Climate change and adaptive land management in southern Africa

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Climate change and adaptive land management in southern Africa

Assessments, changes, challenges, and solutions

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Increasing yields of cereals: benefits derived from intercropping with legumes and from the associated bacteria

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Abstract: Low yield of staple crops such as maize sorghum, pearl millet, and legumes in southern Africa poses a threat to food security, especially in the semi-arid regions of Botswana. The low crop productivity is caused by pests, low fertility, low soil moisture, and high temperatures possibly resulting from climate change. On-farm measurements of N_2 fixation were done in groundnut and Bambara groundnut. Bacteria isolated from their root nodules were assessed as inoculant for cereals under greenhouse conditions. We also determined the most effective sorghum intercrop for reducing yield losses of sorghum caused by stem borers. The results showed that grain legumes fixed N_2 under on-farm conditions. However, amounts of N-fixed on farmers' fields were low because of suboptimal plant densities. Bambara groundnut growing in Lekobeng in Tswapong area fixed the highest amount of nitrogen at 1.3 kg N/ha, a value that is low because of the low planting density used by farmers. Bacteria isolated from legumes showed potential as biofertilisers. For example, in a clayey soil, there were no differences in the number of millet leaves between inoculated and NPK-fed plants. Finally, intercropping of sorghum with legumes led to a reduction in stemborer infestation on sorghum, resulting in higher grain yields for intercrops, about 8.4x more than monocropped sorghum. Taken together, these results show the potential use for legumes and their associated bacteria as biofertilisers in sustainable cropping systems. The data on intercropping will be used to advise farmers on the importance of legume intercrops in controlling stemborers.

Resumo: O baixo rendimento de culturas básicas como o milho, sorgo, milheto e leguminosas na África Austral coloca ameaças à segurança alimentar, em especial nas regiões semi-áridas do Botswana. A baixa produtividade de culturas é causada por pragas, baixa fertilidade, reduzida humidade do solo e elevadas temperaturas, possivelmente devido às alterações climáticas. Em quintas, foram realizadas medições da fixação de N_2 em amendoins e feijão-bambara. Bactérias isoladas dos seus nódulos radiculares foram avaliadas como inoculantes para cereais sob condições de estufa. Também determinámos a intercalação mais eficaz para o sorgo, de modo a reduzir as perdas de rendimento causadas pelas *stemborers* (artrópodes que furam o caule da planta). Os resultados mostraram que as leguminosas fixaram N_2 sob condições agrícolas. No entanto, as quantidades de N fixado nos campos dos agricultores foram baixas devido às densidades insuficientes das plantas. O feijão-bambara em Lekobeng, na área de Tswapong, fixou a maior quantidade de N, 1,3Kg N/ha, um valor que é baixo devido à baixa densidade de plantação utilizada pelos agricultores. As bactérias isoladas de leguminosas mostraram potencial como biofertilizante. Por exemplo, num solo argiloso, não existiram diferenças no número de folhas de milhete entre plantas inoculadas e plantas alimentadas com NPK. Por fim, o cultivo alternado de sorgo com leguminosas levou a uma redução na infestação de *stemborer* no sorgo, levando a maiores rendimentos, cerca de 8,4x mais que em sorgo em monocultura. No conjunto, demonstrámos o potencial uso das leguminosas e suas bactérias, associadas a sistemas de cultivo sustentáveis, como biofertilizantes. Os dados sobre o cultivo alternado serão utilizados para aconselhar os agricultores sobre a importância da intercalação de leguminosas no controlo das *steamborers*.

