--------|----------------|---|-----------------|---|----------------|---|
| | | | Push-pull | Monocrop | Push-pull | Monocrop | Push-pull | Monocrop |
| Stemborer | Foliage | Siaya | 4.25 ± 0.9 | 10.0 ± 2.1 | 4.2 ± 0.7 | 6.1 ± 1.3 | 0.1 ± 0.1 | 0.1 ± 0.1 |
| | | Vihiga | 9.9 ± 2.2 | 24.7 ± 3.0 | 6.4 ± 1.5 | 17.5 ± 2.4 | 0.0 ± 0.0 | 0.7 ± 0.3 |
| | | Migori | 15.6 ± 2.1 | 24.5 ± 2.3 | 3.4 ± 0.7 | 6.1 ± 1.3 | 0.1 ± 0.1 | 0.4 ± 0.2 |
| | | Mean | 9.9 ± 1.2 | 19.7 ± 1.7 | 4.7 ± 0.6 | 13.2 ± 1.2 | 0.1 ± 0.0 | 0.4 ± 0.1 |
| | | p | <.001 | | <.001 | | .010 | |
| | Ear | Siaya | 3.6 ± 0.9 | 2.6 ± 0.7 | 1.5 ± 0.6 | 1.4 ± 0.6 | 0.3 ± 0.2 | 0.6 ± 0.2 |
| | | Vihiga | 1.7 ± 0.6 | 4.8 ± 1.0 | 3.2 ± 0.9 | 19.5 ± 6.6 | 1.5 ± 0.6 | 1.7 ± 0.4 |
| | | Migori | 2.3 ± 0.6 | 3.9 ± 0.7 | 0.6 ± 0.3 | 1.4 ± 0.5 | 0.7 ± 0.2 | 1.2 ± 0.3 |
| | | Mean | 2.5 ± 0.4 | 3.7 ± 0.5 | 1.7 ± 0.4 | 7.0 ± 2.5 | 0.8 ± 0.2 | 1.2 ± 0.2 |
| | | p | .013 | | .011 | | .275 | |
| Fall armyworm | Foliage | Siaya | 17.1 ± 2.5 | 36.3 ± 4.8 | 10.7 ± 1.1 | 12.8 ± 1.5 | 8.9 ± 1.0 | 25.6 ± 3.6 |
| | | Vihiga | 11.2 ± 2.6 | 17.1 ± 3.6 | 16.0 ± 3.1 | 37.6 ± 6.8 | 10.3 ± 1.9 | 32.9 ± 2.9 |
| | | Migori | 22.6 ± 2.8 | 54.5 ± 2.7 | 19.6 ± 4.5 | 12.8 ± 1.5 | 19.3 ± 1.4 | 51.4 ± 2.6 |
| | | Mean | 16.9 ± 1.6 | 36.0 ± 2.9 | 15.4 ± 1.9 | 35.9 ± 3.5 | 13.0 ± 1.1 | 37.0 ± 2.2 |
| | | p | <.001 | | <.001 | | <.001 | |
| | Ear | Siaya | 9.6 ± 1.3 | 23.4 ± 3.7 | 6.4 ± 1.1 | 4.2 ± 0.7 | 15.4 ± 1.6 | 26.3 ± 3.0 |
| | | Vihiga | 3.0 ± 0.7 | 7.9 ± 1.3 | 1.5 ± 0.5 | 2.3 ± 0.9 | 14.2 ± 2.8 | 22.9 ± 3.9 |
| | | Migori | 10.5 ± 1.5 | 25.7 ± 3.6 | 4.3 ± 1.2 | 4.2 ± 0.7 | 24.2 ± 1.5 | 32.5 ± 2.9 |
| | | Mean | 7.7 ± 0.8 | 19.0 ± 2.0 | 4.1 ± 0.6 | 11.3 ± 2.3 | 18.0 ± 1.3 | 27.2 ± 1.9 |
| | | p | <.001 | | <.001 | | <.001 | |

*Note:* Data are mean ± SEM.