Introduction

Suboptimal production of staple crops such as maize, sorghum, and pearl millet and pulses such as *Vigna unguiculata* (L.) Walp (cowpea), *Vigna subterannea* (L.) Verdc. (Bambara groundnut), and *Arachis hypogea* L. (groundnut) is the single most worrisome factor negatively affecting food security in Botswana (Statistics Botswana, 2016). This situation is caused and compounded by a variety of factors including drought, poor soils, pests, and diseases (Khan et al., 2008; Mace et al., 2013). Suboptimal farm management practices include low planting densities; use of unadapted crop varieties that are susceptible to pests, diseases, and parasitic weeds; unfavourable planting dates; and injudicious use of inorganic fertilisers. The use of leguminous plants

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is a possible solution to low soil fertility, especially in low-input farming systems, while resistant varieties can be used to reduce yield losses caused by pests. The relationship between legumes and microorganisms such as bacteria and fungi forms the basis for why legumes are considered important in both natural and agricultural ecosystems. For example, during the biological nitrogen fixation process, bacteria benefit from the carbon energy source fixed by the plant while the plant gets fixed N from the bacteria in return. Biological nitrogen fixation is one of the most studied plant growth–promoting (PGP) processes. Other established plant growth–promoting mechanisms include phosphate solubilisation, iron sequestration through siderophore production and modulating phytohormone levels in the rhizosphere of legumes or non-legumes (Glick, 2012).

Traditionally, soil fertility problems are corrected through fertiliser application whereas those of low soil moisture are solved by irrigation and pests are removed by use of chemical pesticides. Apart from the fact that fertilisers and pesticides are expensive, their application is often associated with exacerbating effects of climate change.

Besides plant growth promotion through the use of various bacteria, intercropping of cereals with legumes reduces pests and consequently improves yields of cereals. For example, sorghum yields per hectare are low and unpredictable, fluctuating largely because of drought, poor soils, and pests (Van den Berg, 1994). Yield losses associated with pests in Africa reportedly range between 25% and 50% (Teetes, 1985). Stemborers are considered major pests of sorghum in many areas where sorghum is produced (Kfir et al., 2002). A survey conducted by Obopile & Mosinkie (2001) showed that famers in Botswana ranked stemborers as the most damaging pests, and losses of up to 10% per larva per plant have been reported (Roome, 1970; Obopile & Mosinkie, 2001). The species of stemborers occurring in Botswana are the spotted stemborer (*Chilo partellus* Swinhoe), the widely distributed African maize stemborer (*Busseola fusca* Fuller), the pink stemborer (*Sesamia calamistis* Hampson) and the sugarcane stemborer (*Eldana saccharina* Walker) (Obopile & Mosinkie, 2001). The objectives of this study were (1) to assess levels of nitrogen fixation and amounts of N_2 fixed by Bambara groundnut and groundnut in farmers' fields in the Okavango delta and Tswapong areas of Botswana, (2) to evaluate the effects of inoculating millet in Botswana with bacteria isolated from root nodules and roots of herbaceous legumes under glasshouse conditions, and (3) to determine the most effective sorghum intercrop that will reduce yield losses caused by stem borers on sorghum.

Materials and methods

Study site and sampling design

The sampling of groundnut and Bambara groundnut plants was done at Xakao (18°18′17″ S; 21°53′16″ E) in the Okavango Delta and at Lekobeng (22°44′16″ S; 27°11′17″ E) in the Tswapong region of Botswana. At each location, four plants were sampled randomly in an area measuring about 20 m x 20 m that was subdivided into 4 strata, with each stratum representing a replication. Therefore, a total of 16 plants were sampled from each field.

Nitrogen fixation measurements

The plants were collected in separate labelled paper bags and brought back to the laboratory, where the roots were gently washed under a stream of water and the root nodules separated from the plants and kept separately at 4°C before root nodule bacteria isolation. Plant shoots were separated from roots and oven dried at 60°C for 48 h and milled to a fine powder. The natural abundance rates of $^{15}N/^{14}N$, %N, and legumes and reference plants were determined by a Thermo Finnigan Delta Plus XP stable light isotope mass spectrometer (Fixon Instrument SPA, Strada Rivolla, Italy). About 2.0 mg of each pulverized sample was weighed in a tin capsule (Elementary Microanalysis LTD, Okehampton, UK) and run against two internal reference plant materials, namely *Nasturtium* sp. and *Vachellia* sp.

The isotopic composition of ^{15}N was measured as the difference in the number of atoms of ^{15}N to ^{14}N in atmospheric N_2 according to Junk & Svec (1958) and Mariotti (1983):

$$\delta^{15}N\ (‰) = \frac{(^{15}N/^{14}N)\ \text{sample-}(^{15}N/^{14}N)\text{standard}}{(^{15}N/^{14}N)\text{standard}} \times 1000$$

The percentage of N derived from the atmosphere (%Ndfa) was calculated according to Shearer & Kohl (1986) as follows:

$$\%Ndfa = \left[\frac{(\delta^{15}Nref) - \delta^{15}Nleg)}{(\delta^{15}Nref - B\ value)}\right] \times 100$$

where δ^{15}Nref is the mean ^{15}N natural abundance of a non-N_2-fixing reference plant, δ^{15}Nleg is the mean ^{15}N natural abundance of the legume (shoot), and the B value is the ^{15}N natural abundance of legume shoots that were totally dependent on biological N_2 fixation for their N nutrition.

The amount of N-fixed was calculated as proposed by Maskey et al. (2001):

$$\text{N-fixed} = (Ndfa/100) \times \text{legume shoot N}$$

After establishing normality, data on $\delta^{15}N$, %Ndfa, %N, and N-fixed were subjected to analysis of variance using STATISTICA version 13.1 (StatSoft Inc., 2016). Where there was statistical significance, means were separated using Fisher's LSD post hoc test.

Characterisation of bacteria

Bacteria that had been isolated (Vincent, 1970) from root nodules of groundnut and Bambara groundnut sampled from different farmers' fields in the Okavango delta and Tswapong area in Botswana were characterised. Bacterial physiological assays such as phosphate solubilisation and cellulase activities were carried out using specific growth media; for example, the Pikovskayas agar (Subba Rao, 1977) was used for phosphate solubilisation and cellulase activity detections (Teather & Wood, 1982).

Using bacteria as biofertiliser

Pennisetum glaucum (L.) R. Br. (pearl millet) was grown under sterile conditions in a greenhouse on three soils including a clayey, a loamy, and a sandy soil. A positive control using NPK fertiliser (2:3:2) and a negative control with

Table 1: Symbiotic traits of *Vigna subterranean* (L.) Verdc. and *Arachis hypogea* L. grown on farmers' fields in the Okavango delta and Tswapong areas of Botswana.

Agro-ecological zones	Farming area	Crop	$\delta^{15}N$ (‰)	%Ndfa	N-fixed ($kg.ha^{-1}$)	Number of plants/ha
Okavango	Xakao	*Vigna subterranean* (L.) Verdc.	1.8 ± 0.3a	50.3 ± 4.4c	0.3 ± 2.1c	230
Tswapong	Lekobeng	*Vigna subterranea* (L.) Verdc.	–1.2 ± 0.2c	96.4 ± 2.7a	1.3 ± 0.1a	213
Tswapong	Lekobeng	*Arachis hypogea* L.	0.5 ± 0.3b	69.3 ± 4.1b	0.8 ± 0.2bc	188
Okavango	Xakao	*Arachis hypogea* L.	0.5 ± 0.3b	71.0 ± 4.5b	1.2 ± 0.2ab	195

neither fertiliser nor inoculation were also included. Treatments were replicated four times. All the soils were sterilised in an autoclave to kill all microorganisms before commencement of experiments. Plants were harvested 45 days after planting by uprooting them with intact roots. They were oven dried at 60°C for 48 hours and they were weighed on a balance to obtain biomass.