## 3.3 | Density of *Aspergillus* and *Fusarium* spp. in maize

*Fusarium* was the most frequently isolated fungal genus across seasons and counties in maize from both push–pull and maize monocrop (Table 3). *Aspergillus*, *Penicillium*, *Acremonium*, and *Verticillium* spp. were also isolated in varying frequencies. The "other" fungi were mainly isolated in low frequencies (<0.01%) and population (<1 cfu/g maize) on average. The total density of fungi (cfu/g) was significantly lower ($p < .05$) in maize samples from the push–pull cropping system than in maize grown as a monocrop in all three cropping seasons (Table 3). The density of *Fusarium* spp. was significantly less under the push–pull cropping system during the 2017 short rainy ($p = .010$) and 2018 long rainy seasons ($p = .004$), while the density of *Aspergillus* spp. did not differ between the cropping systems, across counties and the seasons. Additionally, the overall density of *Fusarium* spp. was also significantly ($p = .002$) less in maize under the push–pull cropping system compared to the monocrop. Mean densities of *Fusarium* and *Aspergillus* spp. were greatest in maize grown during the 2017 short rain cropping season and lowest in maize grown during the 2018 long rain cropping season. A low density of *Fusarium* spp. (0.4–567 cfu/g) and *Aspergillus* spp. (0.2–1,526 cfu/g) was composed of unidentified species within the genera.

The most frequent *Fusarium* and *Aspergillus* spp. were *F. verticillioides* and *A. flavus*, respectively. *F. proliferatum*, *F. subglutinans*, *A. parasiticus*, *A. niger*, *A. ostianus*, *A. fumigatus*, *A. tamarii*, and *A. ochraceous* were isolated in low frequencies. The density of *F. verticillioides* was significantly ($p < .05$) less in maize grown under the push–pull cropping system compared to maize grown as a monocrop during the 2017 short rain and 2018 long rain and across seasons and counties ($p < .05$) (Table 3). In contrast, the density of *A. flavus* was very low and did not differ significantly ($p = .083$) in maize grown under the two cropping systems, even across individual cropping seasons and counties. The density of *F. verticillioides* was significantly ($p = .043$) influenced by the season. The highest mean densities of *F. verticillioides* and *A. flavus* were recorded during the 2017 short rainy season.

## 3.4 | Levels of aflatoxin and fumonisin in harvested maize grain

The proportion of maize samples contaminated with aflatoxin varied greatly among the three cropping seasons (Table 4). Maize grown during the 2018 long rain cropping season had the greatest proportion of samples contaminated with aflatoxin, as compared to the other two seasons. Overall, the proportion of push–pull maize samples contaminated with levels above the KEBS limit

**TABLE 2** Incidence (%) of different types of ear rotsin maize grown under push–pull and monocrop systems during three cropping seasons in three counties of western Kenya