Assessment of pest incidence in sorghum-legume intercropping

A field study to evaluate the effect of intercropping sorghum with legumes on damage caused by stemborer to sorghum was conducted in the 2015/2016 cropping season at Botswana University of Agriculture and Natural Resources in Sebele, Gaborone. Sorghum (*Sorghum bicolar* (L) Moench) was intercropped in an alternate fashion using a ratio of 1 row of sorghum to 2 rows of legume crop. There were four different legumes namely cowpea (*Vigna unguiculata* (L) Walp.), groundnut (*Arachis hypogea* L.), Bambara groundnut (*Vigna subterranea* (L.) Verdc.) and chickpea (*Cicer arietinum* L). The experiment was laid out in randomized complete block design, with four replications making a total to 20 experimental units. The individual plots were 5 m long with spacing between the rows of 0.75 m and between plants 0.30 m. The spacing between each block was 1 m and plots were labeled using pegs. Data on number of larvae per plant, number of stem tunnels, stem tunneling length and grain yield were collected. Suitability of sorghum plants grown under intercropping and monocrop was tested by measuring the content of N in plants. Data on stemborer damage, yield and nitrogen content were analyzed using mixed model and generalized linear model procedures (PROC MIXED and PROC GLM) (SAS Institute, 2008). All means of the data stated above were separated using Fisher's protected LSD test (significance level: $p \leq 0.05$). The relationship between yield and stemborer damage was tested using regression analysis. Yield loss was calculated as (1 – yield as proportion of the maximum yield) * 100 (Catangul et al., 2009). Maximum yield obtained from sorghum groundnut intercrop was used in the calculation of yield losses of different crop combinations. The relationship between nitrogen content (N) and stemborer damage on sorghum was determined using multiple regression analysis, with N as predictor variable and deadhearts, foliar damage, length of tunneling, number of tunnels and number of moth exit holes and number of larvae per plants as dependent variables. The best-fit models were selected based on stepwise procedures and the best subsets regression in Minitab® Release 17 (Minitab Inc., 2017)

Results

Nitrogen fixation and water use efficiency of indigenous herbaceous legumes

Table 1 shows levels of N_2 fixation of grain legumes at farm level in the Okavango Delta and Tswapong regions of Botswana. There were differences in levels of N_2 fixation between the two regions and between Bambara groundnut and groundnut. It is well accepted that levels of $\delta^{15}N$ less than 5‰ (Pule-Meulenberg & Dakora, 2009) indicate that plants are fixing N_2, thus, these crops formed a symbiotic N fixation with rhizobia, with the highest value of 1.8‰. Table 1 also shows that the dependence of the crops on symbiotic fixation was high, ranging between 50% and 96%. Interestingly, on hectare

Table 2: Sorghum yield and mean number of exit holes, number of tunnels, and length of tunnels caused by larvae of *C. partellus.*

Crop combination	Mean no. larvae	Mean no. exit holes	Mean no. tunnels	Mean length of tunnels	Yield (kg/ha)	§Yield loss (%)
Sorghum mono	12.50 ± 1.32a	14.75 ± 2.64a	7.10 ± 0.75a	0.50 ± 0.24a	33.86 ± 18.91b	87.86
Sorghum-cowpea	3.65 ± 0.74b	6.45 ± 1.90b	6.10 ± 0.61a	0.308 ± 0.54b	218.26 ± 46.10a	21.75
Sorghum-groundnut	4.60 ± 1.03b	7.35 ± 1.08b	4.45 ± 0.34b	0.43 ± 0.05a	278.93 ± 32.14a	0.00
Sorghum-Bambara	4.25 ± 0.65b	5.70 ± 1.08b	4.10 ± 0.37b	0.28 ± 0.37b	273.39 ± 77.37a	1.99
Sorghum-chickpea	4.35 ± 0.55b	7.05 ± 1.23b	4.05 ± 0.44b	0.31 ± 0.04b	234.82 ± 82.16a	15.81

§ No yield loss was calculated for groundnut because it was used as the maximum potential yield of sorghum gained by intercropping (see 'Materials and methods' for the formula used).

basis, actual amounts of N-fixed by these crops are very low, between 0.3 kg N/ha fixed by Bambara groundnut in Xakao and 1.3 kgN/ha by Bambara groundnut in Lekobeng, a farm area near Lecheng in the Tswapong region of Botswana. In Lekobeng, Bambara groundnut fixed more N_2 compared to groundnut, whereas the reverse was true in Xakao (Tab. 1).

Isolation and characterisation of bacteria as plant growth–promoting bacteria (PGPB)

In Botswana, after bacterial isolates were authenticated on their homologous hosts, they were all characterised for their ability to solubilise phosphate and for their cellulase activity. Figure 1 shows cultures that have been isolated from cowpea root nodules and assessed for plant growth promotion traits. Figure 1a is an example of a strain that tested positive for phosphatase solubility whereas Figure 1b was positive for cellulase activity. Both traits are detected by plate assay. Some of the strains were tested on millet in a greenhouse study where the effects of a cocktail of bacteria strains were compared to those of a positive control (NPK fertilizer application) and negative control (neither inoculum nor fertilizer application). Figure 2 shows that there were no significant differences in the number of leaves among the treatments for sandy and loamy soils. In a clayey soil, there were no differences in the number of pearl millet leaves between inoculated and NPK-fed plants, but the control plants exhibited a significantly lower number of leaves.

Pest reduction caused by intercropping with legumes

The study showed a significant increase in insect density of gramineous stemborer, *C. partellus,* on sorghum monocrop compared to the intercrops. Intercropping significantly reduced stemborer damage on sorghum compared to sorghum monocrop, where high levels occurred (Tab. 2). A significant reduction in the number of larvae per plant, number of stem tunnels, and stem tunneling length occurred with intercropping compared to monocropping. Grain yield increased significantly where sorghum was intercropped with grain legumes compared

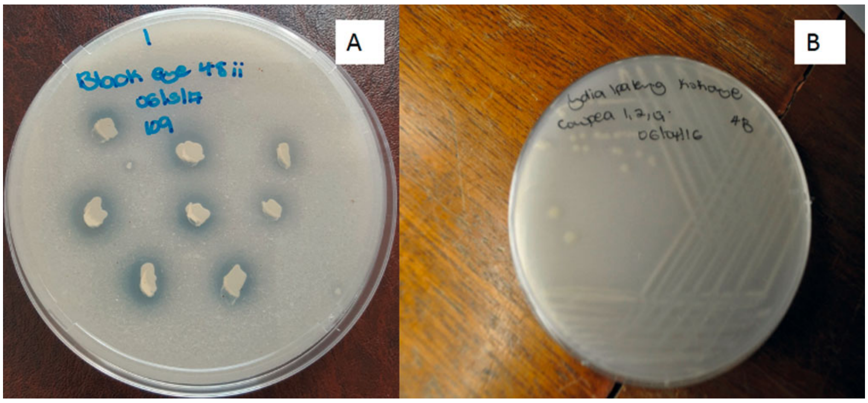

Figure 1: Plant growth promotion traits of bacteria isolated from cowpea root nodules on an agar plate/medium: (a) phosphate solubility (b) cellulase activity.