| Type of ear rot | County | Long rain 2017 | | Short rain 2017 | | Long rain 2018 | |
|---|---|---|---|---|---|---|---|
| | | Push–pull | Monocrop | Push–pull | Monocrop | Push–pull | Monocrop |
| Fusarium | Siaya | 9.0 ± 1.2 | 12.2 ± 3.0 | 2.1 ± 0.3 | 5.8 ± 1.1 | 4.0 ± 0.6 | 12.8 ± 2.2 |
| | Vihiga | 8.8 ± 1.7 | 10.9 ± 2.4 | 5.2 ± 1.2 | 9.7 ± 1.7 | 5.4 ± 0.9 | 10.3 ± 1.8 |
| | Migori | 3.7 ± 1.5 | 8.8 ± 2.0 | 4.4 ± 1.2 | 9.8 ± 2.1 | 3.3 ± 0.8 | 8.3 ± 1.2 |
| | Mean | 7.2 ± 0.9 | 10.5 ± 1.4 | 3.9 ± 0.6 | 8.4 ± 1.0 | 4.3 ± 0.5 | 10.5 ± 1.0 |
| | p | .660 | | <.001 | | <.001 | |
| Penicillium | Siaya | 8.4 ± 2.1 | 4.6 ± 2.0 | 1.2 ± 0.6 | 4.3 ± 2.2 | 5.2 ± 1.9 | 4.3 ± 1.1 |
| | Vihiga | 3.6 ± 1.1 | 5.8 ± 1.8 | 0.8 ± 0.3 | 1.6 ± 0.8 | 6.4 ± 1.5 | 6.6 ± 1.6 |
| | Migori | 5.9 ± 1.7 | 7.4 ± 2.0 | 1.0 ± 0.4 | 0.8 ± 0.5 | 2.4 ± 0.7 | 3.4 ± 0.9 |
| | Mean | 5.9 ± 1.0 | 5.9 ± 1.1 | 1.0 ± 0.3 | 2.3 ± 0.9 | 4.6 ± 0.4 | 4.7 ± 0.7 |
| | p | .540 | | .444 | | .899 | |
| Other | Siaya | 5.2 ± 1.4 | 7.5 ± 2.0 | 4.1 ± 0.6 | 4.2 ± 1.1 | 2.6 ± 0.5 | 6.5 ± 1.3 |
| | Vihiga | 6.9 ± 1.4 | 6.8 ± 1.2 | 1.7 ± 0.4 | 3.1 ± 0.8 | 6.0 ± 1.0 | 2.7 ± 0.7 |
| | Migori | 8.1 ± 1.4 | 10.0 ± 2.2 | 2.9 ± 0.6 | 2.6 ± 0.8 | 3.9 ± 0.8 | 2.8 ± 0.5 |
| | Mean | 6.7 ± 0.8 | 8.2 ± 1.1 | 2.9 ± 0.3 | 3.3 ± 0.6 | 4.2 ± 0.5 | 4.0 ± 0.6 |
| | p | .957 | | .454 | | .768 | |
| Total | Siaya | 23.1 ± 2.8 | 24.6 ± 5.2 | 11.2 ± 1.3 | 16.3 ± 2.6 | 12.7 ± 2.2 | 24.1 ± 3.5 |
| | Vihiga | 19.9 ± 2.6 | 24.3 ± 3.9 | 8.5 ± 1.4 | 15.3 ± 2.0 | 18.2 ± 1.9 | 19.9 ± 2.0 |
| | Migori | 19.2 ± 3.4 | 29.1 ± 3.4 | 11.1 ± 1.5 | 17.9 ± 2.5 | 11.4 ± 1.6 | 16.7 ± 1.6 |
| | Mean | 20.7 ± 1.7 | 26.0 ± 2.5 | 10.2 ± 0.8 | 16.5 ± 1.4 | 14.2 ± 1.1 | 20.2 ± 1.5 |
| | p | .590 | | <.001 | | <.001 | |

Note: Data are mean ± SEM.

**TABLE 3** Populations (cfu/g) of fungi in maize grown under push–pull and maize monocrop during three cropping seasons in three counties of western Kenya

| | Cropping system | LR2017 | SR2017 | LR2018 | Overall |
|---|---|---|---|---|---|
| Total cfu/g | Push–pull | 46,126.1 | 74,187.0 | 16,793.2 | 45,864.9 |
| | Monocrop | 121,162.0 | 184,871.0 | 43,233.9 | 116,085.0 |
| | p | .075 | .013* | .015* | .001* |
| Total Fusarium spp. | Push–pull | 27,737.8 | 44,548.6 | 11,529.0 | 28,033.4 |
| | Monocrop | 47,081.4 | 136,111.0 | 28,139.1 | 69,801.4 |
| | p | .218 | .009* | .110 | .002* |
| F. verticillioides | Push–pull | 25,386.6 | 44,816.4 | 11,461.2 | 27,322.0 |
| | Monocrop | 45,675.0 | 134,881.9 | 27,036.2 | 68,553.5 |
| | p | .216 | .010* | .004* | .001* |

Abbreviations: LR, long rain; SR, short rain.

*Statistically significant (p < .05).

of 10 μg/kg was less than the proportion of contaminated maize grown as a monocrop. There was a significant (p = .001) difference in the proportion of maize samples contaminated with different levels of aflatoxin among the three cropping seasons, but the difference between push–pull and monocrop, as well as among the counties, was not statistically significant. However, there was significantly lower (p < .05) proportion of push–pull samples from

Siaya County contaminated with aflatoxin levels above the KEBS threshold of 10 μg/kg. The 2017 short rainy season had the smallest proportion of samples contaminated with aflatoxin but had the largest proportion of samples contaminated with aflatoxin levels above 10 μg/kg.

There was a greater proportion of maize samples contaminated with fumonisin as compared to the proportion contaminated with

**TABLE 4** Percentage of samples with aflatoxin and fumonisin levels (µg/kg) under different categories in maize grown under push–pull and maize monocrop systems during three cropping seasons in three counties of western Kenya

| Season | County | Cropping system | Aflatoxin | | | Fumonisin | | |
|---|---|---|---|---|---|---|---|---|
| | | | <LOD | ≤1–10[a] | >10 | <LOD | ≤1,000[b] | >1,000 |
| LR2017 | Migori | Push–pull | 42.1 | 52.6 | 5.3 | 47.4 | 21.1 | 31.6 |
| | | Monocrop | 40.0 | 55.0 | 5.0 | 45.0 | 35.0 | 20.0 |
| | Siaya | Push–pull | 50.0 | 50.0 | 0.0 | 15.0 | 45.0 | 40.0 |
| | | Monocrop | 60.0 | 35.0 | 5.0 | 15.0 | 20.0 | 65.0 |
| | Vihiga | Push–pull | 52.6 | 42.1 | 5.3 | 15.8 | 52.6 | 31.6 |
| | | Monocrop | 55.0 | 45.0 | 0.0 | 25.0 | 20.0 | 55.0 |
| | Mean | Push–pull | 48.3 | 48.3 | 3.4 | 25.9 | 39.7 | 34.5 |
| | | Monocrop | 51.7 | 45.0 | 3.3 | 28.3 | 25.0 | 46.7 |
| SR2017 | Migori | Push–pull | 95.0 | 0.0 | 5.0 | 20.0 | 25.0 | 55.0 |
| | | Monocrop | 95.0 | 0.0 | 5.0 | 15.0 | 25.0 | 60.0 |
| | Siaya | Push–pull | 73.7 | 21.1 | 5.3 | 10.5 | 57.9 | 31.6 |
| | | Monocrop | 66.7 | 22.2 | 11.1 | 33.3 | 33.3 | 33.3 |
| | Vihiga | Push–pull | 60.0 | 40.0 | 0.0 | 40.0 | 25.0 | 35.0 |
| | | Monocrop | 47.4 | 47.4 | 5.3 | 21.1 | 15.8 | 63.2 |
| | Mean | Push–pull | 76.3 | 20.3 | 3.4 | 23.7 | 35.6 | 40.7 |
| | | Monocrop | 70.2 | 22.8 | 7.0 | 22.8 | 24.6 | 52.6 |
| LR2018 | Migori | Push–pull | 35.0 | 65.0 | 0.0 | 55.0 | 40.0 | 5.0 |
| | | Monocrop | 35.0 | 65.0 | 0.0 | 35.0 | 35.0 | 30.0 |
| | Siaya | Push–pull | 27.8 | 66.7 | 5.6 | 61.1 | 16.7 | 22.2 |
| | | Monocrop | 44.4 | 44.4 | 11.1 | 11.1 | 50.0 | 38.9 |
| | Vihiga | Push–pull | 20.0 | 75.0 | 5.0 | 35.0 | 45.0 | 20.0 |
| | | Monocrop | 35.0 | 65.0 | 0.0 | 20.0 | 35.0 | 45.0 |
| | Mean | Push–pull | 27.6 | 68.9 | 3.4 | 50.0 | 34.5 | 15.5 |
| | | Monocrop | 37.9 | 58.6 | 3.4 | 22.4 | 39.7 | 37.9 |
| Grand mean | | Push–pull | 50.9 | 45.7 | 3.4 | 33.1 | 36.6 | 30.3 |
| | | Monocrop | 53.1 | 42.3 | 5.6 | 24.6 | 29.7 | 45.7 |

Abbreviations: LOD, lower limit of detection (1 and 100 µg/kg for aflatoxin and fumonisin, respectively); LR, long rain; SR, short rain.

[a]Kenya Bureau of Standards regulatory threshold.