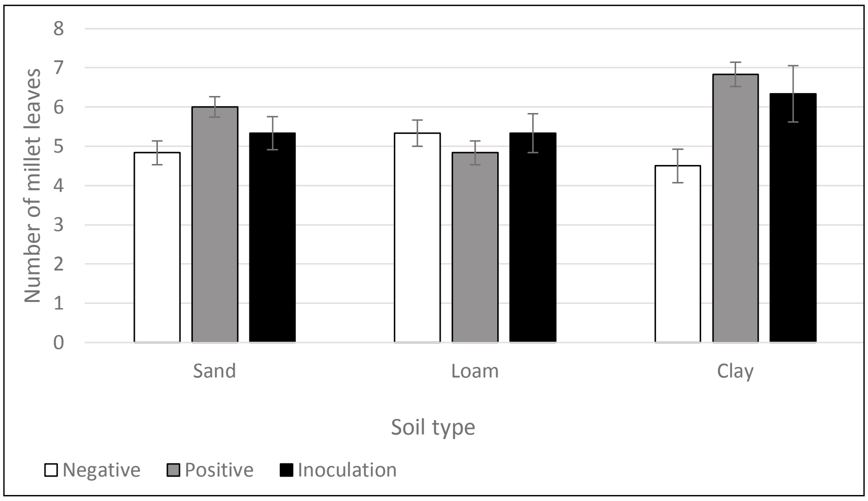

Figure 2: Interactive effect of inoculation and soil type on the number of millet leaves (mean) under greenhouse conditions. The error bars represent standard error (SE).

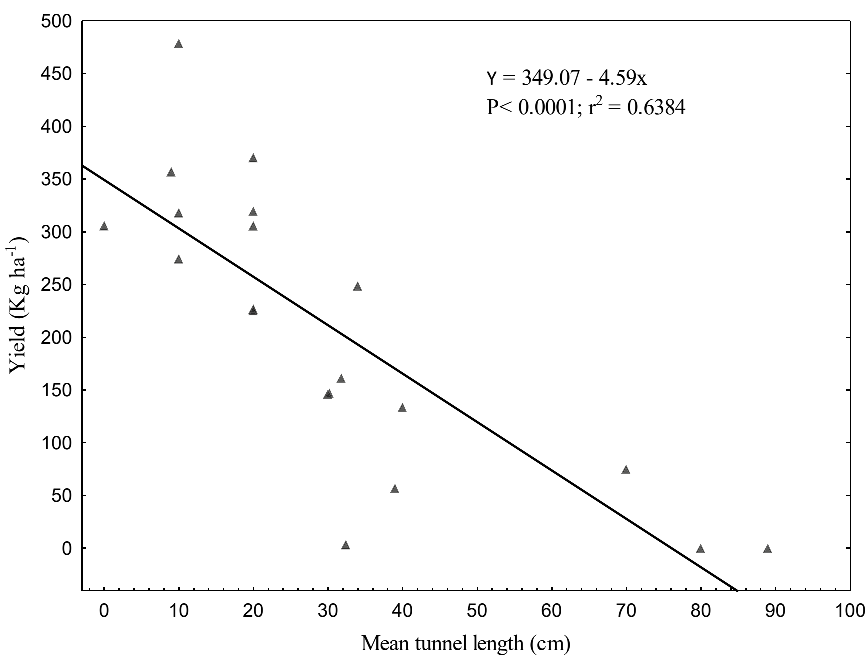

Figure 3: Relationship between sorghum grain yield and mean tunnel length caused by stemborer damage. Yield data were pooled from both mono- and intercropping treatments.

to monocrop (Tab. 2). There was a significant negative relationship between tunnel length, larval density, and yield, indicating that the increase in tunnelling caused by larval feeding significantly reduced yield (Fig. 3). Sorghum monocrop