[b]European Commission regulatory threshold.

aflatoxin. Overall, the proportion of samples contaminated with fumonisin was significantly ($p = .048$) reduced under the push–pull cropping system. The proportion of maize contaminated with fumonisin levels above the European Commission threshold of 1,000 µg/kg was significantly ($p < .001$) lower in maize grown under the push–pull cropping system compared to maize grown as a monocrop. Additionally, there was a significantly ($p = .005$) lower proportion of maize contaminated with different levels of fumonisin in maize grown under the push–pull cropping system during the 2018 long rain cropping season as compared to 2017 long and short rain seasons. The proportion of push–pull samples contaminated with >1,000 µg/kg of fumonisin was significantly ($p = .044$) lower in samples from Vihiga, compared to Migori and Siaya counties.

## 3.5 | Correlation among insect infestation, *Fusarium* ear rot, and mycotoxin levels in maize

Analyses showed that incidence of *Fusarium* ear rot was significantly correlated with the incidence of stemborer ear damage ($r = .167$, $p = .019$) and both FAW foliage ($r = .204$, $p < .001$) and ear damage ($r = .107$, $p = .024$). Accordingly, the population of *F. verticillioides* ($r = .388$, $p < .001$) and fumonisin levels ($r = .429$, $p < .001$) had a significant positive association with the incidence of *Fusarium* ear rot. Similarly, fumonisin levels had significant ($r = .672$, $p < .001$) linear correlation with the density of *F. verticillioides*. The populations of *A. flavus* was also positively and significantly correlated with the levels of aflatoxin ($r = .159$, $p = .001$).

## 4 | DISCUSSION

Insect pests, ear rots, and mycotoxins are major constraints to maize production and use, but feasible management strategies to combat them are limited for resource-constrained smallholder farmers such as those in western Kenya. The current study demonstrated that the push–pull cropping system can contribute to reductions in crop damage and mycotoxin contamination in maize. These results are consistent with previous findings that observed a reduction in the incidence of damage by the two pests under the push–pull cropping system (Khan et al., 2011; Midega et al., 2018). However, the amount of damage by the two pests significantly varied across seasons and study counties, indicating that the extent of damage by the pests depended on multiple factors such as cropping season and agroecology (Manu et al., 2019). Different cropping seasons are characterized by variations in rainfall, temperature, and relative humidity that influence the incidence of diseases, pests, and weeds, and consequently quantity and quality of grain yield. In addition to differences in length, cropping seasons are characterized by different precipitation patterns that influence nutrient mobility and weather events (Das et al., 2010).

To the best of our knowledge, this is the first study that has concurrently assessed the incidence of stemborer and FAW, and their potential impact on maize quality in Kenya. FAW caused significantly more damage than stemborers across the seasons and counties, indicating that the extent of plant damage also greatly depends on the insect pest species of interest. This is probably determined by the population size and feeding habits of the pest. FAW is an aggressive feeder and causes extended skeletonization of leaves and windowed whorls (Goergen et al., 2016) while stemborer infestation causes window-paning marks that are not extended on the leaves (Overholt et al., 2001). However, both pests bore holes in growing maize ears and their moths are nocturnal, which has been reported to make it difficult to manage them through the use of insecticides (Overholt et al., 2001).

Fusarium ear rot was the most prevalent type of ear rot across the seasons, with incidences being significantly reduced in the push–pull cropping system. These results support findings of a preliminary study that reported reduced incidence of maize ear rots in maize under the push–pull cropping system (Owuor et al., 2018). However, the current findings from maize monocrops are inconsistent with observations of previous studies that reported Fusarium ear rot as the third most common type of maize ear rots in the region (Bigirwa et al., 2007). This difference could be attributed to agroecological conditions that are known to vary across seasons, as dominance of fungal species is greatly influenced by climatic conditions (Vacher et al., 2008). Climatic conditions of temperature and moisture are key determinants of presence and extent of infection of maize by ear rot fungi because different fungi require specific conditions of temperature and moisture for growth (Vacher et al., 2008). Moderate temperature and mean monthly precipitation favour the growth of saprophytic fungi. For example, in the current study where Fusarium ear rot was prevalent, characteristic temperatures of the study sites were between 20 and 22°C, which favoured growth of F. verticillioides—whose optimum growth temperature is at 25°C—better than A. flavus, which grows optimally at 30°C (Camardo et al., 2019). These conditions were different to those in the study sites of Bigirwa et al. (2007), which might have been much higher, as suggested by the low altitudes of 900–1,500 m a.s.l. compared to the altitudes in the current study that ranged between 1,140 and 1,900 m a.s.l. The reduction of Fusarium ear rot under the push–pull cropping system could be caused by reduced entry of inocula as a result of reduced insect damage. Another explanation is the possible release of antifungal compounds into the soil by desmodium roots. The chemicals could inhibit spore germination, alter hyphal modifications, and modify the structure of the fungal mycelia, which could reduce the fungal inocula in the soil.