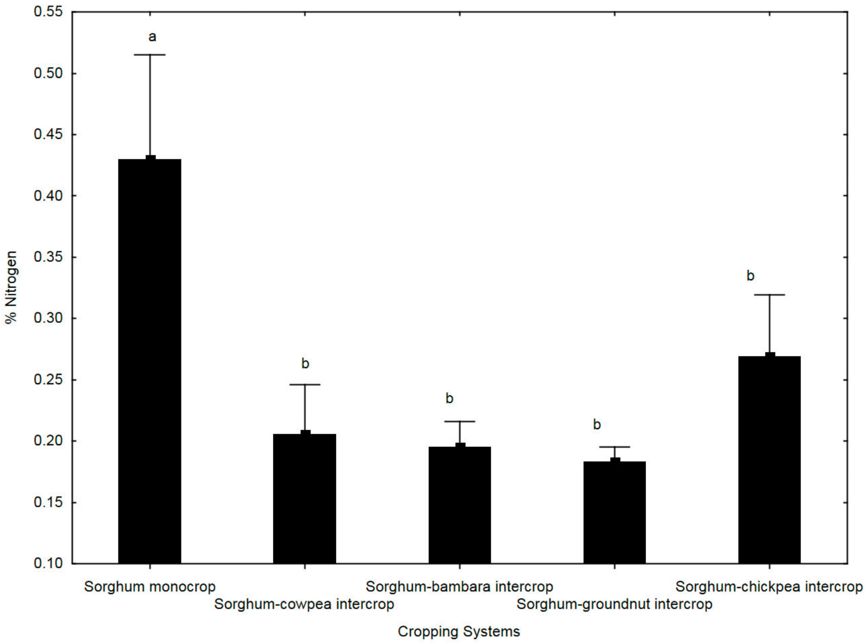

Figure 4: Nutrient composition based on percentage nitrogen content of sorghum plants under intercropping and monocropping, based on means. Letters associated with treatment indicate that a significance occurred ($p \leq 0.05$). The error bars represent standard error (SE).

had the highest yield loss of 87%, significantly higher than in sorghum-legume intercrops.

The percentage of nitrogen content of sorghum plants after physiological maturity was significantly different between crop combinations ($F_{4,52} = 10.27$; $p < 0.0001$) (Fig. 4). The highest nutrient composition was obtained from sorghum monocrop. A significant relationship was observed between the percentage of nitrogen in sorghum and damage by *C. partellus* (number of dead hearts, number of live larvae, number of moth exit holes, and tunnel length) (Tab. 3).

Discussion

Farmers in semi-arid to arid areas such as in Botswana are challenged with less rainfall, declining soil fertility, and cultivation of more marginal land. With the advent of climate change, as predicted by climate change models (Archer et al., 2018), these areas are most likely to receive even less rainfall and experience higher temperatures, worsening the current situation. These areas are characterised by low yields, which make it imperative to develop more sustainable cropping systems. In Botswana, small-scale arable farmers are among the poorest in society. It is important that they have at their disposal appropriate and inexpensive technologies for increasing their crop yields without polluting the environment.

Inoculation of non-legumes

Cereals such as sorghum and millet and pulses such as cowpea and Bambara groundnut dominate the cropping system because of their drought tolerance and the ability of pulses to replenish soil N through symbiotic fixation. Apart from fixing nitrogen biologically, legumes are known to reduce soil erosion (Giller & Cadisch, 1995) and suppress weeds (Exner & Cruse, 1993). Up to 10% of the fixed N can directly benefit a cereal intercrop, and the rest the subsequent one (Dakora & Keya, 1997). In this study, positive results of inoculating non-legumes with root nodule bacteria were obtained. Inorganic fertilisers have been implicated in polluting the environment, especially when used injudiciously. Thus, other reasons to seek alternative ways of improving soil fertility include the fact that nitrogen fertilisers pollute groundwater and increase atmospheric nitric oxide (N_2O), a potent greenhouse gas. Alternative "green" fertilisers are therefore needed to mitigate negative effects of climate change. Inoculating cereals with biofertilisers is a step in the right direction. In this study, although inoculated plants did not perform to the level of NPK-fed plants, there was an advantage compared to control plants when grown on sand and clay. More studies are needed to test different types of bacteria on various cereals under greenhouse and field conditions.