Ear rot fungi mainly gain entry into maize kernels through wounds caused by insect infestation or systemically from the soil through the stalk (Munkvold et al., 1997). Therefore, maize ears under the push–pull cropping system, from which low damage by stemborer and FAW was recorded, had significantly lower ear rot infections. Insects feeding on maize ears either act as vectors of ear rot fungi or open the ear to fungal inocula dispersed by raindrops and wind (Mays, 2015). Maize ear rot fungi also cause infection by entering through the silk as the kernels develop (Thompson et al., 2018). F. verticillioides, F. graminearum, and A. flavus are the most common silk-entering fungal pathogens of maize (Thompson et al., 2018). This could possibly explain why all maize samples including those from the push–pull plots were contaminated with F. verticillioides and A. flavus, which are fumonisin and aflatoxin producers, respectively, even though there was significantly minimal damage to the maize by both stemborer and FAW larvae with the technology.

Overall, F. verticillioides was the most prevalent fungal species in maize samples and the density was significantly lower under the push–pull cropping system than where maize was grown as a monocrop. Like incidence of Fusarium ear rot, the density of F. verticillioides also varied across the cropping seasons. This could be attributed to the effect of environmental factors such as temperature and precipitation on the density of fungal species (Vacher et al., 2008; Manu et al., 2019). Leslie and Summerell (2006) reported that food substrate contaminated with high density of F. verticillioides is unlikely to be contaminated by A. flavus. Moreover, F. verticillioides grows optimally at cooler temperatures (25°C) than A. flavus (30°C) (Camardo et al., 2019). The average temperature for the current study sites ranged from 20 to 22°C, thereby favouring F. verticillioides (Jaetzold et al., 2009). Previous studies have also reported F. verticillioides as the most prevalent fungus isolated in maize (Mukanga et al., 2010). Correlation analysis showed association between density of F. verticillioides and Fusarium ear rot across the three cropping seasons and study sites. Mukanga et al. (2010) and Duan et al. (2016) also reported that F. verticillioides was correlated with Fusarium ear rot in maize. Fumonisin is a field mycotoxin and therefore isolation of F. verticillioides in maize at harvest is an indication of risk of continued contamination of maize with fumonisin during storage.

The lower density of *A. flavus* compared to *F. verticillioides* could possibly explain the high contamination of maize with fumonisin compared to the low contamination with aflatoxin, as was also reported by a previous study (Mutiga *et al.*, 2015). This is in agreement with previous studies that reported co-occurrence of aflatoxin and fumonisin (Mutiga *et al.*, 2015; Guo *et al.*, 2017). The lack of significant differences in aflatoxin contamination of maize under push–pull and maize monocrop systems could have resulted from the low population of *A. flavus* across the seasons, which was also not significantly different between the cropping systems and counties. It is also possible that the climatic conditions of temperature and humidity in the study sites were not conducive for optimal growth of *A. flavus* and aflatoxin production (Camardo *et al.*, 2019). It is also plausible to attribute the high levels of fumonisin and low levels of aflatoxin in the current study to the possibility of a high proportion of toxigenic *F. verticillioides* in the population compared to a high proportion of atoxigenic *A. flavus* population in the harvested grains (authors' unpublished data).

Although the density of *A. flavus* was associated with aflatoxin in the maize samples, there was no visible infection on maize kernels by the fungus. Symptoms caused by *A. flavus* are not always severe in the field, but the fungus can still be present at levels that can contaminate maize with aflatoxins (Mukanga *et al.*, 2010). This is in agreement with findings of a previous study that reported that *Aspergillus* ear rot mainly causes little damage to maize kernels, which therefore cannot be visually observed as discoloration as it might be for other ear rot fungi (Schoeman, 2012). Infection of maize by *F. verticillioides* and *A. flavus* can also be symptomless (Schoeman, 2012; Owuor *et al.*, 2018). Therefore, *A. flavus* was isolated in maize samples even though there were no observations of *Aspergillus* ear rot recorded. The presence of *A. flavus* in very low cfu per gram of maize grains could also possibly explain the absence of visual symptoms of *Aspergillus* ear rots.

The implications of the current study are that the push–pull cropping system significantly reduced the occurrence of maize ear rots, particularly *Fusarium* ear rot, which was consequently associated with a low population of *F. verticillioides*, the main producer of fumonisin. This was suggested by the significant and positive correlation between the incidence of *Fusarium* ear rot and the density of *F. verticillioides*, as well as the correlation between the levels of fumonisin and incidence of *Fusarium* ear rot across the seasons. The association between *Fusarium* ear rots, *F. verticillioides*, and fumonisin was reported during the three cropping seasons.

The mechanism of reduction of *Fusarium* ear rot and fumonisin in maize under the push–pull cropping system was possibly through reduction in maize damage by stemborer and FAW. This observation was supported by the data on incidence of stemborer and FAW infestation, which was significantly lower in maize under the push–pull cropping system, and the significant association between stemborer and FAW damage with the incidence of *Fusarium* ear rot across the three cropping seasons. These findings concur with previous studies that reported a positive association between insect pest damage and the level of mycotoxin contamination of cereals, by reducing the ear rot pathogen inoculum that would infect the maize through wounded ears (Wu, 2007; Mays, 2015).

Reduction of insect damage under the push–pull cropping system is mainly due to the push and pull effects of the technology. Desmodium (push) produces semiochemicals that repel the moths, while at the same time Napier or *Brachiaria* grass (pull) produce chemicals that attract the repelled moths (Khan *et al.*, 2000). The allelochemicals released by desmodium roots condition the soil, such that maize grows more vigorously and produces larger amounts of self-defence volatiles against insects compared to when it is grown as a monocrop (Mutyambai *et al.*, 2019).

Crop production practices that increase grain yield help control mycotoxin contamination of food crops by reducing crop stress, which increases infection by pathogenic fungi (Bruns, 2003). The push–pull cropping system increases grain yields through conservation of moisture, increasing soil organic matter content, reduced insect damage, and availing nutrients such as nitrogen and phosphorus, which increase crop vigour (Khan *et al.*, 2011; Midega *et al.*, 2018). This could be hypothesized as another mechanism by which the push–pull cropping system controls the occurrence of maize ear rots. In addition to increased grain yield, the push–pull technology has other benefits that make the technology economically viable. There is minimum tillage, as well as reduced number of weeding events, hence reduced labour costs. Additionally, desmodium and Napier/*Brachiaria* grass are nutritious fodder for livestock, and because farmers do not have to buy chemicals for insect control, cost of production is low and net return to labour and land is significantly improved (Khan *et al.*, 2008; www.push-pull.net).

In conclusion, the push–pull cropping system reduced the incidence of maize ear rots and associated mycotoxins through effective stemborer and FAW management. Integration of the push–pull cropping system into existing methods of mycotoxin control is therefore recommended. The cropping system is easy to maintain and encourages livestock keeping by resource-constrained smallholder farmers, as the companion plants are an important quality fodder. Removal of maize stovers from the farm after harvest is also recommended because stovers are known to act as a primary source of fungal inocula. It is also important to coat maize seeds for planting with fungicides to reduce systemic infection of the maize under push–pull with ear rot fungi such as *F. verticillioides*. These would reduce infection courts of maize by ear rot fungi, and reduce contamination with aflatoxin and fumonisin to tolerable levels.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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