Intercropping

The results from the intercropping study showed significantly higher *C. partellus* damage in the sorghum monocrop than in the sorghum-legume intercrop. Studies in tropical and temperate zones reported decreased pest densities in diversified cropping systems (Kruess & Tscharntke, 2000). Gahukar (1989) showed that females oviposited eggs on legume crops in the intercropped system and the hatched larvae failed to reach the sorghum plants. This may account for the reduced number of larvae and tunnelling on sorghum in the intercrops in the current study. With alternate row arrangements of host and non-host plants used in our study, the ovipositing female and dispersing larvae

Table 3: Relationship between host quality (%N) and various sorghum damage by *C. partellus*. The independent variable (predictor) was nitrogen content while the stemborer damages were dependent variables (data were pooled across intercrop combinations).

Stemborer damage	Intercept ± SE	Slope ± SE	r^2	*P*
% whorl damage	3.71 ± 3.98	0.470 ± 0.51	0.22	0.421
No. dead hearts	7.69 ± 1.26	0.96 ± 0.16	0.93	0.009
No. live larvae	29.32 ± 3.54	0.98 ± 0.12	0.94	0.004
No. moth exit holes	29.29 ± 2.58	0.98 ± 0.087	0.97	0.001
No. stem tunnels	9.21 ± 3.50	0.84 ± 0.32	0.70	0.078
Tunnel length	0.67 ± 0.22	0.87 ± 0.28	0.76	0.050

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reportedly move easily within rather than between rows, explaining the low density of larvae on sorghum legume intercrop (Chabi-Olaye et al., 2005). The low densities of stemborer larvae on sorghum legume intercrop support the 'disruptive crop hypothesis' in which a second non-host plant species is suggested to affect the ability of the pest to find its proper host plant species through reduced chemical and visual cues and stimuli (Finch & Collier, 2000).

The lower larval densities in the intercropping system may additionally be explained by host plant quality. A study by Baidoo (2004) found that a higher nitrogen content of the stem of maize varieties resulted in a more severe stemborer infestation on these varieties. These results support our findings showing that plants with the highest percentage of nitrogen suffer great damage from the stemborer larvae (Fig. 4). Sorghum monocrop plants had the highest percentage of N and sustained the highest damage levels compared to sorghum-legume intercrops. Elevated nitrogen levels have been found to increase both the survival and the fecundity of stemborers, *Sesamia calamistis* (Setamou et al., 1995).

A significant increase in nutrient content in monocrop sorghum compared to intercrops agree with the host plant quality hypothesis, which states that intercropping negatively affects the host quality and the chemical suitability of the plants for herbivores when compared to the monocultures (Bach, 1981; van Lenteren, 1998). Yield in our study was negatively correlated with stem tunnelling by stemborer, which is known to injure the meristematic tissues of the plant, leading to a reduction in the yield (Bosque-Pérez & Mareck, 1991). In Africa, yield reduction by stemborer feeding and tunnelling can fall between 10% and 100%, depending on the season and status of the plant (Ndemah & Schulthess, 2002). In the current study, yield loss associated with sorghum monocrop was 87.9%, falling within the range reported by van den Berg (2009). Research by (Chabi-Olaye et al., 2005) showed that 3–8 times more stem tunnelling was recorded in maize monocrop, with high stemborer larval densities and yield loss (1.8–3.0 times greater), than in the intercropped system. In our study, larval densities were up to 3 times more in the sorghum monocrop than in the intercrops.

Results from this study have shown that bacteria isolated from the root nodules of legumes have potential as biofertilisers, as shown by positive growth of sorghum and pearl millet when inoculated with bacterial cocktails. Bacterial assays for phosphate solubilisation and cellulase activity confirmed mechanisms of plant growth promotion. Furthermore, intercropping of sorghum with legumes led to a reduction in stemborer infestation on the sorghum, leading to higher yields. In conclusion, our data reveal the potential for the use of microorganisms in sustainable cropping systems as biofertilisers. However, further studies are required to test the bacteria under various scenarios in the field. The data on intercropping that show reduced stemborer infestation under intercropping are directly applicable and should be packaged for use by smallholder farmers, as stemborers are important pests of sorghum in Botswana.

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